

## LEADING ARTICLE

## Role of nerves in enteric infection

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Peripheral and central effects of enteric infection are considered. Nerves play a vital part in the immediate response to enteric infection, promoting pathogen expulsion by orchestrating intestinal secretion and propulsive motor patterns. Laboratory studies indicate that therapeutic agents aimed at modulating the neural response can profoundly alter the outcome of infection. As our understanding of the role of nerves increases, exciting new targets for therapeutic intervention will emerge in both acute and chronic disorders induced by enteric infection.

Anyone who has experienced the severe abdominal cramps, vomiting, and violent diarrhoea associated with infective gastroenteritis will be in no doubt that the nerves in the gut are profoundly affected by enteric infection. They probably also remember the lethargy, nausea, and profound food aversion mediated by effects on the central nervous system (CNS). Both these peripheral and central effects will be considered in this article.

Organisms causing enteric infection have evolved exquisite adaptations to their host, interacting with, and subverting, normal host signalling pathways. The key elements of the host response are vomiting, profuse intestinal secretion, propulsive motor patterns, and behavioural adaptations to avoid future infection. An immediate response is vital in view of the rapid multiplication of enteric organisms; on first encounter acquired immunity has little role. Innate defensive mechanisms including lysozyme, gastric acid, intestinal defensins, and mucus secretion are all important but the simple non-specific clearance of gut contents by vomiting and profuse fluid secretion is a highly efficient way of eliminating organisms, regardless of their specific characteristics. The nervous system is ideally suited to provide such a rapid response and to coordinate the host response, being characterised by speed, plasticity, and learning.

## GUT INNERVATION

The gut is richly innervated containing approximately  $10^8$  neurones, as many as the entire spinal cord. Nerves interacting with enteric infections can be conveniently divided into four groups: (1) intrinsic enteric, (2) extrinsic afferent, (3) extrinsic efferent, and (4) CNS. Intrinsic nerves comprise the bulk of gut nerves. Their cell bodies lie in the submucous and myenteric plexuses, which can be simplistically thought of as controlling mucosal secretory and longitudinal/circular muscle functions, respectively. Predominant excitatory neuromediators are acetylcholine, substance P, 5-hydroxytryptamine (5-HT), and vasoactive intestinal peptide (VIP), with nitric oxide (NO) being the main inhibitory mediator. The extrinsic sensory nerves have cell bodies in the dorsal root ganglia of the spinal cord and the nodose ganglia. Major neuromediators are calcitonin gene related peptide (CGRP), substance P, and glutamine. Their main function is higher order integration of digestion via their input to the brain centres which control such functions as gastric emptying, eating behaviour, pancreaticobiliary secretion, and colonic transit via extrinsic motor nerves to the gut. Postganglionic sympathetic (adrenergic) neurones exert a largely inhibitory influence on secretion and inhibition of this sympathetic "brake" increases the secretory response. Finally, the relevant CNS nerves responding to infection are mainly in the brainstem from where they can influence extrinsic motor nerve output. We can consider the role of these nerves in infection under five major functional headings (see table 1).

## AMPLIFICATION OF LOCAL SECRETORY RESPONSE

The gut mucosa contains numerous free nerve endings whose cell bodies lie in the submucosa and myenteric plexuses. Intrinsic primary afferent nerves (IPANs) have free nerve endings in the mucosa and express a number of receptors, including 5-HT<sub>1p</sub>.<sup>1</sup> They form the afferent arm of reflexes which control secretion and motility. Stimulating them by stroking or puffs of nitrogen elicits secretory responses which involve cholinergic interneurons and cholinergic or VIPergic secretory effector neurones.<sup>2</sup> Infecting organisms stimulate IPANs both directly and indirectly; the

**Table 1** Function of nerves in enteric infection

- Amplification of local secretory response
- Activation of mast and other inflammatory cells
- Inhibition of sympathetic "brake"
- Modulation of gastrointestinal motility and sensitivity
- Modulation of whole host behaviour

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**Abbreviations:** CNS, central nervous system; 5-HT, 5-hydroxytryptamine; VIP, vasoactive intestinal peptide; IPANs, intrinsic primary afferent nerves; CT, cholera toxin; CGRP, calcitonin gene related peptide; TNF- $\alpha$ , tumour necrosis factor  $\alpha$ ; NO, nitric oxide; LPS, lipopolysaccharide; GMC, giant migrating contraction; PAF, platelet activating factor; TLR4, Toll-like receptor 4.

neural networks then spread the secretory response, changing it from a localised response to one which is widespread and capable of flushing the entire gut. This amplification is vital for luminal stimuli to activate the main secretory cells which lie deep in the crypts and hence are not directly exposed to luminal factors. The importance of this amplification can be seen from the effect of VIP antagonists. These block the effector neurones and virtually abolish the secretory response to cholera toxin (CT) in an intact animal,<sup>3</sup> in spite of the fact that the local secretory effect of CT on individual enterocytes, which acts via stimulation of adenyl cyclase, is probably unaffected.

#### Direct action of enteric pathogens on nerves

This is an evolving area. The best studied example at present is CT, a member of the A-B family of heat labile enterotoxins which also include heat labile *Escherichia coli* enterotoxin as well as enterotoxins from *Salmonella*, *Campylobacter jejuni*, *Pseudomonas aeruginosa*, and *Shigella dysenteriae*.<sup>4</sup> CT is composed of one A and five B subunits. The toxic A subunit gains entry to the cell when the B subunit binds to GM<sub>1</sub> ganglioside, a receptor which is present on both enterocytes and nerves. *Vibrio cholerae* facilitates this process by the action of its neuraminidase which converts other gangliosides to GM<sub>1</sub>, thereby increasing the number of receptors. Fluorescein labelled B subunits have been shown to enter VIPergic but not other types of neurones. CT application leads to specific depolarisation and increased excitability of these VIPergic neurones<sup>5</sup> which mediate both secretion and the accompanying vasodilatation needed to allow such profuse secretion. It is likely that other toxins may also similarly activate these largely secretory neurones.

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Impairment of inhibitory M<sub>2</sub> muscarinic receptors by influenza and parainfluenza neuraminidases is another mechanism whereby infection alters neural function, which has been demonstrated in the lung but not so far in the gut. This leads to enhanced vagally mediated bronchoconstriction and postinfectious bronchospasm.<sup>6</sup>

#### Indirect actions

Most secretory responses are indirect enteric pathogens generating a range of molecules including serotonin, prostaglandins, nerve growth factors, histamine, adenosine, and H<sup>+</sup> which act on receptors on nerves to elicit and amplify the secretory response. Enteroendocrine and mast cells are important sources of such mediators.

Enteroendocrine cells, sited with their microvilli protruding into the lumen, are well placed to sample the intestinal lumen and respond to enteric pathogens. More than half of these cells contain serotonin, which is released on exposure to CT. This then acts via 5-HT<sub>2c</sub> receptors to stimulate prostaglandin mediated secretion and via 5-HT<sub>3</sub> and 5-HT<sub>4</sub> receptors to stimulate neural secretory reflexes.<sup>7</sup> CT induced secretion of serotonin requires a rise in intracellular calcium to initiate exocytosis from enteroendocrine cells which explains why both CT induced release of serotonin and CT induced secretion can be blocked by L channel blockers such as nifedipine,<sup>8</sup> while calcium ionophores mimic CT induced secretion of both serotonin and fluid. Prostaglandins are generated by many cells in response to injury and also by macrophages in response to serotonin via 5-HT<sub>2c</sub> receptors. They activate VIPergic and cholinergic secretory nerves and are important in CT induced secretion, which can be substantially reduced by indomethacin in both rats<sup>9</sup> and humans.<sup>10</sup>

Although less well investigated it is clear that rotavirus diarrhoea is also highly dependent on neural reflexes for its

amplification, especially in the later phase when reactive hyperaemia with tissue oedema and vasodilation are prominent before villus tip necrosis develops. At this stage tetrodotoxin, lidocaine, and the ganglion blocker mecamlamine all significantly reduce fluid secretion<sup>11</sup> to an extent suggesting that two thirds of the effect of the virus is mediated via the enteric nervous system.

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Another indirect way in which infection influences nerves throughout the body is via the immune response which, following *Campylobacter* enteritis, may include the development of antibodies to GM<sub>1</sub>, a ganglioside expressed on nerves. Rarely, an ascending polyneuritis or Guillain-Barre syndrome is seen, particularly after infection with certain Penner serotypes whose lipopolysaccharides contain epitopes also found in gangliosides.<sup>12</sup> This “molecular mimicry” may be part of an attempt to evade the host’s immune response.

#### ACTIVATION OF MAST AND OTHER INFLAMMATORY CELLS

Enteric pathogens can activate mast cells in several ways. If the host is immune then pathogen antigens can bind to specific IgE on the mast cell surface to cause release of contents. However, a naive host can also respond via an axonal reflex. This has been well documented in *Clostridium difficile* enteritis. Locally produced toxin A activates nuclear factor κB causing enterocytes and macrophages to produce neutrophil chemoattractants such as interleukin 8, macrophage inflammatory protein 2, prostaglandin E<sub>2</sub>, tumour necrosis factor α (TNF-α), and leukotriene B<sub>4</sub>. However, *C difficile* infection is highly focal and it appears that the neural reflex is important in amplifying and spreading the inflammatory and secretory response. Destruction of extrinsic sensory nerves with capsaicin substantially reduces the toxic effects of *C difficile*, which are also reduced by substance P or CGRP antagonists. This suggests that much of the enteritis in *C difficile* infection depends on substance P, released from extrinsic afferents via an axonal reflex, acting to degranulate mast cells.<sup>13</sup> Mast cell deficient mice have much reduced inflammation and fluid secretion. Furthermore, mast cell stabilisers likewise substantially reduce enteritis in normal mice. Mast cell activation releases many mediators, including histamine, serotonin, prostaglandins, and mast cell tryptase. This latter mediator acts on proteinase activated receptor 2 which is found on extrinsic afferents, causing release of the inflammatory neuropeptides CGRP and further substance P.<sup>14</sup> The impact of released substance P is enhanced by upregulation of substance P receptors on both epithelial cells and lamina propria macrophages in which it enhances TNF-α secretion.<sup>15</sup> This positive feedback amplification allows the host to respond to very small amounts of bacteria in an anticipatory way, allowing clearance of the organism before it has multiplied excessively. Nitregic nerves also appear to exert an inhibitory effect on mast cell degranulation and impairment of these nerves may allow excessive mast cell degranulation. Such a loss of inhibitory nerve input is also important in the impact of inflammatory cytokines.

#### INHIBITION OF SYMPATHETIC “BRAKE”

Sympathetic postganglionic efferent nerves release noradrenaline which hyperpolarises VIP secretomotor nerves thereby reducing VIP release and hence intestinal secretion.<sup>2</sup> Inflammatory mediators generated during infection such as interleukins 1 and 6, TNF-α, and platelet activating factor (PAF) act to inhibit noradrenaline release<sup>16</sup> allowing the massive increases in secretion needed to flush out enteric pathogens.

Such changes may be prolonged; thus even 100 days after infection with *Trichinella spiralis*, long after the parasite has left the gut, noradrenaline release by electrical field stimulation remains depressed at only 50% of normal.<sup>17</sup> The cytokines responsible appear to be locally produced in the myenteric plexus, possibly by enteroglial cells.

## MODULATION OF GASTROINTESTINAL MOTILITY AND SENSITIVITY

### Immediate response

Most enteric infections are associated with inflammation which non-specifically inhibits gastrointestinal muscle contractility, impairing normal mixing activity but allowing intermittent giant migrating contractions (GMCs), which flush the gut, leading to urgent defecation.<sup>18</sup> Bacterial infections also cause systemic exposure to lipopolysaccharide (LPS). This activates resident macrophages within the muscle layers to produce substantial amounts of NO which impairs smooth muscle responsiveness.<sup>19</sup> Studies of motor patterns during infection are rare but those that have been done show that CT, the heat labile enterotoxin of *E coli*, and the Salmonella enterotoxin all induce propulsive clustered migrating contractions as well as profuse secretion in experimental animals.<sup>20, 21</sup> Salmonellosis in humans has similarly been shown to be associated with inhibition of normal intestinal mixing contractions interspersed with episodic GMCs<sup>22</sup> which minimise contact time and contribute to diarrhoea. Possible mediators of these GMCs include prostaglandins, PAF, substance P, and VIP.<sup>23–25</sup> GMCs are characteristically of high amplitude and prolonged and hence are associated with abdominal cramps.

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Additionally there is a sensitising effect whereby afferent firing in response to gut distension is increased by the “inflammatory soup” which is associated with infection.<sup>26</sup> This “soup” includes prostaglandin E<sub>2</sub>, bradykinin, K<sup>+</sup>, and ATP released from damaged cells, which activate nerve endings both directly and indirectly via release of histamine, 5-HT, and nerve growth factor from mast and other cells. Visceral hypersensitivity will render even normal contractions painful and by inhibiting food intake may speed up symptom resolution.

### Long term response

While most of the inflammatory response rapidly subsides, gastrointestinal function may remain disturbed for months and in some cases years. Postinfectious bowel dysfunction is seen in up to 25% of patients following *Campylobacter* enteritis<sup>27, 28</sup> and is characterised by persistent low grade chronic mucosal inflammation<sup>28</sup> and increased numbers of serotonin containing enteroendocrine cells.<sup>29</sup> Persistent changes in gut nerves and muscle are seen in mice following *Trichinella spiralis* infection. This animal model of postinfectious bowel dysfunction has been extensively studied. It is associated with visceral hypersensitivity, muscle wall thickening, and increased responsiveness to cholinergic stimuli. There is an increase in substance P labelled neurones<sup>30</sup> and enhanced afferent firing in response to colorectal distension. Mucosal injury as in chemical colitis has been shown to induce initial nerve loss followed by hyperinnervation, the new nerves showing significant differences in morphology and density.<sup>31</sup> Changes such as this may also underlie the features of the postinfectious syndrome described following bacterial enteritis.<sup>27, 28</sup> These patients with “postinfective irritable bowel syndrome” suffer from diarrhoea and abdominal cramps and

are characterised by accelerated transit and increased sensitivity to visceral distension. These changes may be adaptive to individuals living in areas where bacterial infection is common as they are likely to accelerate transit and hence clearance of organisms from the gut.

## MODULATION OF WHOLE HOST BEHAVIOUR

The CNS responds to infection by recognising possible causes and making associations which profoundly alter subsequent feeding behaviour. The experience of nausea and vomiting induces a profound aversion to any suspect food making similar events less likely in the future. In addition to this learnt response, there are also acute behavioural responses which include thermogenesis, anorexia, lethargy, and withdrawal from normal behaviour, all responses designed to inhibit pathogen survival and divert the host's energy to fighting infection. Many of these behavioural responses are mediated by cytokines such as interleukin 1 $\beta$ , interleukin 6, TNF- $\alpha$ , and interferon which are initially produced locally at the site of inflammation where they stimulate afferent nerves and are later produced in the brain.<sup>32</sup>

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During bacterial enteritis the gut and later the brain are exposed to bacterial LPS which has profound effects on the nervous system. Intraperitoneal LPS activates the vagus<sup>33</sup> and plays a key role in mediating the delay in gastric emptying seen during endotoxaemia.<sup>34</sup> Systemic LPS also accelerates intestinal transit by increasing local production of NO thereby inhibiting normal intestinal motor patterns.<sup>35</sup> Transduction of LPS into a CNS response depends on interaction with Toll-like receptor 4 (TLR4) leading to nuclear factor  $\kappa$ B activation and cytokine production. TLR4 is specifically expressed in microglia in areas of the brain such as the circumventricular organs where the blood brain barrier is absent<sup>36</sup> and where endotoxaemia induces cytokine production and the behavioural responses described above.

## CONCLUSION

As this necessarily brief overview indicates, nerves play a vital part in the immediate response to enteric infection, promoting pathogen expulsion by orchestrating intestinal secretion and propulsive motor patterns. Remodelling of nerves with changed receptor expression following initial damage may also contribute to long term changes in function. Laboratory studies indicate that therapeutic agents aimed at modulating the neural response can profoundly alter the outcome of infection. As our understanding of the role of nerves increases there will undoubtedly be exciting new targets for therapeutic intervention in both acute and chronic disorders induced by enteric infection.

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