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Plasticity of enteric nerve functions in the inflamed and post-inflamed gut

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Abstract

Inflammation of the gut alters the properties of the intrinsic and extrinsic neurons that innervate it. While the mechanisms of neuroplasticity differ amongst the inflammatory models that have been used, amongst various regions of the gut, and between intrinsic versus extrinsic neurons, a number of consistent features have been observed. For example, intrinsic and extrinsic primary afferent neurons become hyperexcitable in response to inflammation, and interneuronal synaptic transmission is facilitated in the enteric circuitry. These changes contribute to alterations in gut function and sensation in the inflamed bowel as well as functional disorders, and these changes persist for weeks beyond the point at which detectable inflammation has subsided. Thus, gaining a more thorough understanding of the mechanisms responsible for inflammation-induced neuroplasticity, and strategies to reverse these changes are clinically relevant goals. The purpose of this review is to summarize our current knowledge regarding neurophysiological changes that occur during and following intestinal inflammation, and to identify and address gaps in our knowledge regarding the role of enteric neuroplasticity in inflammatory and functional gastrointestinal disorders.

Keywords

enteric nervous system; persistent neuroplasticity; visceral afferent

INTRODUCTION

Over the past decade, increasing effort has been directed towards elucidating the roles of the enteric nervous system in various pathophysiological disorders of the gut, including inflammation and infection. It is clear that inflammation leads to changes in neurophysiological, neurochemical and morphological properties of enteric nerves, and it is highly likely that these changes contribute to immediate alterations in gut function. An issue that has received less attention is whether these inflammatory changes persist following the resolution of the initiating inflammatory event. This is clinically relevant because abnormal gut function frequently persists during remission of inflammatory bowel disease (IBD)1⁻3, and IBD and functional gastrointestinal disorders such as irritable bowel syndrome (IBS) are considered by many to be interrelated disorders.2,3

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The theory that a previous inflammatory response to infection, and/or a persistent, barely detectable, increase in immune cells and pro-inflammatory mediators, contributes to altered gut function in IBS is rapidly gaining attention.[2,4,5] According to this model, inflammation-induced changes in the intrinsic and extrinsic neural elements of the bowel persist when the gut appears to be grossly and histopathologically normal, and that these lasting "undetectable" changes are responsible for altered sensitivity, motility and secretion. Here, we provide an overview of the changes that occur in the physiological properties of enteric nerves in the inflamed gut, those changes that persist following recovery from inflammation, and the possible mechanisms that are involved in altering the properties of enteric nerves.

When assessing the body of literature regarding inflammation-induced neuroplasticity in the gut, one must consider the fact that our collective information is derived from a wide variety of experimental conditions. For example, experimental models of inflammation have included administration of chemical irritants such as acetic acid and mustard oil or haptens such as trinitrobenzene sulfonic acid (TNBS), infection with parasitic nematodes such as *Trichinella spiralis* or bacteria such as *citrobacter rodentium*, by the addition of dextran sodium sulfate (DSS) to the drinking water, or by sensitization to antigens such as β-lactoglobulin. Furthermore, the inflammatory responses to a given stimulus can vary among species. For example, TNBS colitis in the rat is associated with a full thickness loss of structural integrity in the inflamed region to the extent that the layers cannot be distinguished.[6] On the other hand, guinea pigs do not survive such an insult, and therefore the TNBS colitis and ileitis that has been studied in guinea pig involves less extensive tissue damage with the inflammatory response centered in the mucosa and an intact muscularis externa.[7] Despite these caveats, a number of common, if not completely consistent, features of inflammation-induced neuroplasticity have been reported, and these are described here.

NEUROPLASTICITY OF INTRINSIC NERVES IN THE INFLAMED AND/OR INFECTED GUT

Electrophysiological studies of inflammation-induced changes in enteric neurons have exclusively involved guinea pig preparations. This is because the guinea pig intestine is relatively easy to dissect into layers, which greatly facilitates the intracellular electrophysiological studies, and because the neuronal elements of enteric neural circuits have been characterized more extensively in the guinea pig than any other species. This allows investigators to evaluate neuroplastic changes in functionally identified neurons.

AH neurons

The type of enteric neuron that is most dramatically affected by inflammatory conditions is the afterhyperpolarizing (AH) neuron. The AH neuron, named for its prolonged afterhyperpolarization (AHP), functions at the afferent end of intrinsic reflex circuits, including those associated with peristalsis, mucosal secretion, and vasodilation.[8–10] These neurons have a number of characteristic electrical and morphological properties. Morphologically, AH neurons have several long processes (Dogiel type II morphology) that project to other ganglia, the circular muscle, and the mucosa. AH neurons are activated by both mechanical and chemical stimuli.[9] They are activated by paracrine mediators, including serotonin (5-HT), released from enteroendocrine cells, by stretch, and by slow synaptic inputs from other enteric neurons. At rest, in a longitudinal muscle-myenteric plexus preparation, the AH neuron is rather inexcitable due to a relatively negative resting membrane potential and its prolonged AHP, which functions to limit action potential firing. However, spontaneous activity is detected in recordings of AH neurons in preparations with intact mucosa.[11,12] Fast synaptic potentials and spontaneous activity are rarely observed in these neurons. While fast excitatory postsynaptic potentials are rarely observed in these

cells, slow excitatory postsynaptic potentials (EPSPs) are a characteristic feature of AH neurons.

Hyperexcitability of the AH neuron is a consistent feature of neuroplasticity in the inflamed bowel. To date, intracellular electrophysiological studies have demonstrated enhanced excitability in the myenteric13 and submucosal14 plexuses of the TNBS-inflamed colon, in the myenteric plexus of the TNBS-inflamed ileum15, and in the small intestine of T. spiralis infected guinea pigs.16,17 In all of these conditions and locations, AH neurons exhibit slower accommodation and a smaller AHP. However, the mechanisms responsible for altered electrical properties vary with the layer of the gut and with the model of inflammation.

In T. spiralis-infected preparations, AH neurons have a more depolarized resting membrane potential and an increased input resistance.16,17 This pattern of changes suggests a decrease in the intermediate conductance, Ca^{2+}-activated K^+ (IK_{Ca}) channel activity in these cells, which would also contribute to the attenuation of the AHP. Christofi and colleagues demonstrated the cAMP-pCREB signaling pathway in jejunal AH neurons is facilitated in T. spiralis infected animals, and that AH neuron hyperexcitability can be reversibly attenuated in these preparations by acute administration of inhibitors of adenylyl cyclase, histamine receptors, cyclooxygenase or leukotrienes.16 This rapid attenuation of excitability *in vitro* indicates that AH neuron hyperexcitability in this model likely involves phosphorylation of ion channels, as opposed to, or possibly in addition to, transcriptional changes.

In TNBS-inflamed preparations, the resting membrane potential of AH neurons is not altered, but various electrophysiological changes have been described in different regions. In colonic submucosal neurons, the duration of the action potential is shorter, and the characteristic shoulder on the repolarizing phase of the action potential is greatly diminished in TNBS-inflamed animals, suggesting that Ca^{2+} channels may be disrupted or decreased in number in these neurons. In colonic myenteric neurons, these features of the action potential are not altered, but there is an increase in the hyperpolarization-activated cation conductance that is activated during the AHP. Another alteration in myenteric AH neuron function in TNBS colitis is that spontaneous activity can be detected in approximately half of the neurons whereas it is rarely observed in control preparations.13 It appears that inflammation-induced hyperexcitability of myenteric AH neurons involves activation of cyclooxygenase-2 (COX-2) and possibly prostaglandins. The TNBS colitis-induced changes in electrical properties are not present in animals that have been treated with a COX-2 inhibitor on days 2–5 post-TNBS and euthanized for electrophysiological examination on day 6.18 Furthermore, the rate of propulsive motility, which is slowed in TNBS-inflamed colons, is significantly improved in TNBS-inflamed colons from animals treated with the COX-2 inhibitor. It is not yet clear, however, whether COX-2 inhibition prevents or reverses the colitis-induced changes in the electrical properties of these neurons.

Changes in synaptic properties have also been reported in AH neurons from inflamed preparations. In *T. spiralis*-inflamed preparations, a significantly higher proportion of jejunal AH neurons exhibit evoked fast EPSPs, as compared to control preparations.17 In the colon, the proportion of AH neurons with detectable evoked fast EPSPs increased from 17% to 74% in TNBS colitis.13 The mechanism for this synaptic plasticity has not yet been determined. We have tested whether fast EPSPs could be evoked in colonic AH neurons from healthy animals in the presence of elevated extracellular Ca^{2+}, with the idea that the synapses existed but were not releasing enough transmitter to detect a response under normal conditions, but no increase in fast synaptic activity was detected (Krauter, Sharkey and Mawe, unpublished observations).

S neurons

The other electrophysiological class of enteric neuron, designated S neurons, includes those cells that behave as interneurons and motor neurons, as well as some neurons that appear to have a sensory role because they can be activated by mechanical stimulation.8,19 S neurons have a single long process with several short processes emanating from their cell body (Dogiel type I morphology), they are slowly accommodating, and fast EPSPs can be elicited in these neurons in response to focal stimulation of interganglionic fiber tracts.

In the TNBS-inflamed colon, no significant changes are detected in the electrical properties of submucosal S neurons or in myenteric S neurons when they are examined as a single population13,14; however, when myenteric S neurons are divided into those with ascending versus those with descending projection patterns, S neurons with ascending projections exhibit increased excitability.13 TNBS colitis-induced changes in these neurons include an increase in the number of action potentials during a depolarizing current pulse and in the proportions of S neurons exhibiting anodal break action potentials (a measure of excitability) or spontaneous activity. In the TNBS-inflamed ileum, about half of Dogiel type I neurons, which presumably exhibit S neuron electrical properties in healthy preparations, exhibit many electrical features reminiscent of AH neurons.15 They have a more hyperpolarized resting membrane potential than control S neurons, a tetrodotoxin-insensitive action potential, and a prolonged AHP that is sensitive to the IK_{Ca} channel blocker, TRAM-34. Therefore, in the TNBS-inflamed ileum, a dramatic phenotypic transformation is occurring in a large proportion of the S neurons. The physiological ramifications of these changes are not yet known.

Synaptic plasticity in the inflamed gut

In the colon, TNBS colitis is associated with a significant increase in the amplitude of the fast EPSP in submucosal as well as myenteric S neurons. In the submucosal plexus, synaptic facilitation involves a pharmacological conversion of the synaptic events in S neurons from being purely mediated by acetylcholine acting at nicotinic receptors, to being mediated by activation of nicotinic as well as purinergic P2X and/or serotonin $5\text{-}HT_3$ receptors due to the release of ATP and 5-HT from presynaptic nerve terminals.14 In the myenteric plexus, inflammation-induced synaptic facilitation is not accompanied by alterations in the pharmacological profile of EPSPs, and no changes in synaptic density are detected by electron microscopy.20 On the other hand, there is evidence for a presynaptic increase in PKA activity, possibly leading to an inhibition of BK-type K^+ channels, and an increase in the readily releasable pool of transmitter vesicles.20 In the TNBS-inflamed ileum, no changes in the synaptic potentials of Dogiel type I neurons have been detected, including those that exhibit the electrical properties of AH neurons during inflammation, as well as those that retain the properties of S neurons. 15

Based on the results summarized above, it is clear that synaptic transmission within myenteric and submucosal ganglia is facilitated in the colon in response to TNBS inflammation, and in AH neurons of guinea pigs with *T. spiralis*-induced jejunitis. In TNBS colitis, the mechanisms that are responsible for the augmented synaptic responses are presynaptic and involve increased transmitter release. However, this is in contrast to results from transmitter release studies involving *T. spiralis*-inflamed tissue samples in response to electrical field stimulation. In a series of studies, Collins and colleagues demonstrated that release of radiolabeled acetylcholine and norepinephrine is decreased in rat longitudinal muscle-myenteric plexus preparations 6 days following *T. spiralis* infection, when the tissue is actively inflamed, and that interleukin 1β plays a role in this response.21 The divergent findings from these sets of studies may be due to differences in sites of release, with the

electrophysiological studies measuring inter-neuronal transmission, and the field stimulation studies measuring release from motor neurons.

Decreased transmitter release has also been observed in antigen-induced responses in the intestines. In tissue from guinea pigs sensitized to cow's milk, application of β-lactoglobulin results in a suppression of fast EPSPs as well as noradrenergic transmission in ileal submucosal ganglia.22 A similar suppression of fast EPSPs occurs in response to application of *Trichinella* antigen in colonic submucosal neurons of guinea pigs that had been infected 6–8 weeks prior with *T. spiralis*.23 These synaptic inhibitory responses to antigen exposure involve mast cell degranulation and presynaptic inhibition mediated by H_3 histamine receptors. Collectively, these reports of both inflammation-induced increases and decreases in neurotransmitter release underscore the need for caution when extrapolating results from one species or inflammatory model to others, and they also indicate that changes involving nerve terminals, in addition to those involving somatic excitability, are key players in the neuroplastic transformations that occur in response to inflammation.

NEUROPLASTICITY OF INTRINSIC NERVES IN THE POST-INFLAMED OR POST-INFECTED GUT

Long-term electrophysiological studies have been conducted with tissues from TNBS-inflamed and *T. spiralis*-infected animals, and it is clear that inflammation-induced neuroplasticity persists in these conditions. In a study of *T. spiralis*-infected animals, Christofi and colleagues evaluated the electrical properties of jejunal AH neurons 35 days post-infection, when the nematode has been cleared from the gut.16 In these preparations, the cAMP-pCREB pathway remained facilitated and the AH neurons were still hyperexcitable. It is important to note, however, that at this time point, several indicators of active inflammation remain elevated, including myeloperoxidase, mast cell tryptase and prostaglandin levels. Therefore, neuroplastic changes may persist due to a sustained activation of receptors for pro-inflammatory mediators leading to changes in channel function and/or transcriptional changes in these cells. AH neuron hyperexcitability can be acutely attenuated in *T. spiralis* infected/inflamed preparations at the 6 day post-infection time point by application of inhibitors of adenylyl cyclase, histamine receptors, cyclooxygenase or leukotrienes *in vitro*16. This indicates that acute regulation, possibly involving phosphorylation, is involved in inflammation-induced neuroplasticity in *T. spiralis* infected preparation, but the CREB phosphorylation, which has also been detected16, indicates that transcriptional changes are also likely to occur in this model.

Inflammation-induced plasticity of enteric neurons also persists in TNBS colitis, but in this model, persistent changes are detected weeks after the inflammation has resolved. Gross damage scores of guinea pig colons return to the control level 14 days after TNBS administration, and myeloperoxidase levels return to basal levels within 28 days following TNBS.24,25 At the 8 week post-TNBS time point, which is at least 4 weeks following the resolution of inflammation, inflammation-induced changes are still detected in submucosal and myenteric ganglia.24,25 In both sets of ganglia, AH neurons remain hyperexcitable and fast EPSPs remain facilitated. At the 8 week post-TNBS time point, when AH neuron excitability and synaptic potentials are still enhanced, PGE_2 levels are normal.24 Since AH neuron excitability is normal when COX-2 is inhibited during active inflammation18, and inflammation has been resolved for at least 4 weeks at the 8 week post-TNBS time point, it is likely that inflammation-induced neuroplasticity is imprinted in the enteric nervous system during active inflammation, and likely involves transcriptional changes. This is supported by the finding that motility remains disrupted even in animals that have been treated with a COX-2 inhibitor for four days prior to euthanasia at the 8 week post-TNBS time point24, whereas COX-2 inhibition restores motility when administered during active

inflammation. Neuronally mediated colonic secretion also remains attenuated 56 days following TNBS administration in guinea pigs.25

Sensitization to antigens is another form of persistent change involving neuroimmune interactions. As described above, synaptic activity is suppressed in response to antigen exposure in tissue from milk fed guinea pigs and guinea pigs that have recovered from *T. spiralis* infection. In addition, AH neurons are depolarized and become hyperexcitable.22,23 In these sensitization models, the memory is stored by mast cells, which degranulate in response to antigen exposure, and it is their secretory products that mediate the neuronal responses.

NEUROPLASTICITY IN EXTRINSIC PRIMARY SENSORY NERVES IN THE INFLAMED GUT

Neurophysiological changes in the extrinsic primary afferent nerves of the inflamed gut

Inflammation of the GI tract is associated with changes in the electrical properties of spinal afferent neurons that project from the gut to the spinal cord, and these changes could result in visceral hypersensitivity. Electrophysiological studies of dorsal root ganglion (DRG) neurons have shown that TNBS ileitis leads to neuronal hyperexcitability in mouse and guinea pig neurons that project to the ileum.26,27 This is manifested as a decrease in the action potential threshold, an increase in the number of action potentials generated during a depolarizing current pulse, and an increase in spontaneous activity. These changes were limited to neurons projecting to the inflamed organ indicating that humoral factors are not responsible for this neuroplasticity. Hyperexcitability of extrinsic afferent neurons in response to TNBS-induced inflammation and experimental gastric ulceration is associated with an increase in TTX-resistant Na^{2+} currents and a decrease in the A-type and delayed rectifier K^+ currents.26,28–30 It is likely that the TTX-resistant channel, $Na_V1.8$ contributes to inflammation-induced hyperexcitability because DRG neurons from mice lacking this channel did not become hyperexcitable in mice with jejunitis initiated by *Nippostrongylus brasiliensis* infection whereas afferent neurons from wildtype and $Na_V1.9^{-/-}$ mice were hyperexcitable.31 An unavoidable limitation of these studies is that the recordings are obtained from enzymatically isolated neuronal cell bodies, and while these findings likely reflect changes that contribute to enhanced firing properties of these neurons, additional inflammation-induced changes in these neurons enhance the probability of activation of the peripheral terminals of these neurons.

Recently, considerable attention has been directed towards elucidating the role of transient receptor potential (TRP) cation channels in inflammation-induced neuroplasticity of extrinsic afferent nerve fibers in the gut. Of notable interest are the vanilloid channels TRPV1 and TRPV4, and the ankyrin channel, TRPA1. These proteins are particularly interesting because their activation thresholds for stimuli such as temperature, pH and osmolarity are greatly affected by pro-inflammatory signaling molecules in the local environment, including proteases (acting via protease-activated receptors), bradykinin, and prostaglandin E_2.32,33 This could help explain how the sensitivity of afferent fibers to chemical and mechanical stimuli is shifted in pathophysiological conditions.

Changes in TRPV channels in the extrinsic primary afferent nerves of the inflamed gut

The founding member of the TRPV subfamily, TRPV1, is involved in the development of inflammation and in the inflammation-induced sensitization of visceral afferent neurons. The TRPV1 channel is expressed primarily by unmyelinated, nociceptive primary afferent neurons.34 Activation of TRPV1 channels in peripheral terminals of afferent nerves leads to local release of neuroactive peptides substance P and CGRP, resulting in a neurogenic

inflammatory response. Consistent with this concept, inhibition of TRPV1 has an anti-inflammatory effect on TNBS-induced colitis.35 On the other hand, TRPV1 has also been shown to have a protective role in inflammatory models.36 For example, dinitrobenzene sulfonic acid colitis is more extensive in TRPV1$^{-/-}$ mice than in wildtype littermates37, and TRPV1 activation ameliorates DSS-induced colitis in mice.38

Results from a number of studies indicate that TRPV1 activation and upregulation are involved in hypersensitivity in the inflamed gut. In the rat, TNBS colitis leads to higher TRPV1 protein39 and transcript40 levels in lumbosacral DRG, and a higher proportion of intestine-projecting DRG neurons that are TRPV1-immunoreactive.35,40,41 In addition, TRPV1 antagonists inhibit much or all of the inflammation-induced hypersensitivity of afferent nerves to colorectal distention.35,40,41 Further support for a role of TRPV1 in visceral hypersensitivity comes from a study by Jones and colleagues demonstrating that TRPV1$^{-/-}$ mice are less sensitive to distension or a pro-inflammatory cocktail than control mice, and afferent fiber sensitivity in control mice is attenuated by the TRPV1 antagonist, capsazepine.42

Data from human studies also support a potential role for TRPV1 receptors in visceral hypersensitivity. An increase in the density of TRPV1-immunoreactive nerve fibers is detected in intestinal biopsies from individuals with IBD.43 Interestingly, a recent study also demonstrated a 3-fold increase in TRPV1-immunoreactive nerve fibers in biopsies from individuals with IBS as compared to controls.44 The contingent of patients in this study represented a combination of IBS with diarrhea, constipation or alternating symptoms. Histological analysis of H&E stained sections did not reveal inflammation, but the samples did contain increases lymphocytes and mast cells, as revealed by CD3 and kit immunohistochemistry, respectively, so it is possible that pro-inflammatory mediators contribute to the neuroplasticity.

The TRPA1 and TRPV4 channels also may be involved in visceral hypersensitivity. Elevated TRPA1 protein levels are detected in lumbosacral DRGs in rats with TNBS colitis39, and TRPA1 transcript levels are increased in mice with mustard oil-induced colitis.45 Furthermore, intrathecal injection of TRPA1 antisense oligonucleotides reduced TRPA1 expression in the DRG and attenuated colitis-induced hyperalgesia to mechanical and chemical stimuli.39 In the case of TRPV4, this channel mediates the PAR2-induced sensitization of colonic afferent nerves46, it is expressed in visceral afferent neurons47, and mechanosensory responses are augmented by TRPV4 stimulation in colonic afferent nerves.47

It is not yet completely clear how these changes in afferent neuron channel expression and functions are triggered, but a recent study by Qiao, Grider and colleagues has begun to shed light on this issue.48 They found that in rats with TNBS colitis, protein and RNA levels for brain derived neurotrophic factor are elevated in DRGs from animals with colitis. Furthermore, in studies of extracellular signal-regulated kinase (ERK) expression and distribution, they found that phospho-ERK 1 and 2 expression is increased in the DRGs, and phospho-ERK immunoreactivity is increased in the superficial dorsal horn. Increased ERK 1/2 expression has been reported in other nociceptive systems, and somatic and visceral hypersensitivity can be alleviated by the MEK/ERK inhibition. In a related study, Galan and colleagues demonstrated that hyperalgesia induced by colonic administration of mustard oil or capsaicin is associated with increased ERK 1/2 expression and was blocked by the inhibitor of ERK phosphorylation, U0126.49 Collectively, these findings suggest that ERK phosphorylation may be involved in triggering transcriptional changes that contribute to hyperexcitability and hypersensitivity.

NEUROPLASTICITY IN EXTRINSIC PRIMARY SENSORY NERVES IN THE POST-INFLAMED GUT

Studies of extrinsic afferent nerve function in inflammation models indicate that visceral hypersensitivity to mechanical and/or chemical stimuli persists beyond the recovery from inflammation. Persistent, post-inflammatory hyperalgesia to colorectal distension and/or luminal capsaicin administration has been demonstrated in *T. spiralis* infected mice50 and in TNBS-colitis in rats.35,51 In single fiber recordings from rat lumbar splanchnic afferent nerves, Coldwell and colleagues found that in DSS colitis a higher proportion of nerve fibers responded to 5-HT and they responded more robustly, and that 5-HT was more potent and effective in the inflamed preparations.52 This enhanced responsiveness to 5-HT was maintained following recovery from inflammation. Persistent, post-inflammatory hypersensitivity to 5-HT has also been reported in mice infected with *T. spiralis*.53

It is possible that TRPV1 contributes to persistent hypersensitivity of visceral afferent nerves following recovery from inflammation, but the relationship has not been completely resolved. Miranda and colleagues demonstrated that the TRPV1 antagonist, JYL1421 significantly decreased augmented visceromotor reflex responses to colorectal distention; however TRPV1 blockade had no effect on the responsiveness to intraluminal capsaicin, and TRPV1 immunoreactivity in DRGs had returned to normal at the post-inflammatory time point.35 It also appears that the inflammation-induced increase in TRPV1 expression reverses with the resolution of inflammation in DSS-treated mice. Eijkelkamp and colleagues reported that colonic TRPV1 protein levels are elevated during active inflammation, but return to the control level at a post-inflammatory time point in which capsaicin-induced responses are still facilitated.54 These authors proposed that a facilitation of TRPV1 signaling might persist at the post-inflammatory time point even though the TRPV1 expression levels have returned to a normal level. When administered intracolonically, the yeast protein-carbohydrate derivative, Zymosan, causes persistent increases in visceromotor responses in the absence of an inflammatory response in wildtype mice, but not in $TRPV1^{-/-}$ mice, suggesting that TRPV1 channels do contribute to long-term hypersensitivity.55

TTX-resistant Na^{2+} channels $Na_V1.8$ and/or $Na_V1.9$ could contribute to persistent, post-inflammatory hyperexcitability. As described above, an increase in TTX-resistant Na^{2+} current expression is detected in extrinsic primary afferent neurons in response to inflammation.26 In transgenic mice with jejunitis induced by infection with *N brasiliensis* hyperexcitability is detected during inflammation and after resolution of inflammation in $Na_V1.9^{-/-}$ mice, but is absent in $Na_V1.8^{-/-}$ mice. 31 Therefore, it is likely that $Na_V1.8$ plays a critical role in the persistent post-inflammatory hyperexcitability of DRG neurons.

MANY QUESTIONS REMAIN

Over the past decade, significant progress has been made in our efforts to elucidate what changes are occurring in the intrinsic neurons and extrinsic primary afferent neurons in response to inflammation and infection. This review has concentrated primarily on changes related to the neurophysiological properties of these neurons, and certain common features are evident from the various models that have been studied, but more questions have been raised than have been answered. Brief descriptions of a number of these issues are described below.

How are neuroplastic changes induced in sensory neurons that innervate the gut?

Intrinsic (AH) and extrinsic sensory neurons become hyperexcitable in response to inflammation and infection of various kinds. They exhibit various changes in channel

activity that lead to lower thresholds for somatic activation of action potentials and slower accommodation. These neurons send processes into the lamina propria, where the inflammatory response is centered, and are therefore directly exposed to inflammatory mediators and other factors such the neurotrophin brain-derived neurotrophic factor (BDNF), which could influence ion channel expression and function. Identifying the triggers for neuroplastic changes in these neurons, and how these signals are transmitted from the mucosal processes to the neuronal cell bodies is an important goal. In the case of colonic myenteric AH neurons, we know that COX-2 activation is a critical element in the initiation of plasticity of these neurons in TNBS colitis18, but we cannot be sure of where this fits into the cascade of events that leads to inflammation-induced changes. Prolonged exposure to PGE_2 in organ culture mimics the effects of TNBS colitis56, but it is not yet clear whether COX-2 products act directly on AH neurons *in vivo*, and in which cells COX-2 is activated to affect these neurons. Also, while it is clear that the peripheral terminals of extrinsic sensory neurons exhibit inflammation-induced hypersensitivity, it is not yet known whether AH neurons are more sensitive to activation in response to physiological stimuli in the inflamed bowel.

Why does neuroplasticity persist following recovery from inflammation?

Another common feature of inflammation and infection-induced neuroplasticity is that the changes in these neurons are long-lasting. Hyperexcitability of extrinsic and intrinsic sensory neurons, as well as synaptic facilitation in enteric circuits, persist for at least weeks following inflammation. This aspect of inflammation-induced neuroplasticity clearly could have implications with regard to altered gut function during periods of remission from IBD, and with regard to functional disorders of the gastrointestinal tract. This underscores the need to gain a better understanding of the triggers that lead to neuroplastic changes, and to determine why the system does not return to the normal steady state once these triggers have been removed. It is as though a switch has been turned on, but it needs a yet to be identified signal to turn it back to the off position. Hopefully progress will be made in this area as we elucidate the transcriptional changes that are occurring in these neurons in response to inflammation. Questions remaining include: What genes are being activated or inactivated? How are these genes activated? And how can these genes be influenced to restore their level of activity to normal?

It is worth mentioning that in IBS, a condition in which the inflammatory state may be debatable, it appears that soluble mediators within the tissue can contribute to visceral sensitivity. Extracts from biopsy samples from individuals with IBS leads to visceral hypersensitivity and extrinsic sensory neuron activation that involve protease activity.57,58

What are the physiological implications of enhanced neuronal excitability and synaptic activity in the inflamed gut?

Now that key neuroplastic changes that are induced by inflammation in the intestines have been identified, it is important to determine how these changes influence the regulated functions of the gut regulated by the enteric nervous system, namely motility, secretion and vasodilation. Intuitively, one would expect that inflammation-induced AH neuron hyperexcitability and synaptic facilitation would lead to enhancement of all of these gut functions; however, propulsive motility is slowed in TNBS colitis.59 It is possible that the spontaneous activity and excitability of AH neurons could increase background noise in the myenteric circuitry, resulting in a form of “attention deficit disorder” in the enteric nervous system and decrease the precision of neuronal signaling in the gut. This would result in a decreased ability to generate a strong pressure gradient at the site of stimulation. Indeed, administration of an IK_{Ca} channel blocker *in vivo* disrupts propulsive motility in the rat small inteistine.60 We have tested this concept by activating the neurons in a guinea pig

distal colon preparation with the Na^+ channel opener, veratridine, and evaluating fecal pellet propulsion. At a concentration of 1 μM, veratridine causes myenteric neurons to fire repetitively61, and the rate of fecal pellet propulsion is significantly decreased (Linden, Sharkey and Mawe, unpublished). This effect would likely be augmented by an increase in neurotransmitter release, and as described above, synaptic transmission is facilitated in the inflamed colon. Therefore, while alterations in interstitial cells of Cajal and smooth muscle likely contribute to changes in motility, it is likely that changes that increase neuronal activity *decrease* the fidelity of enteric neural signaling and therefore contribute to dysmotility in colitis.62 A next step will be to evaluate the net output from the myenteric plexus to the smooth muscle in the inflamed colon to further test the validity of the attention deficit model.

The effects of increased neuronal activity and synaptic facilitation on secretory and vasodilatory reflexes likely differ from those in motor reflexes. The motor reflex is relatively complex consisting of sensory neurons, ascending and descending interneurons, and excitatory and inhibitory motor neurons that must act in concert to promote coordinated motor activity. Secretory and vasodilatory reflexes, on the other hand, can consist simply of a sensory neuron and motor neuron. Therefore, it is likely that these reflexes are facilitated during active inflammation, which along with malabsorption would lead to diarrhea, a characteristic feature of both IBD and infectious enteritis.63

What is the mechanism of altered gut function at sites distant from the inflamed region?

Experimental studies show that there are functional changes that occur in the gut in regions that are remote from a site of localized inflammation.64,65,66 In the submucosal plexus of the colon from animals with ileitis, in which the colon was not inflamed, many of the functional changes described in the inflamed colon have recently been observed. For example, AH neurons display hyperexcitability and there is a facilitation of the synaptic input to S neurons.64 These findings suggest that a local immune and/or inflammatory event in one region of the bowel may cause changes not restricted to the site of inflammation. At this point it is not clear if these changes are due to circulating immune cells or mediators such as neurotrophins, or if local inflammation causes changes in the afferent neural output from the gut which then leads to feedforward effects through the synaptic circuitry of the sympathetic or parasympathetic nervous systems. Evidence consistent with a neuronal mechanism has been described. Blandizzi and colleagues65 demonstrated an upregulation of inhibitory presynaptic α_2 adrenoceptors on sympathetic nerves of the ileum in animals with colitis, which leads to reduced small bowel transit. Similarly, De Schepper and colleagues66 showed that colitis leads to alterations in gastric emptying in rats treated with TNBS. However, in this case the functional effects were blocked by sectioning the pelvic afferent pathway from the colon, implicating that a pathway involving CNS circuitry may be involved. In related human studies, alterations in neuronal chemical coding patterns have been detected in non-inflamed regions of colon from individuals with ulcerative colitis or Crohn's disease.67,68

Many aspects of this issue remain to be resolved. For example, is there an alteration in intestinal host defense functions, such as secretion and blood flow, as well as changes in motility? Considerable work is required to identify the pathways and mediators of these distant effects of localized inflammation, but these studies will shed light on the regulatory control and integration of gut function at many levels.

What is the best inflammation model to use?

The best animal model for studying inflammation-induced neuroplasticity is the model that provides the feasibility for the investigator to answer their question, and at the same time

most closely resembles the human pathological condition. While progress is being made, and common features of inflammation-induced neuroplasticity are being identified, the compromises that are made by investigators for the sake of feasibility consistently raise questions as to the validity and/or relevance of these findings. For example, studies of alterations in ion currents of extrinsic sensory neurons are typically conducted with enzymatically dissociated neurons that have had their processes detached, and the vast majority of work on the physiological properties of enteric neurons has been done in the guinea pig, which limits the molecular approaches that can be applied. Hopefully, we will be able to apply the findings obtained with these preparations to other models including, wherever possible, human tissue. This approach has been accomplished to some degree with regard to alterations in mucosal serotonin signaling69, and as testable models for inflammation-induced neuroplasticity are developed, it will be interesting to see whether they are validated in human tissue. Enhanced interactions between neurobiologists and immunologists would also aid our efforts to resolve the intricacies of neuroimmune interactions in the gut.

When is chronic inflammation really chronic in animal models?

Various animal models of inflammation are described as representing acute or chronic inflammatory responses, or both. For example, the TNBS model comprises an acute phase lasting 24–48 hr that is associated with the mucosal disruption, and a chronic phase caused by T cell activation that is self-limiting and occurs from day 3 to three or four weeks after TNBS administration. The question is, when studies are conducted at post-TNBS day 6 or 7, during the peak of “chronic” inflammation, does this really reflect chronic condition in an individual who has been suffering from IBD for several years? For example, while visceral hypersensitivity is a property of various animal models of inflammation, including TNBS, as well as acute inflammatory responses in humans, pain is not necessarily a characteristic symptom of chronic IBD. It has been proposed that over the long term, anti-nociceptive mechanisms may prevent or diminish visceral hyperalgesia.70 Collins and colleagues have investigated this issue in mice with DSS colitis.71 They found that mice with mild and acute DSS colitis exhibit augmented responsiveness to colorectal distension, but mice with chronic DSS colitis, induced repetitively over a 12-week period, do not exhibit hypersensitivity and have elevated β-endorphin and μ opioid receptor levels. The time-course disparity, with longer durations allowing for compensatory changes, could also explain why 5-HT content and EC cell numbers are increased during the chronic phase of TNBS colitis and ileitis, whereas many studies report that they are reduced in the mucosa of individuals with IBD.69 On the other hand, serotonin transporter (SERT) expression is reduced in all animal models of inflammation studied to date as well as IBD. It is possible that 5-HT is down-regulated over the long term in response to decreased SERT expression and an associated increase in 5-HT availability.

CONCLUDING REMARKS

The focus of this review was to describe the changes in the physiological properties of enteric nerves in response to inflammation, and in subsequent recovery. Clearly, neurophysiolgical changes are only part of the picture regarding inflammation-induced changes in gastrointestinal function. For example, overall changes in gastrointestinal function likely involve neuroplasticity, as well as changes in the structure and function of enteric glia, autonomic input to the gut, interstitial cells of Cajal, smooth muscle, epithelium, and the vascular bed. That said, now that we have identified a number of consistent features of inflammation-induced neuroplasticity, and we have begun to understand the mechanisms that are involved, we are in position to explore the effects of these types of changes on gut function, and whether normal gut function can be restored when neuroplasticity is prevented or reversed.

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