The enteric nervous system in intestinal inflammation

KEITH A SHARKEY PhD, EDWARD J PARR BSc

KA SHARKEY, EJ PARR. The enteric nervous system in intestinal inflammation. *Can J Gastroenterol* 1996;10(5):335-341. Since about the 1950s nerves in the wall of the intestine have been postulated to play a role in the pathogenesis of inflammatory bowel disease (IBD). Human and animal studies examining the role of nerves in intestinal inflammation are the focus of this review. Consideration is given to two possible ways that nerves are involved in IBD. First, nerves may play a role in the development or maintenance of inflammation through local release of transmitters. Second, once initiated (by whatever means), the processes of inflammation may disrupt the normal pattern of innervation and the interactions of nerves and their target tissues. Many of the functional disturbances observed in IBD are likely due to an alteration in the enteric nervous system either structurally through disruptions of nerve-target relationships or by modifications of neurotransmitters or their receptors. Finally, it appears that the enteric nervous system may be a potential therapeutic target in IBD and that neuroactive drugs acting locally can represent useful agents in the management of this disease.

Key Words: Capsaicin, Crohn's disease, Enteric nervous system, Inflammation, Neurogenic inflammation, Peptides, Substance P, Ulcerative colitis, Vasoactive intestinal polypeptide

Le système nerveux entérique dans l'inflammation intestinale

RÉSUMÉ : Depuis environ 1950, on estime que les nerfs de la paroi intestinale jouent un rôle dans la pathogenèse de la maladie inflammatoire de l'intestin (MII). Des études sur des sujets humains et sur des animaux se sont penchées sur le rôle des nerfs dans l'inflammation intestinale. Le présent article en fait le bilan. Les nerfs peuvent influencer de deux façons sur les MII. Tout d'abord, les nerfs jouent un rôle dans l'installation ou le maintien de l'inflammation par la libération locale de neurotransmetteurs. Ensuite, une fois déclenchés (peu importe de quelle façon), les processus inflammatoires peuvent perturber le mode normal d'innervation et d'interaction des nerfs et de leurs tissus cibles. De nombreuses anomalies fonctionnelles observées dans les MII sont probablement dues à une altération du système nerveux entérique, soit sur le plan de la structure à cause de la perturbation des liens entre les nerfs et leurs cibles, soit sur le plan des modifications affectant les neurotransmetteurs ou leurs récepteurs. Finalement, il semble que le système nerveux entérique puisse être une cible thérapeutique dans les MII et que les médicaments neuroactifs à action locale puissent être utiles dans le traitement de cette maladie.

Since about the 1950s nerves in the wall of the intestine have been postulated to play a role in the pathogenesis of inflammatory bowel disease (IBD). Storsteen and colleagues (1) were the first to demonstrate that enteric neurons were involved in this process by providing histological evidence for an increased number of myenteric ganglion cells in chronic ulcerative colitis. Similar observations were also made in Crohn's disease (2). Extrinsic nerves innervating the bowel have also been implicated in IBD based not only on the common observation of abdominal pain in IBD,

but also on clinical observations that vagotomy and pelvic nerve lesions could modify, and in some cases improve, the outcome of IBD (3-5). Additionally, autonomic neuropathy has been described in IBD and might contribute to IBD symptoms (6,7). Although surgical denervation is no longer used for the therapeutic management of IBD, the role of nerves in this multifactorial disease has become established. Human and animal studies examining the role of nerves in intestinal inflammation are the focus of this review. Consid-

Neuroscience and Gastrointestinal Research Groups, Department of Medical Physiology, The University of Calgary, Calgary, Alberta

Correspondence and reprints: Dr Keith Sharkey, Department of Physiology and Biophysics, The University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta T2N 4N1. Telephone 403-220-4601, fax 403-283-8731, e-mail ksharkey@acs.ucalgary.ca

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Figure 1 Fluorescence micrographs of substance P immunoreactivity (SP-IR) in whole mount preparations of the myenteric plexus (A and C) and submucosa (B and D) from the guinea-pig ileum. **A,B** Sham-operated controls. **C,D** Twenty-four hours after a single intraluminal injection of trinitrobenzene sulphonate. Note that 24 h after the induction of inflammation there was a substantial reduction in SP-IR in the submucosa, particularly that associated with blood vessels. No reduction, and possibly a slight increase, in SP-IR was observed in the myenteric plexus. Full details are provided in reference 22. Scale bar: 100 μ m. Reproduced with permission from reference 22

eration will be given to two possible ways that nerves are involved in IBD. First, nerves may play a role in the development or maintenance of inflammation through local release of transmitters. Second, once initiated (by whatever means), the processes of inflammation may disrupt the normal pattern of innervation and the interactions of nerves and their target tissues. Finally, the role of the central nervous system in modulation of immune function must be acknowledged, but it is outside the scope of this review to consider this aspect of the nervous regulation of inflammation.

INNERVATION OF THE GASTROINTESTINAL TRACT

The gastrointestinal tract is innervated by extrinsic sympathetic and parasympathetic autonomic nerves, primary afferent fibres that follow the course of the autonomic nerves and enteric nerves (8). Although extrinsic nerves innervating the bowel are thought of as largely efferent, the majority are afferent (9). These nerves respond to noxious mechanical and chemical stimulation, and are mostly sensitive to the selective sensory neurotoxin capsaicin (9-11). These nerves contain a number of biologically active peptides, in particular substance P and calcitonin gene-related peptide (CGRP) (12). The local release of these peptides from the peripheral terminals of primary afferents gives rise to neurogenic inflammation (13), which causes effects such as local vasodilation, plasma protein extravasation and mast cell degranulation. The contribution that primary afferents make to inflammatory processes in the gastrointestinal tract is not clear due to, in part, the complexity of intestinal innervation and the fact that many transmitters found in primary afferents are also present in enteric nerves.

The enteric nervous system (ENS) consists of two interconnected ganglionated plexuses and an extensive fibre and terminal network that innervates all components of the bowel wall (8). The ganglionated plexuses lie between the longitudinal and circular muscle layers (myenteric plexus) and in the submucosa (submucous plexus). The observation that a single enteric neuron may contain more than one transmitter or neuron-specific marker has led to the concept of chemical coding of enteric neurons (14). This process has been so successful in the guinea-pig ileum that apparently all neurons in both plexuses have been accounted for (14-16). However, because this detailed coding has not been fully explored in many other regions or in other species, its true value or applicability is limited. Chemical coding has recently been extended further by combining studies based on the projections of identified neurons, and coupling these combinations with their electrophysiological classification (15-17). Taken together, a structural and functional mapping of the guinea-pig ileum has been achieved. Thus, by visualizing identified cell types, inferences can be made about their function, and changes in the number or proportion of given cells may indicate altered function.

Neurons in the ENS are in a unique environment. They are regularly distorted by peristaltic contractions, the wall of the gut contains a resident population of inflammatory cells that contribute to a physiological state of basal (low level) inflammation and there is constant remodelling of the mucosa due to epithelial sloughing, which includes remodelling of the neural connections. This environment leads to a cell phenotype that differs substantially from other autonomic neurons. For example, work from the author's laboratory has recently shown that enteric neurons constitutively express high levels of the protein B-50 (18) and certain proto-oncogene products such as *fos* and *myc* (19,20).

ENTERIC INNERVATION IN INFLAMMATION – ANIMAL STUDIES

Peptides: Much of the work in animal models of gastrointestinal inflammation has focused on substance P because of its role as a mediator of neurogenic inflammation (21). Using a hapten-induced model of ileitis (intraluminal injection of trinitrobenzene sulphonate [TNBS]) it was shown that the pattern of substance P immunoreactivity in the submucosa of the guinea-pig ileum is modified (22). Substance P in the ileum is in primary afferent and enteric nerves; the former (detected on the basis of their location around blood vessels) appears particularly affected. There is an initial reduction in substance P by 24 h (Figure 1) consistent with sustained release, followed by a gradual increase in the intensity and density of immunoreactive nerves and an increased quantity of substance P, first in enteric nerves and later in primary afferent nerves until the pattern of innervation returns to normal 21 to 30 days later.

Substance P and CGRP in rabbit colitis or ferret jejunitis/ileitis models were found to be reduced up to 48 h after induction of inflammation (23,24). In the rabbit it was also found that the density and intensity of immunoreactivity

were reduced, although the overall pattern of nerves was similar in inflamed and control animals (24).

In jejunitis due to *Trichinella spiralis* infection, Swain et al (25) showed that substance P levels were increased in the myenteric plexus up to six days postinfection. This increase was abolished in capsaicin-treated rats, indicating a primary afferent origin of substance P. It was also abolished in congenitally athymic rats, which suggests that T lymphocytes play some role in the increase, probably through release of cytokines. Hurst and co-workers (26) followed up that observation to demonstrate that interleukin-1-beta was involved in the increase in substance P, which was found to be exclusively neuronal (26). The cellular mechanisms and inflammatory cell types underlying this neuroimmune interaction in the myenteric plexus have yet to be elucidated, and whether similar mechanisms apply to the submucous plexus is unknown.

Sharkey and Ihns (27) have provided preliminary evidence that the number of vasoactive intestinal polypeptide (VIP) immunoreactive neurons in the submucous plexus of the guinea-pig were not affected in TNBS-induced ileitis and that VIP-containing neurons in the myenteric plexus were actually increased. Similarly, Kishimoto et al (28) observed an increased VIP immunoreactivity in neurons and fibres in both plexuses of the colon and an elevated content of VIP in dextran sulphate-induced colitis in rats. Interestingly, these increases are consistent with preliminary reports of increased VIP mRNA in IBD in humans (29).

Capsaicin-sensitive nerves: In intestinal inflammation there are only a few studies that have examined the role of capsaicin-sensitive nerves (12). In rats with colitis induced with TNBS, acetic acid or dextran sulphate sodium, neonatal capsaicin treatment augments damage after induction of inflammation (30-33). This suggests that primary afferent nerves are involved in aspects of restoration of mucosal integrity in 'chronic' inflammation; however, further confirmation and extension of these data are required.

In rats sensitized to egg albumin, neonatal capsaicin treatment reduced the short circuit current responses (an indicator of active chloride secretion) to antigen challenge by about 50% (34). This implies a role for these nerves in the regulation of secretory events in inflammation. Because a mast cell stabilizer also blocked secretion induced by antigen challenge, some of the effects of nerves may be mediated through a nerve-mast cell interaction (34). Secretion is an important physiological defence mechanism to luminal irritants (or inflammatory agents). MacNaughton et al (35) examined some aspects of the neural control of secretory events and showed that mast cells are essential for nerve-mediated chloride secretion in the rat ileum in uninflamed tissues. Another important secretory product is mucin, which acts as a physical defence mechanism and whose release is enhanced in inflammation (36). It has now been demonstrated that capsaicin-sensitive nerves are involved in the neural regulation of mucin secretion (37,38).

Transmitters and receptors: The role of neurotransmitter receptors in inflammation, which until recently lacked at-

tention, was examined by Martinolle and colleagues (30). They examined the regulation of adrenergic receptors on smooth muscle/myenteric plexus preparations of guinea-pig jejunum and found that α_1 - and α_2 -adrenergic receptors were upregulated 10 days after induction of jejunitis with TNBS, whereas beta-adrenergic receptors were down-regulated. Those authors suggested that motility disturbances seen in small bowel inflammation might be related to this inverse receptor regulation.

Finally, a few studies have investigated transmitter release from the ENS or its electrophysiological consequences. Both acetylcholine and noradrenaline release are depressed in trichinella-infected rats (40,41). The depression observed in infected animals was mimicked in both cases by preincubation of tissues with interleukin-1-beta, which suggests that it may be the inflammatory mediator of this effect (42). This suggestion is further supported by studies in which an interleukin-1 receptor antagonist was given to infected rats. In this case noradrenaline release was enhanced compared with that in infected controls (42). In contrast to the depressed release of acetylcholine seen in rats, enhanced sensitivity to applied acetylcholine – potentially compensating for the reduced release – was observed in neurons in the myenteric plexus of trichinella-infected guinea-pigs (43). Although it appears that this is not due to marked changes in electrical properties of the neurons, there was a trend towards enhanced excitability of neuronal membranes. In addition, neurons from infected animals showed a marked increase in responsiveness to histamine (43). This led Wood (44) and Frieling (45) to suggest that histamine, released from mast cells, is responsible for many of the observed pathophysiological effects of inflammation through actions at the level of the enteric plexuses.

ENTERIC INNERVATION IN INFLAMMATION – HUMAN STUDIES

The original observation of increased ganglion cells in the ENS in IBD not only established a neural involvement in these diseases but also initiated a controversy: how can there be more enteric neurons if they are postmitotic? This has not been resolved, but evidence suggests that adult enteric neurons (at least in animals) have a remarkable plasticity of form and the capacity for growth and active DNA synthesis (46,47). Although cell division in adult neurons seems unlikely, it cannot be completely excluded as a possibility; a study in humans found that the number of centrioles in neurons of the submucous plexus was increased in patients with Crohn's disease (48), which may indicate mitosis. Another possible explanation for the observed enteric neuronal hyperplasia is that this disorder is established early in development but does not become clinically apparent until much later in life. This implies that IBD is directly related to a developmental defect that results in abnormal proliferation and survival of enteric neuronal precursors or both. A third possible explanation is that there are precursor cells in enteric ganglia capable of proliferation into neurons. Whether this is true is unknown, but it should be considered given ob-

servations of the presence of stem cells in the adult central nervous system (49).

In addition to neuronal hyperplasia, ganglion cell and axonal degeneration and necrosis have been observed in IBD (50-52). The pathological abnormalities of IBD appear to depend on the type of IBD (Crohn's disease versus ulcerative colitis), the region of the intestinal wall affected (mucosa versus muscle) and whether the tissue was from a site of active disease (50-52). The damage observed may be immunologically mediated because neurons (and glia) in tissues from Crohn's disease display the major histocompatibility class II antigen on their surface (53). Because this display is important for antigen presentation to T lymphocytes, it may be linked to nerve response to these inflammatory cells; however, its significance is unclear. Also at the cellular level in Crohn's disease, it appears that there is up-regulation of nerve growth factor in coarse nerve fibres in actively inflamed regions of bowel (54). This may reflect remodelling or regrowth of nerves or their response to inflammatory stimuli.

Several studies examining neuropeptides in the bowel in IBD have found both quantitative and qualitative changes in the innervation. The best studied peptides are VIP and substance P. In the earliest reports, VIP immunoreactive fibres were found to be coarser, thicker and more intensely stained in Crohn's disease compared with tissues from ulcerative colitis or controls (55,56). Associated with this was an increase in VIP content in full wall thickness and biopsy specimens from Crohn's disease. Later studies have not been able to confirm these findings. In one study, VIP immunoreactive nerves were found to be coarser in Crohn's disease compared with controls, but the levels of VIP were not elevated in the ileum and were significantly reduced in the colon (57). In other studies, VIP immunoreactive nerves were reduced in the colon in Crohn's disease (58-60) and ulcerative colitis (60), as was the content of VIP measured by radioimmunoassay (61). Why such differences have been found between studies is not completely clear, but regional variation, disease state and sampling may all contribute. However, real differences may exist because in some of these studies substance P was also assessed and far fewer discrepancies were noted. In support of the studies that show a reduction in VIP, one report measuring plasma levels of VIP showed a significant positive correlation between VIP levels and disease activity (62). In active disease VIP levels nearly doubled. This increase may indicate a massive and sustained release of VIP in active disease which is consistent with reduced levels detected in tissues, although other explanations are possible.

Substance P appears to be increased in the mucosa in ulcerative colitis as determined by radioimmunoassay (61, 63,64) and by immunohistochemistry (58). In one study this increase was shown to correlate with the extent of inflammation, indicating a potential role of this peptide in ulcerative colitis pathogenesis (64). On the other hand, substance P appears to be relatively unaffected in Crohn's disease (57, 61). However, it has been demonstrated that receptors for

substance P were up-regulated on blood vessels and lymphoid follicles in Crohn's disease and ulcerative colitis (65).

A few studies have also examined other peptidergic neuronal systems. Enkephalin and bombesin appear to be reduced in Crohn's disease but increased in ulcerative colitis (61). Calcitonin gene-related peptide was reported to be reduced in the muscle layers of resected specimens in Crohn's disease (30). Somatostatin-containing neurons in the submucous plexus were reduced in Crohn's disease but not ulcerative colitis, and this finding was unrelated to the degree of inflammation (66). Reduced levels of somatostatin have been seen in IBD (67), but these reflect both neuronal and enteroendocrine cell pools of the peptide and are thus hard to interpret. Catecholamine-containing nerves are apparently increased in ulcerative colitis (68,69), but tissue catecholamine levels were in one study unaffected (68) and in another increased (70). Finally, there is a preliminary report of up-regulation of nitric oxide synthase in neurons of the submucous and myenteric plexuses in Crohn's disease (71).

INFLAMMATORY CELLS AS TARGETS FOR THE ENTERIC AND AUTONOMIC NERVOUS SYSTEMS

Mast cells and nerves are found in association with villi of the small intestine (72,73). Stead (74) showed that, in rats infected with a nematode there is remodelling of nerve fibres in the villi associated with changes in mast cell density of the tissue. This implies a structural and presumable functional nerve-mast cell relationship in the bowel, which is supported by studies described above that demonstrate the importance of mast cells in nerve-mediated secretory events. The role of mast cells in models of IBD such as TNBS-treated animals is less clear and their relationships with nerves have yet to be examined. Because the submucosa is an important interface of nerves, mast cells and blood vessels, all of which may play a role in neurogenic inflammation, the relationships of these structures in this region in inflammation deserves examination. In a recent ultrastructural study in IBD patients, Dvorak et al (75) demonstrated that nerve-mast cell associations were significantly increased (as were mast cells) in ulcerative colitis tissues compared with Crohn's disease or control tissues. However, this study showed that the distances between nerves and mast cells were greater than those described in other animal or human studies (72,73). This does not imply that nerves and mast cells were not linked functionally, and further examination of this issue is required.

Other cells, such as macrophages, plasma cells and eosinophils, are probably also involved in neurally mediated responses and are associated with nerves. Macrophages are present in the ENS, and eosinophils and plasma cells have been shown to be innervated in human and animal gastrointestinal tract (76-78).

Another cell type of relevance to inflammation is the lymphocyte. The gastrointestinal tract is a rich source of lymphocytes and indeed plays a vital role in exposure to antigens and in lymphocyte recirculation (79,80). Ottaway (81) was the first to show a structural and functional link between

VIP innervation and lymphocyte function. Substance P also affects lymphocyte function and can increase lymphocyte movement through peripheral lymph nodes. In preliminary experiments Sharkey and co-workers (82) demonstrated that substance P and CGRP can increase lymphocyte movement through the gastrointestinal tract (82). Similarly, capsaicin-treated rats have been shown to respond less vigorously to an antigenic challenge, and substance P can reverse this effect (83,84). Taken together these findings suggest that neuropeptides may prime cells of the immune system to respond to a challenge, such as inflammatory stimulus, and hence are able to modulate the functional capacity of the immune system.

MODULATION OF INFLAMMATION USING NEUROACTIVE COMPOUNDS

Can nerves initiate or maintain the inflammatory state once it has been initiated? This is an important question because it may lead to novel therapeutic strategies for IBD management. Recent evidence in humans and animals broadly supports the idea of nervous involvement in the inflammatory process. In 1985 the α_2 -adrenergic agonist clonidine was used clinically in the treatment of ulcerative colitis (85). Although the results of this study looked promising, others have reported less successful results (86,87), and the side effects of this compound probably preclude it is a widely useful agent. Another compound of interest is nicotine. Treatment of ulcerative colitis patients with nicotine gum or transdermal nicotine patches was found to be effective in some patients (88,89). As with the clonidine studies, the site of action of nicotine has not been determined. Given the enormous number of actions of nicotine centrally

and peripherally it is unclear to what extent a peripheral neuromodulatory action is a factor in its effects.

Another class of drugs that have been reported to be beneficial are local anesthetics. Björck et al (90,91) reported that topical lidocaine improves the histological and clinical appearance of proctitis and proctosigmoiditis. Their findings were confirmed and extended by McCafferty and colleagues (92) in a controlled study of the effects of lidocaine in TNBS-induced colitis in rats. McCafferty et al observed that pretreatment of rats with lidocaine reduced the severity of colitis and that lidocaine treatment after inflammation was also effective in reducing disease severity. It is clear that local anesthetics can have actions other than to block conduction of nervous impulses although this mechanism is an appealing one for future studies.

SUMMARY

The role of the ENS in intestinal inflammation has been established. At this stage there are more questions than answers regarding how ENS is involved and to what extent alterations in structure or neuronal phenotype contribute to IBD pathogenesis. Many of the functional disturbances observed in IBD are likely due to an alteration in the ENS, either structurally through disruptions of nerve-target relationships or by modifications of neurotransmitters or their receptors. Careful studies that investigate the effects of intestinal inflammation on the ENS at the cellular and molecular level are required to achieve a complete understanding of these complex diseases. Finally, it appears that the ENS may be a potential therapeutic target in IBD and that neuroactive drugs that act locally can represent useful agents in IBD management.

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