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Role of vasoactive intestinal peptide and inflammatory mediators in enteric neuronal plasticity

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Abstract Complex circuits involving both local intrinsic neurones (i.e. enteric nervous system; ENS) and extrinsic neurones achieve nervous control of digestive functions. The ENS is comprised of many functionally different types of neurons: sensory neurones, interneurons and secreto-motor neurons. Each neuronal population is required to manifest local reflex behavior and is central to the regulation of both motor and secretory activities. It must be emphasized, however, that not only muscle and secretory cells but also other intestinal cells are targeted by enteric neurones, i.e. endocrine cells, interstitial cells of Cajal, immune cells, blood vessels and enteric glia. In addition to the ENS the gastrointestinal tract receives an extrinsic innervation by sympathetic, parasympathetic and sensory fibres. Neuronal projections from the intestine to prevertebral ganglia also exist. Taken together, the picture of a complex nervous regulation of digestive functions highly integrated with the central nervous system and the rest of the autonomic nervous system has emerged. The ENS is adaptive and plastic, but also vulnerable, system and ENS disturbances may be of pathogenic importance in functional bowel disease. In particular the interplay between the enteric neurones and the immune cells is suggested to be of crucial importance. The review discusses possible roles of the mediators vasoactive intestinal peptide (VIP) and prostanoids in ENS plasticity in response to injury and inflammation.

Keywords cytokines, inflammation, myenteric plexus, prostaglandins.

INTRODUCTION

Enteric neuronal plasticity is an essential adaptive response to various injuries or functional changes within the gastrointestinal tract. It is a complex process involving alterations in neuronal excitability, neurotransmitter expression and/or structural rearrangements. These changes can occur transiently in response to an acute stimulus or become permanently encrypted into the enteric neural circuitry following severe injury or a chronic pathological process. Vasoactive intestinal peptide (VIP) and prostanoids have emerged as important mediators of neuronal plasticity. These mediators are prime examples of the bidirectional communication between the immune and enteric nervous systems. Below we highlight a limited number of studies, which demonstrate the roles of these two mediators in regulating an adaptive plastic response of the enteric nervous system (ENS).

VASOACTIVE INTESTINAL PEPTIDE

Changes in the expression of neurotransmitters have been found to be a common event in neuronal plasticity (for a review see Ekblad *et al.* 1999¹). VIP is one of the most readily upregulated transmitters and this occurs during several intestinal pathophysiological situations (Fig. 1), such as blockade of axonal transport by colchicine or axotomy,² transplantation or primary culture of isolated ganglia,^{2,3} intestinal surgery⁴ and intestinal hypertrophy.⁵ An increased VIP expression has also been reported in myenteric ganglia in patients with Crohn's disease.⁶ Recently, VIP given intraperitoneally to mice with trinitro-benzenesulfonic acid (TNBS)-induced colitis reduced the clinical as well as the histopathological signs of the inflammatory response.⁷ This was achieved by down-regulating proinflammatory cytokines [tumour necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6 and IL-12] and by increasing the production of the anti-inflammatory agent IL-10. In

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Figure 1 Upper panel: whole mounts of myenteric ganglia demonstrating autoradiographic labelling of VIP mRNA in nerve cell bodies by *in situ* hybridization in control and colchicine-treated rat small intestine. The number of neurones labelled for VIP mRNA markedly increase after colchicine treatment. Lower panel: myenteric neurones after 4 days in culture, subjected to double staining with nitric oxide synthase (NOS) and VIP. The number of myenteric neurones expressing VIP increase during culturing and this VIP upregulation colocalizes within NOS-containing neurones.

addition it is noteworthy that intestinal inflammation induces secretion of cytokines such as IL-1, TNF- α , interferon (IFN)- γ and transforming growth factor (TGF)- β ⁸ from the myenteric ganglia (the exact identity of the cytokine-secreting cells, neurones or glia, have not yet been established). Taken together, these data suggest an important role for VIP as an anti-inflammatory neuropeptide, denoting that the enteric nervous system can have a profound effect on gastrointestinal immunology.

VIP has also been found to be important in neuroprotection in both central^{9,10} and enteric neurones.¹¹ In the central nervous system (CNS) the neurotrophic effect of VIP has been suggested to be mediated via release of a variety of cytokines (IL-1 α , IL-1 β , IL-3, IL-6, TNF- α)¹² chemokines (RANTES and macrophage inflammatory protein-1 α) and growth factors such as neurotrophin-3¹³ and activity-dependent neurotrophic factor¹⁴ from astrocytes. VIP has also been shown to release nitric oxide in cocultured rat cerebral cortical

cells and glia.¹⁵ Therapeutic administration of VIP or the related pituitary adenylate cyclase-activated peptide (PACAP) after CNS trauma, as well as in neurodegenerative disorders such as Parkinson's disease and multiple sclerosis, has been suggested to possibly prevent neuronal cell death.¹⁶ This suggestion is based on the findings that VIP and PACAP inhibit the production of proinflammatory mediators from activated microglia.

In cultured myenteric neurones both VIP and nitric oxide (NO) promote neuronal survival while VIP antiserum or NO synthase (NOS) inhibition enhance neuronal cell death,¹¹ indicating that ENS and CNS utilize similar mechanisms for neuroprotection. VIP belongs to a large family of structurally related peptides that also include secretin, growth hormone-releasing factor and PACAP. Like VIP, PACAP is considered an important neurotransmitter within the ENS.¹⁷ Three VIP/PACAP receptors have been cloned:¹⁸ PAC₁ with high affinity for PACAP, but with low affinity for VIP,

VPAC₁ and VPAC₂. VPAC₁ and VPAC₂ receptors have approximately equal affinity for both VIP and PACAP. In addition pharmacological characterization of intestinal VIP/PACAP receptors has revealed the existence of a 'VIP-specific' (activated by VIP but not by PACAP) as well as a PACAP-27-preferring receptor.¹⁷ The expression of PACAP is, in analogy to VIP, readily upregulated in enteric neurones in a number of experimental models such as axonal transport blockade by axotomy or colchicine treatment² and during hypertrophic growth of the intestine.⁵ The VIP/PACAP receptor(s) involved in promoting neuronal survival is still unsettled. In contrast to VIP, PACAP has not been found to promote survival of cultured myenteric neurones.¹⁹ This suggests, as both VPAC₁ and VPAC₂ have a high affinity for both VIP and PACAP while PAC₁ is activated almost exclusively by PACAP, the presence of a 'VIP-specific' receptor mediating neuroprotection in adult myenteric neurones. In this context it is notable that an as yet uncharacterized VIP-preferring receptor has also been suggested to mediate the VIP-induced cytokine release from astrocytes.¹²

VIP has emerged as a multifactorial enteric neurotransmitter. It has long been recognized as an important neurotransmitter in the ENS for its potential to induce intestinal secretion and relaxation, but now also for its roles in neuroprotection, growth regulation and additionally as a potent anti-inflammatory peptide. The precise mechanisms, by which these various effects of VIP are mechanistically accomplished, are far from understood, but will provide fertile ground for important future studies.

INFLAMMATORY MEDIATORS

Inflammatory modulation of the gastrointestinal nervous system is gradually becoming a well-recognized phenomenon. Various products released from resident and/or infiltrating leucocytes have been demonstrated to sensitize or even directly activate myenteric neurones and intestinal primary afferent neurones. Reactive radicals, such as superoxide, peroxynitrite, lipid peroxide and hydrogen peroxide generated by leucocytes during inflammation or ischaemia, have been shown to alter guinea-pig colonic AH/type-2 myenteric neurones²⁰ as well as cause enteric neuronal damage.²¹ Additionally, reactive radicals have been reported to stimulate afferent splanchnic C fibre units.²²

Resident leucocytes within the gastrointestinal wall, such as mast cells and muscularis macrophages, when activated play a significant role in neuronal plasticity following injury and a changing environ-

ment. The mast cell product bradykinin has been shown to facilitate the enteric release of acetylcholine,^{23,24} and to increase the excitability of myenteric neurones²⁵ and intestinal primary afferents.^{26–28} Interestingly, the neuromodulatory effects of bradykinin appear to be mediated in large part through the release of prostanoids^{23,24,29,30} via stimulation of the Bk-2 receptor that is coupled to mobilization of both extracellular and intracellular calcium stores in enteric neurones.²⁹

Gradually, evidence is being amassed that indicates that resident muscularis macrophages play an important role in modulating enteric neuromuscular activities. As would be expected of this resident phagocyte, muscularis macrophages are prolific secretors of cytokines, chemokines and mediators that alter motility.^{31–35} The cytokines IL-1 β and IL-6, potentially from muscularis macrophages,^{30,33,34,36–38} have also been shown to directly enhance the excitability of both AH- and S-type myenteric neurones,^{39,40} decrease the amplitude of fast excitatory postsynaptic potentials⁴⁰ and modulate the enteric release of norepinephrine.⁴¹ It has been shown that TNF binding protein and IL-1 receptor antagonism limits lipopolysaccharide (LPS)-induced iNOS expression within the gut wall, and thus moderates sepsis related ileus.³⁸ Additionally, this treatment, by limiting cytokine-neuronal interactions, could also potentially ameliorate endotoxin induced ileus.

In addition to cytokines, various leucocyte populations involved in the intestinal inflammatory response secrete prostanoids from the highly inducible enzyme cyclooxygenase-2 (COX-2). Cyclooxygenases are key enzymes that produce prostaglandins, catalysing the conversion of arachidonic acid to prostaglandins. Three cyclooxygenase (COX) isoforms have been identified and are referred to as COX-1, COX-2 and COX-3,^{42,43} COX-1 and COX-3 are produced constitutively, whereas COX-2 is an inducible enzyme known to be upregulated in many inflammatory states.⁴⁴ COX-2 has been shown to be expressed abundantly during sepsis, inflammatory bowel disease, surgery and transplantation.^{32,33,45–47} It follows that this synthase would be induced by reactive radicals, cytokines, and growth factors.⁴⁸ Resident muscularis macrophages, a main leucocyte population involved in the inflammatory response during sepsis and postoperative ileus,^{33,37,45} secrete significant motility altering amounts of prostaglandins.^{34,45}

It has been shown conclusively that prostaglandins, through the induction of COX-2, play a major causative role in initiating and maintaining ileus after intestinal surgery.³³ In this inflamed bowel state

and in others, prostaglandins could hypothetically modulate intestinal motility by four distinct mechanisms. First, prostanoids are proinflammatory and participate in generating the complex inflammatory milieu within the muscularis externa. We have shown that selective COX-2 inhibition given as a pharmacological pretreatment restrains the development of the molecular and cellular inflammatory response within the surgically manipulated muscularis.³³ Secondly, the local generation of prostaglandins within the muscularis by macrophages appears to have a direct inhibitory effect on inflamed smooth muscle contractility, because when COX-2 is acutely inhibited the suppression in circular muscle contractility is relieved from tonic prostanoid inhibition.^{33,45}

The third and fourth proposed mechanisms demonstrate the involvement of prostaglandins in enteric neuronal plasticity and, thus, illustrate an immunoneuronal interaction within the ENS. Given the demonstrated direct smooth muscle effects of prostaglandins during intestinal inflammation, intuitively the ENS and primary afferent nerve endings within the inflamed muscularis would also be exposed to pathological high levels of prostanoids. Hence, the effect of PGE₂ has been studied on myenteric and sensory afferent neurones. Recently, it has been shown that the acute application of PGE₂ depolarizes both AH and S-type colonic neurones with little effect on input resistance or electrical excitability. Prolonged exposure, however, also caused an enhancement in excitability. Hence, these results suggest that PGE₂ can play a role in altered motility during inflammatory states by evoking changes in the electrical properties of myenteric neurones. Additionally, prostaglandins have been shown to alter mucosal secretory responses.^{49–51} Finally, prostaglandins, secreted during inflammation, have been shown to enhance extrinsic primary afferent nerve firing.⁵² Again, PGE₂ in particular has been demonstrated to have complex effects on primary intestinal afferents.^{53,54,54} Furthermore, it has been demonstrated that intestinal surgical manipulation of the gut markedly increases spinal *c-fos* expression for a prolonged period postoperatively and that COX-2 inhibition significantly diminishes this prolonged increase in primary afferent activity.⁵⁵ It has been hypothesized that this reflects heightened local primary afferent activation, which would initiate subsequently sympathetic inhibitory motor reflexes to the gut, leading to an immunoneuronal component of inflammatory mediated ileus. Thus, these data demonstrate that prostaglandins generated by the inducible COX-2 provide a crucial link between intestinal inflammatory

mechanisms and the development of neuroplastic responses within the inflamed gut wall.

CONCLUDING REMARKS

Gastrointestinal dysfunction, which often has a component of dysmotility, accompanies most gastrointestinal diseases. Gastrointestinal activities are controlled, to a great extent, by enteric nerves and thus many functional bowel disorders probably have their origin in neuropathological changes of the ENS. We are gradually acquiring a fairly good understanding of ENS organization and function, and even though it belongs to the autonomic nervous system it strikingly resembles the CNS in neurochemistry, neuronal circuitry, mechanisms for long-term potentiation, glial elements and lack of collagen. By analogy with CNS neurological disorders, it may be expected that the ENS will also malfunction and be mechanistically at the root of various gastrointestinal diseases, and accumulating evidence supports this hypothesis. Hence, the pathogenesis and pathophysiology of several intestinal disorders are suggested to involve injury- or inflammation-induced neurodegeneration. In response to and in order to counteract these various injurious mechanisms, the ENS has developed adaptive plasticity and neuroprotective mechanisms as key features to maintain intestinal function. Advancements in our knowledge on the identity, role and regulation of the various mediators involved in plasticity and protection will undoubtedly be followed by clinical applications.

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