Role of Neuropeptides in Inflammatory Bowel Disease

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Abstract: Inflammatory bowel disease (IBD) is a chronic, relapsing condition involving complex interactions between genes and the environment. The mechanisms triggering the initial attack and relapses, however, are not well understood. In the past several years the enteric nervous system (ENS) has been implicated in the pathophysiology of IBD. Both the ENS and the central nervous system (CNS) can amplify or modulate aspects of intestinal inflammation through secretion of neuropeptides that serve as a link between the ENS and CNS. Neuropeptides are defined as any peptide released from the nervous system that serves as an intercellular signaling molecule. Neuropeptides thought to play a potentially key role in IBD include substance P, corticotropin-releasing hormone, neurotensin, vasoactive intestinal peptide, mu-opioid receptor agonists, and galanin. This review focuses on the role of these neuropeptides in the pathophysiology of IBD and discusses the cell types and mechanisms involved in this process. The available evidence that neuropeptide blockade may be considered a therapeutic approach in both Crohn's disease and ulcerative colitis will also be discussed.

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Inflammatory bowel disease (IBD) is a chronic, relapsing condition involving complex interactions between genes and the environment.[1,2] The mechanisms triggering the initial attack and relapses, however, are not well understood. In the past several years the enteric nervous system (ENS) has been implicated by several groups in the pathophysiology of IBD.[3,4] The ENS is an extensive and diffuse network of millions of sensory neurons, interneurons, and motor neurons, which control a variety of functions within the gastrointestinal (GI) tract.[5] Unlike the various other nervous systems in the body, the ENS can work without central input from the brain and is often considered "the brain-in-the gut."

The central nervous system (CNS) may also amplify or modulate several aspects of intestinal inflammation through stimulation of the hypothalamic–pituitary axis (HPA)[6–8] and secretion of neuropeptides that serve as a major link between the ENS and CNS. Neuropeptides are defined as peptides released from the nervous system that serve as intercellular signaling molecules. In this complex system, secretion of a single neuropeptide can be influenced by other neuropeptides, neurotransmitters, cytokines, hormones, and drugs, which in turn interact with specific receptors in several cell types to generate or influence central and peripheral responses. Conversely, when released at the nerve endings in the intestine, neuropeptides diffuse into surrounding tissues and bind to their corresponding receptors, affecting nearby muscle, epithelium, endothelium, and immune cells.[9] Consequently, organs with a high density of neuropeptide receptors, such as the intestine, are particularly responsive to neuropeptides and their effects.[10] This review focuses on the pivotal role of neuropeptides in the pathophysiology of IBD and, as such, whether neuropeptide blockade may be considered a therapeutic approach in both Crohn's disease (CD) and ulcerative colitis (UC).

IMMUNE DYSREGULATION, NEUROPEPTIDES, AND IBD

Immune System and the Enteric Nervous System

It is well established that the immune system plays a key role in the pathophysiology of IBD and that CD and UC have distinct immune profiles that are linked to their specific pathogenesis. In general terms, CD is characterized by inflammation associated with a Th1 response,[11–13] while UC is based on a Th2 inflammatory response.[12,14,15] More recently, the regulatory role of the neuroenteric immune axis in intestinal inflammation is gaining recognition.[16–18] The mechanism by which the neuroendocrine system communicates with the immune system, creating a "neuro-immune dialogue," is through the release of cytokines and neuropeptides to end organ receptors.[19] While the major source of neuropeptides are neurons, immune cells such as lymphocytes, macrophages, and enteroendocrine cells are also responsible for neuropeptide synthesis in the GI tract. Additionally, several immune sources for substance P (SP), vasoactive intestinal

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peptide (VIP), and corticotropin-releasing hormone (CRH) serve as both targets and effectors.[16] Neuropeptides directly influence a number of immune functions, including immunoglobulin production, lymphocyte mitogenesis, chemotaxis, phagocytosis, neutrophil lysosomal release and migration, and homing patterns of immune effector cells.[19,20] Several studies also show that nerve–mast cell communication plays a major role in the pathophysiology of intestinal inflammation.[20]

The immune system uses several pathways and sites of entry to communicate with the brain and activate the HPA.[21,22] Inflammatory cells are located in the lamina propria of the gut, in close contact with enteric nerves, demonstrating an anatomical connection between enteric nerves and inflammatory cells.[15] Evidence of peripheral nerve fibers showing close anatomical association and functional communication with immune cells has not only been documented for lymphoid organs, but also for tertiary sites like mucosal surfaces. Neuroimmune communication is also facilitated via enteroglia, which are abundant and in close proximity to nerves, immune cells, and blood capillaries, enabling them to act as immunomodulatory cells in the ENS.[23] Enteroglia are also able to respond to neurotransmitters, putting them in a prime position to modulate gut immune response based on the balance of neural input signals.[24]

Nervous System, Immune System, and IBD

Functional communication between enteric nerves with hormones and neuropeptides has also been shown to play a major role in the pathophysiology of IBD. Clinically, this connection has been suspected for some time. One of several interesting anecdotal reports involves a man with previously refractory UC who went into complete remission following a Brown-Sequard paralysis at the level of C5.[25] Animal studies have shown similar connections. Thus, colitis in rats is exacerbated if sensory nerves are disrupted by pretreatment with capsaicin. Additionally, targeted ablation of enteric glial cells in mice leads to fulminant jejuno-ileitis,[26] underscoring the importance of these cells in the maintenance of the enteric mucosa and suggesting an important role for them in the pathophysiology of IBD.

Neural involvement in IBD is illustrated by colonic nerve damage and changes in mucosal innervation and neuropeptide expression during intestinal inflammation.[27,28] Neuronal changes seen in CD include hyperplasia of the ganglion cells, extensive axonal degeneration, changes in gut neuropeptide content, and infiltration of the myenteric plexus with plasma cells, mast cells, and lymphocytes.[29,30] Patients with long-standing UC have similar but less evident alterations. These changes are seen in both humans and experimental animal models.[18,31,32]

Changes in the ENS and extrinsic innervation in a formalin-induced colitis model in rats demonstrated an increase in c-fos, a marker of neuronal activation, in myenteric neurons, enteric glia, as well as in the brainstem and spinal cord.[33,34] These observations led to studies showing a link between the immune and nervous systems in IBD. Experimental colitis in guinea pigs, for example, is associated with a 20% loss of myenteric neurons primarily associated with infiltration of neutrophils, eosinophils, and lymphocytes in myenteric ganglia.[18] While the mechanism of indiscriminate neuronal loss in experimental colitis is not completely understood, administration of antineutrophil serum in 2,4,6 trinitrobenzene sulfonic acid (TNBS)-induced colitis results in decreased inflammation and attenuates the loss of myenteric neurons,[18] and steroids also cause a significant decrease in cell loss in TNBS-mediated colitis.[35]

The neuroenteric immune axis is a complex system consisting of different cell types and neuropeptides and their receptors that interact at multiple levels, making it difficult to navigate.[16] Neurotransmitters contained in neurons of the ENS are capable of effecting multiple inflammatory cells,[36] while lymphocytes and other inflammatory cells express receptors for hormones and neuropeptides of the HPA.[36] Moreover, different neuropeptides are often colocalized in the same neurons and regulate similar functions or activate similar or identical pathways. This review will focus on the functions of the most well-studied individual neuropeptides and peptide hormones for their potential roles in the pathophysiology of IBD. The mechanism(s) of action of these peptides as they relate to IBD pathogenesis will also be discussed.

SUBSTANCE P AND ITS NEUROKININ-1 RECEPTOR IN INTESTINAL INFLAMMATION AND IBD

SP, an 11 amino acid peptide originally isolated by Chang and Leeman[37] from bovine pituitary, is widely distributed in the CNS and the periphery.[16] The gut is one of the most abundant sources of SP in the body, where it is expressed in the myenteric and submucosal plexi, as well as in the dorsal root ganglia and intrinsic and extrinsic sensory neurons.[38–40] Immune cells such as monocytes,[41] lamina propria macrophages,[42] eosinophils,[43] and lymphocytes[44] also express SP. The effects of SP are mediated by 3 receptors belonging to the G-protein receptor superfamily, neurokinin (NK)-1, -2, and -3. The NK-1 receptor is the high-affinity receptor for SP, while NK-2 and NK-3 bind with much lower affinity to this peptide (see Table 1).[45] NK-1 receptors are expressed abundantly in the GI tract and the colon in multiple cell types, including nerves of the ENS,[40,45] smooth muscle,[39] immune,[10,42] endothelial,[46] and epithelial cells.[47,48] Since primarily NK-1, but not NK-2 or NK-3, receptors have been linked with intestinal inflammation and IBD, focus in this review will be given to SP-NK-1 receptor interactions.

TABLE 1. Neuropeptides, Their Receptors, and Major Locations in the Intestine and Colon

Neuropeptide	Receptors	Main Neuropeptide Locations in the Gut
Substance P (SP)	Neurokinin-1[a], 2, 3	Myenteric and submucosal plexus of the gut, dorsal root ganglia, intrinsic and extrinsic sensory neurons, monocytes, lamina propria macrophages, eosinophils, lymphocytes
Corticotropin-releasing hormone (CRH) Urocortins I, II, III (UI, II and III)	Corticotropin-releasing hormone receptor I and II CRH: CRHR1[a] UI:CRHR1 = CRHR2 U11 and 111:CRHR2[a]	Enterochromaffin cells, myofibroblasts, autonomic ganglia, and extrinsic nerve cells
Neurotensin (NT)	Neurotensin Receptor I[a] and II	Throughout the intestine: N cells localized among epithelial cells of the jejunum and ileum
Vasoactive intestinal peptide (VIP)	VPAC1 and VPAC2	All layers of the colon, with highest concentration in the myenteric plexus: primarily localized to neurons, but also in T cells and eosinophils. Also abundant in smooth muscle sphincters of the lower esophagus, ampulla of Vater and the rectum.
Opioids	Mu-opioid receptor (MOR)	MOR locations: mesenteric and submucosal plexi in the ileum and colon
Galanin	Galanin receptor 1(Gal1R),[a] Gal2R, Gal3R	Enteric nerve terminals

[a]High-affinity receptor.

Expression of SP and NK-1 Receptors in IBD

Several studies point to an important role for SP and the NK-1 receptor in the pathophysiology of IBD. Increased SP expression has been observed in both tissue[49–51] and nerve fibers in the colons of patients with UC.[40,52] Increased SP levels in UC patients are also correlated with disease activity,[40] most evidently in mild to moderate colitis,[52] while in severe UC the density of SP immunoreactive nerves appears to be decreased.[53,54] Other studies, however, failed to demonstrate altered SP immunoreactivity in the colonic or rectal mucosa in UC patients versus controls.[19,45,55] Contradictory results have also been produced in biopsy tissues from CD patients. Mucosal SP levels have been found to be significantly increased,[50] decreased,[51] or no different[56] between CD and control patients. Although the reason for these contradictory results is not clear, use of tissues from different stages of the disease, different drug modalities of the subject population, or technical approaches may play a role.

The expression of NK-1 receptors has also been observed to vary at virtually all levels of the intestine, mostly based on whether samples are taken from inflamed versus noninflamed tissue and from CD versus UC patients.[40] In certain studies evaluating UC and CD biopsy specimens, receptor binding sites for SP are elevated in arterioles, venules, and lymph nodules,[57] as well as in enteric neurons, submucosal blood vessels, lamina propria inflammatory cells, and epithelial cells,[48] suggesting that the SP-NK-1 receptor system might represent a major immunoregulatory circuit involved in IBD. However, in other studies expression of NK-1 receptors in UC is confined to pathologically active colon biopsies, while NK-1 receptor upregulation in CD is evident in both pathologically positive and negative samples.[58] Upregulation of NK-1 receptors in macroscopically uninvolved tissues may help explain the high risk for recurrence after surgical resection of CD, as well as the well-known propensity of CD to relapse after drug-induced remission.[48] Additionally, increased NK-1 receptor binding sites are observed on enteric neurons of patients with both active and nonactive CD, but not on enteric neurons of UC patients.[58] More pronounced NK-1 receptor expression in the myenteric plexus and enteric neurons in CD versus UC might indicate different neural mechanisms involved in the pathophysiology of the 2 entities.[58] However, these results have also been conflicting. Renzi et al[48] found no difference in the extent of NK-1 receptor upregulation in CD versus UC patients.

Role of SP in Experimental Colitis

Studies with animal models strongly suggest a functional role for SP and its high-affinity receptor in the pathophysiology of IBD. SP levels are increased in the peripheral blood of rats with Dextran sulfate sodium (DSS) colitis.[15] SP immunoreactivity is decreased in the entire colon early after intracolonic TNBS administration, but increased SP expression is evident in the circular muscle 7 days after the induction of colitis, suggesting its possible involvement in tissue repair mechanisms.[59] Injection of an NK-1 receptor antagonist to rats decreases colonic inflammation and oxidative

stress in response to DSS,[60] and affecting both colitis severity and contractility alterations 14 days after intracolonic TNBS administration.[61] Additionally, experimental colitis can be significantly suppressed with SR 140333, a specific NK-1 receptor antagonist.[62] NK-1 receptors, both at the mRNA and protein levels, are upregulated in an ileal pouch/anal anastomosis model in rats and administration of an NK-1 receptor antagonist inhibited development and clinical signs of pouchitis in this model.[63] A dramatic early increase in NK-1 receptors is also observed in the rat ileal mucosa during *Clostridium difficile* toxin A inflammation with particularly increased expression in intestinal epithelial cells[47] being observed in patients with *C. difficile*-associated colitis,[64] an infection associated with relapses in IBD patients.[65]

The mechanism of NK-1 receptor upregulation during intestinal inflammation is likely associated with increased cytokine expression, a consistent intestinal response in IBD patients.[66] Thus, in human macrophages the proinflammatory cytokines IL-1β and TNFα increase expression of NK-1 receptors via a mechanism involving the transcription nuclear factor κB (NF-κB), via NF-κB binding sites present in the promoter region of the human NK-1 receptor (see Fig. 1).[67] Consistent with this observation, NF-κB also modulates NK-1 receptor expression in T cells in response to IL-12 and IL-18 stimulation, while the cytokine IL-10, a downregulator of the Th1 response, blocks this activation.[68] Interestingly, NF-κB plays a major role in the pathogenesis of the inflammatory response in IBD,[69] and NF-κB activation during DSS colitis in rats precedes upregulation of NK-1 receptors,[70] further suggesting an important role of the NF-κB system in SP-NK-1 receptor interactions during colonic inflammation.

Another potential SP-NK-1R mechanism was revealed in a study evaluating colonic mucosa explants from IBD patients that showed increased mucosal mast cell mediator secretion in response to SP compared to normal colonic mucosa.[71] This indicated a functional SP–mast cell interaction in IBD. Supporting this concept, Riegler et al[72] reported that SP, via NK-1R, stimulates histamine release from normal human colonic mucosa and these mediators modulate chloride secretion in response to SP in this tissue.

Studies with genetically engineered animals lacking NK-1 receptors provided direct evidence for the importance of these receptors in the development and progression of IBD-like colitis as well as in other forms of intestinal inflammation. For example, NK-1R-deficient mice have substantially less histologic inflammation, neutrophil accumulation, and mediator release during the acute phase of TNBS-induced colitis,[73] and have diminished inflammatory diarrhea in response to *C. difficile* toxin A.[73] IL-10 knockout mice have diminished intestinal inflammation in response to the nonsteroidal antiinflammatory drug (NSAID) peroxicam when administered with an NK-1R antagonist, even after inflammation is established.[74] Studies with NK-1R-deficient mice also underscored the importance of NK-1 receptors in TNFα-dependent neutrophil accumulation in experimental cutaneous inflammation[75] and antigen-elicited IFN-gamma production from T cells.[76] Conversely, compared to wildtype, mice lacking neutral endopeptidase (NEP), an enzyme that degrades SP, have substantially worse TNBS colitis and toxin A-associated ileitis that can be reversed by administration of recombinant NEP or a specific NK-1 receptor antagonist.[62,77] Mast cell-deficient mice also have reduced intestinal secretion and inflammation thought, in good part, to be due to SP-NK-1 receptor-dependent pathways.[78]

IBD, Adipose Tissue, and NK-1 Receptors

Patients with CD accumulate intraabdominal fat from the onset of the disease, indicating that mesenteric obesity may be an important IBD feature.[79] Fat hypertrophy and fat wrapping on the bowel, commonly known as "creeping fat," have long been considered a hallmark of CD and correlate significantly with the degree of transmural inflammation.[79] Moreover, intraabdominal fat is characterized by increased expression of PPAR-γ and TNF-α,[80] suggesting that mesenteric depots could participate in the inflammatory response via release of proinflammatory cytokines. Recent results indicate that mucosal inflammatory changes following intracolonic administration of TNBS in mice is associated with increased expression of proinflammatory cytokines and the NK-1 receptor in the proximal mesenteric fat depots.[81] Moreover, human mesenteric preadipocytes express NK-1R, both at the mRNA and protein levels, and SP exposure leads to increased NK-1 receptor and IL-8 expression and protein secretion, indicating that mesenteric fat depots may participate in intestinal inflammatory responses via SP-NK-1 receptor-related pathways.[81]

Cellular Mechanisms of SP-Dependent Proinflammatory Responses

Studies in SP-NK-1R interactions in cellular systems indicate that SP can exert direct proinflammatory responses in target cells, including secretion of IL-1β,[82] IL-6,[83] and the potent chemoattractants IL-8[81,84] and TNF-α.[42] A major molecular mechanism for SP-dependent induction of proinflammatory genes is represented by activation of the NF-κB system.[84–86] The SP-NF-κB signaling pathway also involves activation of MAP kinases and the Rho family of proteins[83,84] and phosphorylation and activation of PKCδ (see Fig. 1).[87] SP-NK-1 receptor coupling in human colonocytes also stimulates COX-2 gene expression and PGE2 release, an effect mediated via activation of JAK2 and PKCΘ and STAT binding sites on the promoter region of the COX-2 gene.[88] COX-2 expression is also elevated in colon of mice during experimental colitis, and this is normalized by administration of a specific NK-1R antagonist,[88] while SP, via NK-1R, stimulates prostaglan-

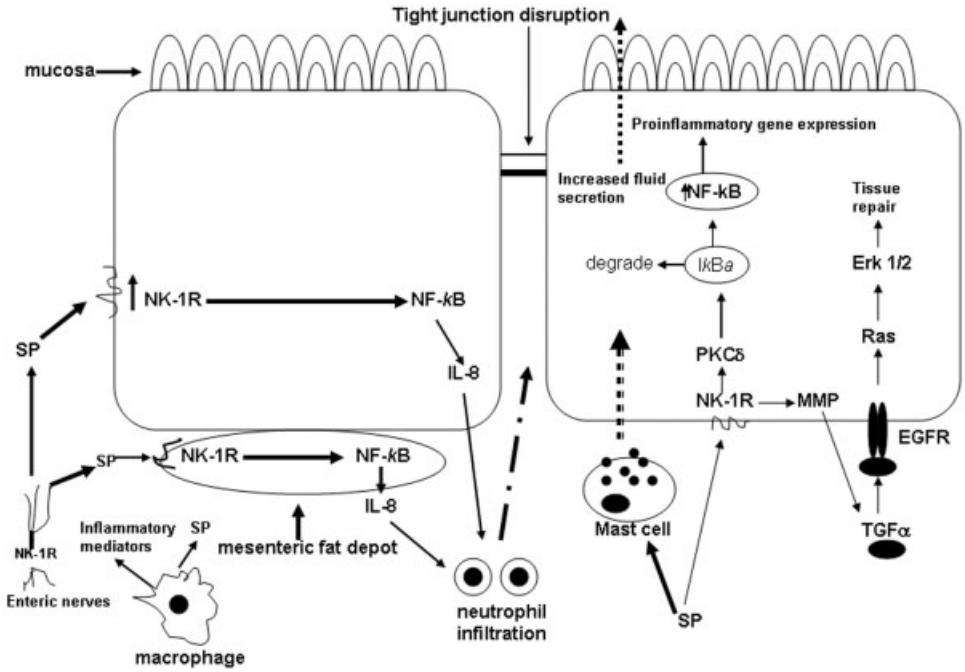

FIGURE 1. Major effects of substance P (SP) on neuroimmune signaling during IBD-induced intestinal inflammation and repair. SP is released from various areas of the gut, including, but not limited to, the myenteric and submucosal plexi and enteric nerves. Once released, SP binds to NK-1R on colonic epithelial cells activating both proinflammatory and tissue repair/cell proliferation pathways. SP can activate protein kinase C δ, causing degradation of IκBα and activation of the NF-κB system with resulting increased synthesis of NF-κB-driven proinflammatory genes, such as interleukin-8 (IL-8). IL-8 and other potent chemoattractants released from epithelial and lamina propria cells cause neutrophil infiltration and release of potent neutrophil mediators. At the same time, SP can also activate pathways important in tissue repair where, by binding to NK-1R, SP induces matrix metalloproteinase (MMP) activity that causes release of TGFα, which subsequently binds to the epidermal growth factor receptor, activating the pathway leading to cell proliferation and tissue repair. Colitis is also found to induce upregulation of the NK-1 receptors as well as an upregulation of proinflammatory cytokines, including IL-8, presumably through the NF-κB system in both the colon and the mesenteric fat depots. Many inflammatory cells are also affected in IBD. Shown above are mast cells that have increased mucosal mast cell mediator secretion in response to SP compared to normal colonic mucosa. Additionally, macrophages play a dual role in being able to secrete SP and also secrete proinflammatory cytokines necessary to upregulate the NK-1R.

din release from normal human colonic mucosa that is involved in chloride secretion in response to SP.[72]

SP-NK-1 Receptor Interactions Promote Mucosal Healing

In addition to its proinflammatory actions in cellular systems and experimental IBD models, recent studies indicate that SP and the NK-1 receptor may play a protective role in mucosal regeneration and healing in the recovery phase of colitis. For example, mice genetically deficient in NK-1 receptors have enhanced histologic and clinical signs of colitis, elevated mucosal cytokine production, and increased mortality in both TNBS and DSS colitis models.[73] These responses are associated with an SP-dependent epidermal growth factor receptor (EGFR) transactivation leading to cell proliferation in colonic fibroblasts[73] and human colonic epithelial cells.[89] SP also triggers cell mitosis in other cell types, including T cells,[90] human astrocytoma,[91] and smooth muscle cells.[92] In addition, NK-1 receptor coupling by SP in human colonocytes leads to a prompt release of matrix metalloproteinases and secretion of TGFα, but not other EGFR ligands, leading to EGFR and MAPK activation and cell proliferation (see Fig. 1).[89]

Therapeutic Implications for NK-1 Receptor Antagonism in IBD

The ability of proinflammatory cytokines to modulate expression of NK-1 receptors on colonic mucosa cells, the increased expression of SP seen in some studies of IBD patients, and the evidence presented here involving animal models of IBD intestinal inflammation suggests that these receptors represent a potential therapeutic target for the treatment of IBD. NK-1 receptor antagonists are expected to decrease SP-mediated activation and mediator release from several immune and inflammatory cell types, as well as reduce ion secretion, intestinal permeability, and the increased colonic motility frequently seen in IBD patients. Development of highly specific neurokinin receptor antago-

nists by several major pharmaceutical companies and their current use in rheumatoid arthritis, depression, anxiety, and chemotherapy-associated emesis opens up the possibility for their potential use in intestinal inflammation and IBD. However, results from preliminary human clinical trials assessing the utility of these antagonists for treatment of IBD are currently not available. Moreover, the dual role of NK-1 receptors in contributing to 2 opposing intestinal processes, namely inflammation and tissue repair, following colitis raises the possibility that their effectiveness may be limited to particular IBD subgroups. Clearly, controlled clinical studies are needed to further examine their potential uses in this area.

ROLE OF CENTRAL AND PERIPHERAL CRH AND UROCORTINS IN INTESTINAL INFLAMMATION

CRH is a 41 amino acid neuropeptide[93] that functions as the major physiologic adrenocorticotropin hormone (ACTH) secretagogue in the human body, leading to the release of corticosteroids.[93,94] CRH is known to play a pivotal role in the coordination of endocrine, behavioral, and immune responses in a variety of psychological conditions, such as stress and depression.[95,96] Several other members of the CRH family of peptides have been recently recognized, including Urocortins I, II, and III, with various degrees of homology to CRH. The biological effects of CRH and urocortins (Ucn) are mediated by 2 different G-protein-coupled receptors, CRHR1 and CRHR2, with substantial sequence similarities,[97] but distinct affinities for mammalian CRH and specific urocortins. Thus, UcnI binds with high affinity to both CRH receptors, while CRH has higher affinity for CRHR1 than CRHR2 (see Table 1).[97] In contrast, UcnII and UcnIII bind selectively to CRHR2.[98] Interestingly, these 2 classes of receptors are also distributed differentially in the brain and the periphery, indicating mediation of different biological functions.

Central CRH and Modulation of Intestinal Inflammation

Centrally, CRH is found in the spinal cord and is synthesized by neurons in the brain[99] with its main source in the paraventricular nucleus (PVN) of the hypothalamus. CRH and the urocortins are also expressed in the periphery and most prominently in the GI tract,[100] where they are produced by enterochromaffin cells,[101] myofibroblasts, autonomic ganglia, and extrinsic nerve cells.[102,103] Stress has long been implicated in the progression and exacerbations of IBD, and several studies indicate that central CRH and its receptors might participate in IBD-related responses. For example, intracolonic administration of TNBS induces similar degrees of colitis in rat strains with hypo (Lewis/N) and hyper (Fischer344/N) CRH responses. Colitis is inhibited by intracerebroventricular (icv) injections of CRH in both rat strains, while increased colonic inflammation is observed by central injection of astressin, a CRH receptor type 1 and 2 antagonist.[104] Central CRH also reduces the proinflammatory action of stress in this model of colitis, indicating a protective role in the stress-associated aggravation of experimental colitis.[104] However, another study failed to show involvement of CRH in stress-induced aggravation of experimental colitis.[105] Increased CRH is also present in magnocellular hypothalamic neurons during TNBS-associated colitis.[106] In this model, enhanced activity in brain nuclei involved in the autonomic, behavioral, and neuroendocrine responses is noted that is associated with activation of CRH and CRHR1, but not CRHR2 mRNA transcripts in the PVN of the hypothalamus.[107] Acute colitis also leads to increased CRH expression in the PVN and this effect is maintained following recovery from colitis.[108]

CRH Is a Proinflammatory Neuropeptide in the Intestine

CRH also acts on specific receptors in peripheral sites of the immune system, influencing peripheral inflammation,[109] as well as several immune functions,[110] by acting directly on immune and inflammatory cells.[7] CRH, related peptides, and their receptors have been convincingly implicated in gastric motor function,[111] intestinal and colonic motility, and mucosal function.[112] Recent evidence from animal studies also indicates that CRH might play a role in intestinal inflammation. Wlk et al[113] reported a significant increase in CRH and both of its receptors in mouse ileum injected with toxin A from *C. difficile*. Peripheral injection of CRH receptor antagonists directed against both receptors, or specifically against CRHR1, dramatically reduced intestinal secretion and inflammation in response to this toxin.[113] Interestingly, mice genetically deficient in CRH have diminished intestinal responses to *C. difficile* toxin A,[114] while CRH silencing in the ileum using RNA interference also resulted in reduced secretion and neutrophil activation in response to toxin A.[115]

CRHR2 and its specific ligand, UcnII, also appear to mediate proinflammatory responses in the gut. CRHR2 null mice have substantially reduced intestinal inflammation and lower intestinal levels of chemoattractants keratinocyte derived chemokine and monocyte chemoattractant protein-1 following intraluminal exposure to *C. difficile* toxin A. This effect can be reproduced by administration of astressin 2B, a selective CRHR2 antagonist, prior to toxin A exposure, in wildtype mice.[116] Additionally, human colonic epithelial HT-29 cells express CRHR2α mRNA.[116] Thus, CRHR2 mediates intestinal inflammatory responses via release of proinflammatory mediators at the colonocyte level. Together, these results indicate an important role for CRH and the CRH receptor family in the development and progression of acute enterotoxin-mediated intestinal inflammation.

CRH and IBD Models

Recent results indicate that the CRH system is also involved in the inflammatory response associated with IBD. Increased CRH mRNA and protein expression is present in the rat cecum after peptidoglycan-induced colitis in inflammatory, mesenchymal, and neuronal cells.[117] Increased numbers of CRH immunoreactive cells are also present in macrophages and enterochromaffin cells in the colonic mucosa of human IBD patients.[101,118,119] Colonic biopsies from patients with active UC show significantly increased CRH immunoreactive lamina propria mononuclear cells and macrophages.[119] Additionally, Ucn expression is increased in colonic lamina propria mononuclear cells in UC patients and positively correlated with the severity of colitis.[120] Ucn treatment also protects mice against chronic TNBS colitis and reduces Th1 cytokine levels in this model.[121] These studies indicate that CRH and its family of peptides may play a proinflammatory as well as a protective role in the development of colitis.

The peripheral mechanisms by which CRH and Ucns might be involved in the development and progression of colitis are not entirely clear. However, based on the available evidence discussed above, the type of inflammatory stimulus, intestinal cell phenotype, type of CRH receptor, and phase of colitis (acute versus chronic) all appear to play a role. Clearly, CRH and UcnII possess direct proinflammatory responses in target cells. CRH can stimulate activation of NF-κB,[122] a transcription factor associated with activation of proinflammatory genes and IBD pathogenesis. UcnII stimulates expression of the proinflammatory chemoattractants IL-8 and MCP-1 in colonocytes.[116] However, in contrast, UcnII also enhances IL-10 and decreases TNFα secretion in murine RAW264.7 macrophages,[123] and UcnI and II, via CRHR2, induce macrophage apoptosis,[124] all potent antiinflammatory responses. Moreover, treatment of murine macrophages with CRH and Ucn I and II results in CRHR2-mediated upregulation of TLR4 expression,[125] an important pathogen recognition receptor that plays a key role in the pathophysiology of IBD.[126] Together, these results suggest that CRH and related peptides may represent an important component of innate immunity and play a role in the therapy of IBD. Clearly, however, more research needs to be done before application of CRH receptor blockade in IBD can be considered.

NEUROTENSIN IN INTESTINAL INFLAMMATION AND IBD

NT and Its Receptors in the GI Tract

Neurotensin (NT) is a 13 amino acid peptide[127] synthesized in large amounts throughout the length of the intestine and in smaller amounts in the brain.[128,129] In the small intestine NT is produced by specialized cells, called N cells, that are localized among epithelial cells of the jejunum and ileum.[130] NT mediates its functions by binding to 2 specific G-protein coupled receptors, NT receptor 1 (NTR1)[131] and 2 (NTR2).[132] Studies with nonpeptide NTR1 antagonists indicate that this receptor subtype mediates most of the intestinal responses of NT. NTR1 is present in the animal and human intestine,[133,134] colonic adenocarcinoma cells,[135] and in nontransformed human colonocytes (see Table 1).[136]

Expression of intestinal NT and NTR1 is altered during different disease states. For example, the small intestine of celiac disease patients secretes increased levels of NT.[137] Colonic NTR1 mRNA is reduced during immobilization stress in rats,[138] a response associated with increased circulating NT.[139] In contrast, increased colonic NTR1 and NT expression was evident during intestinal secretion and neutrophil infiltration in response to *C. difficile* toxin A in the rat ileum before secretory and inflammatory changes in the rat colon were evident.[133] Colonic NT and NTR1 mRNA and protein expression are also elevated following DSS administration, after inflammatory changes in this animal model are established.[134] Most importantly, Brun et al[134] showed the presence of a small number of NTR1-positive cells in normal colonic mucosa and increased expression of these receptors at the protein and mRNA levels in colonic biopsies from UC patients. NTR-1-positive cells were also primarily expressed in colonic epithelial crypt cells as well as in cells in the colonic lamina propria in IBD patients.[134]

NT Mediates Inflammatory Responses in the Small Intestine and Colon

A limited number of studies indicate that NT is involved in inflammatory responses in the gut. The first functional evidence was reported by Castagliuolo et al,[138] who showed that peripheral injection of the NTR1 antagonist SR 48692 inhibited colonic fluid secretion, permeability to mannitol, neutrophil transmigration, and mast cell activation in response to *C. difficile* toxin A. Experiments with colonic explants also indicate that this proinflammatory effect is in part SP-dependent,[138] suggesting a functional communication between these 2 peptides in the development of colitis. The proinflammatory effects of NT may involve direct interactions of this peptide with several intestinal cell types, including colonic epithelial cells. Thus, in colonic epithelial cells NT-NTR1 interactions stimulate transcription of the potent chemoattractant IL-8 and this effect involves activation of the transcription factor NF-κB via mobilization of calcium stores.[136] NT-induced NF-κB activation and IL-8 gene transcription involves activation of PKCα,[140] the Rho family of small GTP-binding proteins RhoA, Rac1, and Cdc42,[141] as well as Ras.[136] Interestingly, activation of the NF-κB system is also associated with NT gene expression in the gut[142] indicating that this transcription factor is closely related to NT–NTR1 signaling in the inflammatory response in the gut.

The ability of NT to activate several types of immune and inflammatory cells might be central to its modulating

activities in intestinal inflammation. Thus, NT degranulates mast cells in the human jejunum,[143] and NTR1 mediates mast cell mediator release from mast cells,[144] including those located in the colon.[133] NT interacts with neutrophils affecting several neutrophil functions, including locomotion, phagocytosis, and adherence.[145] NT also increases chemotaxis of lymphocytes[146] and interacts with macrophages, stimulating their phagocytic activity,[147] and production of IL-1β.[148] NT, via NTR1, binds to human umbilical vascular endothelial cells,[149] stimulating release of prostacyclin[150] and enhancing their migration, implicating a role for NT in angiogenesis.[151] A preliminary report by Castagliuolo et al[152] showed that microvascular endothelial cells in the colonic mucosa of patients with IBD express NTR1, suggesting that NT may affect endothelial cell function during intestinal inflammation.

NT as a Healing Peptide in Colitis

Recent results indicate that NT might promote healing in chronic colitis. For example, daily administration of an NTR1 antagonist worsened inflammatory responses following DSS administration in mice, while injection of NT had a healing effect.[134] NTR1 antagonism prevents mucosal healing during the repair phase of colitis, likely via mechanisms involving NT-induced stimulation of epithelial cell migration and release of prostaglandins.[134] Interestingly, studies demonstrated that NT is able to promote growth of the intestinal and colonic mucosa.[153,154] One of the mechanisms mediating NT-induced restoration and repair of intestinal damage via cell proliferation involves a signaling pathway associated with metalloproteinase-dependent tyrosine phosphorylation of EGFR at the colonocyte level.[155] EGFR promotes mucosal restitution following colonic inflammation and is found to be elevated in tissues from IBD patients.[156] In colonocytes, NT-NTR1 coupling causes release of matrix metalloproteinases from the cell membrane that stimulate release of the EGFR ligand transforming growth factor-α (TGFα).[155] Interestingly, TGFα release and EGFR phosphorylation and activation also mediates NT-induced MAP kinase activation and IL-8 transcription in colonic epithelial cells,[155] suggesting that this signaling pathway triggers both proinflammatory and healing NTR1-associated responses during colitis. Several other pathways activated by NT can also participate in colonic mucosal healing and restitution. For example, NT stimulates intestinal epithelial cell migration in wounded colonocyte monolayers and stimulates COX-2 gene expression and prostaglandin release.[134] Interestingly, prostaglandin blockade also mediates chloride secretion from native human colon in response to NT.[157] Moreover, NT triggers adenosine release from native human colonic mucosa[157] and activation of adenosine receptors decreases colonic inflammation.[158] Stimulation of mucin release by NT from colonic goblet cells in vitro[159] and in vivo[138] might also protect the intestinal epithelial cell barrier during colitis.

Taken together, the evidence presented here strongly suggests that the NT–NTR1 interaction promotes acute intestinal inflammation, but also augments healing during the repair phase of IBD colitis. Many of the effects and signaling pathways activated by NT involve colonic epithelial cells. However, NT also mediates several IBD-associated symptoms, including small and large intestinal motility[160,161] and stimulation of chloride secretion[157] in humans. However, since NT and its receptors can be localized in the CNS and along the length of the GI tract, and NT can directly activate immune, inflammatory, epithelial, and neuronal cells, the mechanisms by which this peptide participates in IBD pathophysiology might be difficult to completely elucidate. These considerations further underscore the need for better understanding of the cellular and molecular mechanisms involved in NT-associated signaling pathways before treatment options can be determined.[162]

VASOACTIVE INTESTINAL PEPTIDE (VIP) IN IBD

VIP is a 28 amino acid neuropeptide[163] located in the central and peripheral nervous systems, including in the GI tract.[164] All layers of the colon contain VIP, with the highest concentration in the myenteric plexus.[19] Although primarily localized to neurons, immunomodulatory cell types such as T cells and eosinophils[165] also produce VIP.[166] VIP exerts its actions by binding to 2 G-protein-coupled VIP receptors, VPAC1 and VPAC2, which are also shared by PACAP (see Table 1).[167]

VIP exhibits broad significance in intestinal physiology. Functionally, VIP was originally identified in the GI tract and named for its potent vasodilator actions.[168] It is also known to govern regulation of motility, inhibit the peristaltic reflex in the circular smooth muscle layer, control intestinal blood flow, and modulate the immune system.[19,40,169] In the GI tract it is abundant on the smooth muscle sphincters of the lower esophagus, ampulla of Vater, and the rectum, where it regulates sphincter relaxation.[170] VIP is released from nerve terminals with enzymes of the nitric oxide (NO) synthesis cascade in the myenteric plexus[171] and, together, these 2 peptides are believed to be the primary components of nonadrenergic, noncholinergic nerve transmission in the gut.[5]

VIP Possesses Immunomodulatory Functions

VIP has established antiinflammatory[172] and immune cell function[19] activities. VIP is produced by Th2 cells following antigenic stimulation[172] and inhibits Th1 responses[172] by inducing Th2 cytokines in CD4 cells and inhibiting induction of Th1 cytokines.[16] Th2 effectors are the sole source of lymphocyte-derived VIP, further suggesting that this is part of a Th2-polarizing loop.[16] Moreover, VIP present in nerve fibers in lymphoid organs provides a potential neural

pathway for stimulation of this cytokine shift.[16] Aside from polarizing Th2 cells, VIP possesses other potent antiinflammatory effects including inhibition of leukocyte migration[172] and migration of lymphocytes,[173] and decreased NK-cell activity.[174] VIP also induces human IgA1 and IgA2 production,[175] and modulates IgA production by intestinal lamina propria lymphocytes.[176]

Several pieces of evidence indicate that VIP participates in the pathophysiology of colitis and IBD. Treatment with VIP after the onset of TNBS-induced colitis in Balb/c mice reduced the clinical and histopathologic severity of colitis as well as Th1 cytokine levels, indicating its potential use in the therapy of CD.[13] However, using the same strain of mice, another group failed to reproduce a similar VIP response, even when VIP was given prior to TNBS administration.[172] Interestingly, VIP treatment reduced TLR2 and TLR4 levels on colonic macrophages, dendritic cells, and CD4 and CD8 T lymphocytes of TNBS-administered mice.[177] However, the functional consequences of this VIP response were not reported in that study.[177] Similar conflicting results were also evident when human IBD tissue biopsies were examined for VIP expression. Bishop et al[178] reported a more than 200% increase in VIP content in colonic nerves of CD patients compared to UC patients or controls. A similar increase in VIP concentrations was also evident in rectal biopsies from CD, but not UC patients.[179] Moreover, VIP coding of enteric neurons in the submucosal plexus was increased in the rectum of CD patients.[169] Two other studies also reported no significant differences in VIP expression in the colon of UC patients.[52,180] In contrast, Mazumdar and Das[50] found decreased expression of VIP immunoreactivity in the colon of both CD and UC patients compared to controls, while 2 other studies also showed a significant decrease of rectal and colonic VIP in UC and CD patients.[19,54] Although, based on its potent in vitro immunomodulatory activities, VIP might represent a potential candidate for treatment of IBD, the evidence discussed above does not, at this time, support such use. Moreover, VIP administration has potential side effects, such as hypotension and diarrhea, when given at high doses. One clinical area where VIP might serve a current role is as a parameter in gauging activity in IBD. In 115 patients with IBD, serum VIP levels, measured by specific radioimmunoassay, showed a strong, positive correlation with clinical activity both at baseline and follow-up with nearly 2-fold increases in VIP concentration during active periods of disease.[181]

MU OPIOID RECEPTORS AND COLITIS

The mu-opioid receptor (MOR) is a G-protein-coupled receptor with several subtypes[182] widely expressed in the CNS[183] and peripheral tissues, including the intestine, where they are localized to the mesenteric and submucosal plexi in the ileum and colon (see Table 1).[183–185] Activation of MOR by endogenous and exogenous agonists has diverse effects on immune and physiologic processes such as pain relief, bacterial infections, suppression of the immune response, euphoria, and addiction.[186,187] The MOR is involved in the homeostatic regulation of immunologic and inflammatory reactions, suggesting a potential role in immune regulation in IBD. The ability of MOR-activating peptides to affect intestinal motility,[188] gastric secretion,[189] immune,[189] and inflammatory[190] responses also suggests that abnormal content of such peptides in the GI tract and the blood may influence IBD pathophysiology.[191] Several studies using tissue from IBD patients underlie this possibility. Thus, a significant increase in MOR expression is present in ileal and colonic tissue from patients with CD[183] and a 30-fold increase is seen in inflamed versus noninflamed colons of UC patients.[183]

Recent studies with animal models of intestinal inflammation and IBD strongly support an antiinflammatory role for MOR in IBD. Pol et al[192] showed a significant increase of MOR expression in neurons of the myenteric plexus during experimental intestinal inflammation, via a mechanism likely involving nitric oxide.[193] Intestinal inflammation also increases the inhibitory potency of MOR agonists on gut secretion and permeability in response to croton oil administration[194] by activating MOR.[195] Administration of selective synthetic MOR agonists prevented inflammation in hapten-induced and T-cell-dependent experimental models of colitis and this effect was abolished by administration of the opioid antagonist Nalaxone.[196] Similarly, antisense oligodeoxynucleotides against mu and delta-opioid receptor mRNA efficiently block the intestinal effects of opioids during experimental inflammation.[197] Moreover, compared to wild-type, MOR-deficient mice were prone to increased colonic inflammation, with a 50% mortality after 3 days of TNBS administration.[196]

The mechanism of action of MOR in intestinal inflammation appears to involve effects on the immune system. In humans, MOR was expressed in ileal and colonic enteric neurons as well as in immunocytes such as myeloid cells and $CD4^+$ and $CD8^+$ T cells.[183] MOR expression is also significantly enhanced by cytokines and repressed by NF-κB inhibition, consistent with in vitro data showing NF-κB and STAT binding sites on the human MOR gene promoter that control MOR gene expression.[187] Moreover, administration of the opioid agonist DALDA in colonic mucosal explants of IBD patients decreased TNFα mRNA expression, while selective peripheral MOR agonists were able to locally modulate TNFα production,[183] indicating that opioid agonists might have a role in IBD treatment. Another mechanism may involve B-endorphins, since B-endorphins downregulate inflammatory responses in an MOR-dependent manner.[191] In mouse experimental colitis MOR exerts an antiinflammatory effect in the colon through regulation of cytokine production and T-cell proliferation.[196]

Altogether, the data suggest that MOR appears to exhibit antiinflammatory effects in IBD via modulation of NF-κB and the immune system. However, more work needs to be done looking at human studies before more definitive conclusions can be drawn.

GALANIN AND INTESTINAL INFLAMMATION

Galanin is a 30 amino acid peptide[198] widely distributed in the enteric nerve terminals lining the GI tract.[199,200] Galanin is secreted by enteric nerves[201] and mediates its effects by binding to 3 different G-protein-coupled receptor subtypes identified as Galanin Receptor 1 (Gal1R), Gal2R, or Gal3R.[200,202] While GI smooth muscle cells[200] express all 3 receptor subtypes, Gal1R is the only receptor found on colonic epithelial cells (see Table 1).[200] Galanin is best known for its ability to alter smooth muscle contractility and regulate intestinal motility.[203] However, galanin's other known intestinal functions include fluid secretion[203] and alterations of intestinal ion flux.[201] Four animal studies examining galanin's role in modulation of intestinal secretion[201] showed that galanin has variable effects on ion secretion. Galanin activation of Gal1R in T84 colonic cells results in a rapid and transitory increase in chloride secretion.[201] Further, infection of T84 cells with pathogens known to activate the NF-κB pathway augmented Gal1R expression and chloride secretion.[203] A few studies have evaluated galanin's role in IBD in humans and animals. There is grossly increased expression of Gal1r seen in colonic tissue from UC and CD patients.[203] Additionally, DSS murine colitis activates NF-κB, therefore inducing Gal1R expression in epithelial cells lining the mouse colon.[200] Further, concomitant administration of the NF-κB inhibitor dexamethasone attenuated activation of this transcription factor by inhibiting this increase in Gal1R expression.[200] Intracolonic administration of galanin antibody showed a similar effect, decreasing DSS-induced intestinal fluid accumulation.[200] Mechanistically, cloning of the Gal1R gene[200] has revealed multiple recognition sites for the inflammation associated transcription factor NF-κB.[204]

Putting together the above observations, galanin's prominent role in intestinal fluid secretion and its connection to NF-κB, a critical participant in IBD, links the diarrhea associated with it to a NF-κB-mediated increase in Gal1R expression.[200] This suggests that identification of a Gal1R-specific antagonist may result in new pharmacologic therapies for the treatment of diarrhea resulting from the inflammation witnessed in IBD.[199]

CONCLUSIONS

IBD is an extremely complex illness involving multiple levels of interaction between the neural, immune, and endocrine systems. Neuropeptides serve as a viable link between all 3 systems. The literature in recent years has supported a prominent role for the ENS and neuropeptides as a pathway between the CNS and the ENS in the pathophysiology of intestinal inflammation and IBD. The neuroendocrine system communicates with the immune system through release of cytokines and neuropeptides to end-organ receptors and immune cells. Additionally, neuropeptides directly influence a large number of immune functions. The close anatomical association and functional communication between peripheral nerves and lymphoid organs and mucosal surfaces further supports this concept. We focused on the functions of the most well-studied individual neuropeptides and peptide hormones for their potential roles in the pathophysiology of IBD. Their key role is demonstrated by the fact that all appear to modulate disease activity through activation of NF-κB, a transcription factor associated with activation of proinflammatory genes and IBD pathogenesis. However, the complexity of these systems and their interactions, conflicting study results, and opposing mechanisms of action of several of the neuropeptides discussed warrants further research in this field. Further clarification of the molecular mechanisms of neuropeptides and their effects on human disease may yield treatment options in the future.

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