Implications of Tachykinins and Calcitonin Gene-Related Peptide in Inflammatory Bowel Disease

Peter Holzer
Department of Experimental and Clinical Pharmacology, University of Graz, Austria

Key Words
Gastro-intestinal tract
Tachykinins
Substance P (SP)
Neurokinin A (NKA)
Calcitonin gene-related peptide (CGRP)
Tachykinin receptor antagonists
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Primary afferent neurons
Inflammatory bowel disease
Motor disturbances
Diarrhoea
Inflammation
Hypersensitivity
Pain

Abstract
Calcitonin gene-related peptide (CGRP) and the preprotachykinin A gene-derived peptides substance P (SP) and neurokinin A (NKA) are expressed in extrinsic primary afferent nerve fibres and intrinsic enteric neurons of the gut. The actions of tachykinins on the digestive effector systems are mediated by three different types of tachykinin receptor, termed NK₁, NK₂ and NK₃ receptors, while the gastro-intestinal actions of CGRP are brought about by CGRP₁ and possibly other CGRP receptors. These neuropeptide transmitters are expressed by enteric neurons, intestinal muscle, epithelium and vascular system in a cell-specific manner and enable SP, NKA and CGRP to influence motility, electrolyte and fluid secretion, vascular and immune functions in a peptide- and region-specific fashion. Inflammatory disorders of various aetiology involve changes in the peptidergic innervation of the gut, and inflammatory bowel disease is associated with NK₁ receptor upregulation in intestinal blood vessels and lymphoid structures. Some of these alterations are reproduced in experimental models of gastro-intestinal disease, and there is mounting evidence that an imbalanced function of peptidergic neurons contributes to motor, secretory, vascular and immunological disturbances in intestinal anaphylaxis, infection and inflammation. In a therapeutic perspective it seems conceivable that tachykinin and CGRP receptors antagonists can be employed as spasmylocytic, antidiarrhoeal, anti-inflammatory and antinociceptive drugs.

Introduction
The regulation of intestinal functions by extrinsic afferent neurons, extrinsic efferent neurons of the sympathetic and parasympathetic autonomic nervous system and the intrinsic neurons of the enteric nervous system is increasingly appreciated. Delineation of their chemical coding has revealed that the neurons in the gut express, apart from the classical transmitters acetylcholine and noradrenaline, a variety of neuropeptides including the tachykinins substance P (SP) and neurokinin A (NKA), calcitonin gene-related peptide (CGRP), vaso-active intestinal polypeptide (VIP) and neuropeptide Y [1]. Nerve activity leads to exocytotic release of the peptides which by interacting with specific receptors on postjunctional neurons or effector cells participate in the control of gastro-intestinal motility, secretion, circulation and tissue homoeostasis.

After elucidation of the physiology and pharmacology of tachykinins and other neuropeptide systems in the gut is now becoming evident that gastro-intestinal inflammation and other disorders of the digestive system are related to imbalanced function of certain peptidergic neurons. The hypothesis that neuropeptides have a bearing on gastro-intestinal functions has been put forward for a number of gastrointestinal disorders including inflammatory bowel disease (IBD). The objective of this review is to describe the role of tachykinins and the CGRP system in inflammation and to discuss the potential therapeutic implications of targeting tachykinin and CGRP receptors in inflammatory bowel disease.
Fig. 1. Projections of SP-immunoreactive neurons within the guinea pig intestine, with information on the co-existence with other neuropeptides or neuronal markers. BV = Blood vessel; CB = calbindin; ChAT = choline acetyltransferase; CM = circular muscle; CR = calretinin; DRG = dorsal root ganglion; DYN = dynorphin; ENK = enkephalin; LM = longitudinal muscle; MM = muscularis mucosae; MP = myenteric plexus; MU = mucosa; NF = neurofilament protein; PVG = prevertebral ganglion; SMP = submucosal plexus.

tro-intestinal disease has been most advanced for SP, NKA and CGRP, and it is with the pathophysiological implications of these peptides that the current article is primarily concerned with. I will first give a brief outline of the cellular systems that express these peptides and the physiological functions which they are thought to serve in the digestive system. Thereafter I will discuss the evidence that associates these peptides with pathological states of the gastro-intestinal tract and summarize those conditions in which neuropeptides emerge as a promising target for novel therapeutic interventions.

Expression and Release of SP, NKA and CGRP in the Gut

As other regulatory peptides, the tachykinins SP and NKA are derived from larger precursor peptides, the preprotachykinins (PPT), which are encoded by two different PPT genes [2]. The PPT-A gene encodes both SP and NKA, whereas the PPT-B gene encodes neurokinin B only [2]. The primary RNA transcript of the PPT-A gene is alternatively spliced to produce four different forms of PPT-A messenger ribonucleic acid (mRNA), termed α-PPT, β-PPT, γ-PPT and δ-PPT [3]. SP can be produced by translation of all four PPT-A mRNAs, while sequences coding for NKA are found in β-PPT and γ-PPT mRNA only. Since the PPT-B gene does not seem to be expressed within the digestive system to any appreciable degree, the predominant tachykinins in the gut of mammals are SP and NKA [3]. Whereas SP (undecapeptide) and NKA (decapeptide) are short peptides whose sequence is conserved across mammals, CGRP is a 37-amino-acid peptide whose sequence varies slightly among different mammalian species [4, 5]. Importantly, CGRP exists in two forms as CGRP-α, which is generated by transcription and alternative splicing of the calcitonin/CGRP-α gene, and CGRP-β, which is encoded by the CGRP-β gene [4, 5].

In the gastro-intestinal system, tachykinins and CGRP are primarily expressed in intrinsic enteric neurons and extrinsic primary afferent nerve fibres. The quantitatively most important source of tachykinins is the enteric nervous system [3] which has its cell bodies in the myenteric and submucosal (submucous) plexuses and supplies all gastro-intestinal effector systems (fig. 1). In the guinea pig small intestine which has been studied in most detail, it is important to note that most enteric SP-positive neurons co-express choline acetyltransferase (fig. 1) and that hence tachykinins are cotransmitters of cholinergic enteric neurons [1]. Neuro-anatomical tracing studies [1, 6, 7] have identified several classes of enteric SP neurons in the guinea pig intestine, which differ with regard to morphology, chemical coding and/or projection (fig. 1). CGRP is likewise expressed in enteric neurons of the myenteric and submucosal plexuses, although it is less abundant than SP and NKA [1, 4, 8].

The other important source of CGRP, SP and NKA in the gut is extrinsic afferent neurons which differ from intrinsic enteric neurons with regard to origin, chemical coding, sensitivity to the excitotoxin capsaicin and projec-
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and NK3 receptors, have been cloned, identified to have 7

system. NKA from presumably extrinsic afferents in the digestive

ification of the tissue [18, 19] can release CGRP, SP and

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cation of CGRP and SP is a characteristic of many spinal

expressed in extrinsic afferents is CGRP-

mucosa, to the submucosal and myenteric nerve plexuses

and to the circular and longitudinal muscle layers (fig. 1).

While CGRP and tachykinins do not occur in the same

enteric neurons of the rodent and canine gut, co-expression

of CGRP and SP is a characteristic of many spinal afferents [1, 10, 14, 15]. In addition, the identity of CGRP
differs inasmuch as most of the CGRP expressed in

afferents [1, 10, 14, 15]. In addition, the identity of CGRP

depolarized [3, 4, 9]. The calcium dependency of the release process points to an exocytotic mecha-

anism. Peptide release from extrinsic afferents can specifically be elicited by the excitotoxin capsaicin [3, 4, 9],
because receptors for this drug (vanilloid receptors) are exclusively expressed on spinal and trigeminal sensory

neurons [17]. In the context of this article it is particularly worth noting that inflammatory mediators [3, 9] and acid-

ification of the tissue [18, 19] can release CGRP, SP and NKA from presumably extrinsic afferents in the digestive

system.

Physiological Implications of SP, NKA and CGRP in the Gut

Neuropeptide Receptors

The functional relevance of SP, NKA and CGRP in the gut is underlined by the cell-selective expression of specific peptide receptors on gastro-intestinal effector systems. Three receptors for tachykinins, termed NK1, NK2 and NK3 receptors, have been cloned, identified to have 7 transmembrane spanning segments and found to be coupled to G proteins and the phospho-inositide signalling

pathway [20, 21]. Although NK1 receptors are considered to be SP-preferring, NK2 receptors NKA-preferring and

NK3 receptors NKB-preferring receptors, SP, NKA and NKB are full agonists at all three tachykinin receptors. They can be differentiated, however, by receptor-selective agonists and a variety of peptide-derived or non-peptide antagonists. The activity of tachykinins at their receptors is regulated by agonist-induced receptor internalization and the activity of membrane-bound proteases such as neutral endopeptidase EC 3.4.24.11 [3, 21]. There is pharmacological evidence for a multiplicity of CGRP receptors as well [5], but only one receptor termed CGRP1 receptor, coupled to the adenylyl cyclase signalling system and antagonized by CGRP8-37, has thus far been cloned [22].

Actions of SP, NKA and CGRP on Gastro-Intestinal Motility

Although SP was discovered as a gut-contracting peptide, it is now evident that tachykinins can both stimulate and inhibit gastro-intestinal motility, the net response depending on the type and site of tachykinin receptors that are activated (fig. 2). Nerve-independent facilitation of motor activity can be brought about by NK1 receptors expressed on interstitial cells of Cajal [23–26] and NK2 receptors located on gastro-intestinal smooth muscle cells [24, 27]. NK3 receptors are largely confined to enteric neurons [24, 28] and predominantly mediate cholinergic contraction of the intestinal musculature [3]. However, some NK3 receptors and in particular NK1 receptors [23–26, 28] are also present on inhibitory motor pathways within the enteric nervous system and thus enable SP and NKA to depress motor activity [29, 30] and peristalsis [31] via release of the inhibitory transmitters nitric oxide and VIP. These motor actions of tachykinins have a bearing on the enteric control of gastro-intestinal motility. SP and NKA participate in ascending motor pathways which mediate contraction in response to mucosal stroking or distension of the intestinal wall [3] and synergize with acetylcholine in the relay and execution of the enteric motor programme of peristalsis [32, 33]. This synergistic action, with acetylcholine overriding the action of SP and NKA under physiological conditions, needs to be borne in mind when the implications of tachykinins in gastro-intestinal regulation are considered as a potential target for thera-

dic intervention.

CGRP can also contract the gut via stimulation of enteric neurons, but its prominent motor action is muscle relaxation which arises from a direct action on smooth muscle cells [4]. There is still scarce information to attrib-

ute CGRP a role in the enteric control of gastro-intesti-
Fig. 2 Localization of tachykinin NK₁, NK₂, and NK₃ receptors on neurons, interstitial cells (IC) and muscle cells in the guinea pig intestine. Circles depict neuronal somata. ATP = Adenosine triphosphate; CM = circular muscle; LM = longitudinal muscle; M₃ = muscarinic type 3 acetylcholine receptor; MP = myenteric plexus; N = nicotinic acetylcholine receptor; NO = nitric oxide.

Fig. 3. Implication of tachykinins and tachykinin NK₁, NK₂ and NK₃ receptors in intestinal secretomotor reflexes. Chemical stimuli from the lumen may act on enterochromaffin (EC) cells to release 5-hydroxytryptamine (5-HT) which stimulates enteric sensory neurons (S) of a secretomotor reflex. Circles depict neuronal somata. ACh = Acetylcholine; E = epithelium; EP = enteric nerve plexuses; M = muscarinic acetylcholine receptor; N = nicotinic acetylcholine receptor; NO = nitric oxide; TK = tachykinin.

Tachykinin motility, but there is increasing evidence that under pathological conditions CGRP is released from extrinsic afferent nerve fibres in the gut and causes cessation of propulsive motor activity.

Actions of SP, NKA and CGRP on Gastro-Intestinal Ion and Fluid Transport

Intestinal iron and fluid secretion can be stimulated or inhibited by CGRP, yet it is not known whether these secretory effects are of physiological relevance [4]. The situation, though, is different in the stomach where accumulation of acid in the gastric lumen releases CGRP from extrinsic afferent nerve fibres which represent a negative feedback system in gastric secretory control [19]. This implication of CGRP is consistent with the peptide’s high activity in depressing basal and secretagogue-induced acid secretion, an action that is brought about by CGRP₁ receptors and involves release of somatostatin, whereas the release of acetylcholine, gastrin and histamine is inhibited [4, 34]. In contrast, the variable effects of tachykinins on the gastric secretion of acid, bicarbonate and pepsinogen have not yet been elucidated with regard to their functional significance [35]. There is, however, mounting evidence that tachykinins regulate the secretory activity of the small and large intestine [35]. Stimulation of NK₂ receptors on enterocytes [27] and of NK₁ and NK₃ receptors on enteric neurons [24, 25, 28, 36] enhances electrolyte and fluid secretion, and it appears as if tachykinins play a messenger role in enteric secretory reflex pathways (fig. 3). The available evidence suggests that SP and NKA released from intrinsic sensory neurons contribute to the activation of cholinergic and noncholinergic...
secretomotor neurons which by releasing acetylcholine, VIP and nitric oxide cause ion and fluid secretion [35].

**Vascular and Immunological Actions of SP, NKA and CGRP in the Gut**

Both CGRP and tachykinins are vaso-active peptides. CGRP is a particularly potent vasodilator and has been identified as the non-adrenergic non-cholinergic transmitter by which peri-arterial nerve stimulation dilates the mesenteric arteries of several species including man [9]. CGRP is likewise very active in enhancing blood flow in the gastric mucosa of rat, guinea pig and rabbit while its dilator actions in the intestinal mucosa have been studied less systematically [4, 9, 34]. When released from extrinsic afferent nerve fibres in the acid-threatened stomach, CGRP causes a nitric-oxide-dependent vasodilatation which serves a defensive role in the face of pending acid injury to the gastric mucosa [34]. CGRP does not seem to participate in the physiological regulation of gastric blood flow and comes into play under pathological conditions only [35]. SP and NKA may induce vasodilatation or vasoconstriction in the gut, the type of action depending on the vascular bed and species under study [35]. Dilatation of vessels is typically mediated by NK₁ receptors, whereas all three tachykinin receptor types may give rise to vasoconstriction [35]. It is, however, little known whether the effects of tachykinins on gastro-intestinal blood flow are of pathophysiological relevance, which is also true for the NK₁-receptor-mediated increase in venular permeability [35]. This reaction, which is consistently seen in the mouse gut only [37], facilitates the extravasation of macromolecules, fluid and neutrophil leucocytes which, like mast cells, may also directly be stimulated by SP [9]. It remains to be examined whether tachykinins are responsible for the ability of afferent neuron stimulation to increase myeloperoxidase activity and release of interleukin 1 and prostaglandin E₂ in the guinea pig gallbladder [38].

**Pathological Implications of SP, NKA and CGRP in the Gut**

**Changes in the Expression of Neuropeptides and Neuropeptide Receptors in Gastro-Intestinal Disease**

Gastro-intestinal disorders involving mucosal infection, inflammation or ulceration can be associated with changes in the peptidergic innervation of the gut. As summarized in table 1, the alterations of neuropeptide expression are variable, and in many cases it is not known whether they are primary of secondary to the disease and whether they reflect changes in the transcriptional, trans-
lational or metabolic fate of the neuropeptides, changes in nerve activity or changes in peptide release [35]. To shed more light on these issues, several experimental studies have attempted to reproduce disease-related changes in gastro-intestinal neuropeptide systems (table 1) and thus to establish experimental models with which to study the pathophysiological mechanisms behind the observed neuropeptide perturbations.

Although some of the experimentally induced alterations mirror those seen in inflammatory bowel disease (table 1), the results are conclusive only when changes in the neuropeptide tissue levels have been related to changes in gene transcription or peptide release. Thus, colitis evoked by trinitrobenzene sulphonic acid (TNBSA) in the rat leads to increased transcription of β-PPT mRNA [39] while the tissue levels of SP and CGRP are reduced, which points to enhanced release of neuropeptides during the initial phase of the inflammatory reaction [40, 41]. SP and CGRP are likewise depleted from the rabbit colon affected by immune-complex-induced inflammation [42] although in this case the expression of β-PPT mRNA remains unaltered [43]. Inflammation-induced release of SP is indicated by elevated concentrations of SP in rat blood plasma, which accompany the increase in SP synthesis in myenteric neurons following dextran-sulphate-induced colitis [44] and the decrease in intestinal SP levels caused by γ-irradiation [45]. Infection with Salmonella dublin leads to upregulation of β- and γ-PPT mRNA in macrophages of gastro-intestinal lymphoid organs [46, 47], and macrophages in the lamina propria of the rat ileum treated with Clostridium difficile toxin A release greater amounts of SP than macrophages from normal ileum [48].

Of considerable potential is the observation that inflammatory bowel disease [49–51] and pseudomembranous colitis due to C. difficile infection [52] are associated with upregulation and ectopic expression of NK₁ receptors on intestinal blood vessels and lymphoid structures. While NK₁ receptor upregulation in ulcerative colitis is confined to active, pathologically positive specimens of the colon, the ectopic expression of NK₁ receptors in Crohn’s disease is seen in pathologically positive and negative samples of the intestine [51]. The functional implication of NK₁ receptors in the disease process remains to be elucidated as does the source of tachykinins which may potentially be targeted at the upregulated and ectopically expressed receptors. Although S. dublin infection enhances the expression of NK₁ receptor mRNA in macrophages of lymphoid organs [46], the upregulation of tachykinin receptors reported for inflammatory bowel disease has not yet been observed in experimentally induced inflammation of the gut. On the contrary, TNBSA-induced colitis in the rat decreases NK₁ and NK₂ receptor mRNA expression in vasculature, muscle and nerve [53], a change that is thought to reflect a consequence, not cause, of the inflammatory reaction [54].

**Implications of Neuropeptides in Gastro-Intestinal Motor Disturbances**

There is mounting evidence that SP, NKA, CGRP and VIP play a pathophysiological role in the derangement of gastro-intestinal motor activity induced by intestinal anaphylaxis, infection, inflammation, trauma and stress. From the data summarized in table 2 it appears as if extrinsic afferents releasing tachykinins and CGRP are of particular importance, since these neurons are sensitive to tissue irritation and injury. There are two distinct ways by which extrinsic afferents can contribute to motor dysfunction. On the one hand, neuropeptides released from their peripheral terminals in the gastro-intestinal wall are likely to interfere with motility, as the disturbance of peristalsis caused by capsaicin-evoked afferent neuron stimulation in the guinea pig isolated ileum (fig. 4) involves CGRP [55]. The gastro-intestinal motor dysfunctions caused by oesophageal acidification [56], anaphylaxis [57, 58] and local inflammation [59–61] seem also to be brought about by enhanced neuropeptide release from afferent neurons within the gut. On the other hand, afferent neurons will participate in autonomic intestino-intestinal reflexes in which SP, NKA and CGRP released from the central endings of afferent neurons in the spinal cord or brainstem mediate transmission to the efferent reflex arc. Such a central role is most probably reflected by the contribution which tachykinins and CGRP make to emesis, the peritoneogastric reflex, the rectocolonic reflex and postoperative ileus following abdominal surgery (table 2). In addition, neuropeptides may participate in short-loop sympathetic reflexes which are relayed by prevertebral ganglia, because the sympathetic neurons in these ganglia receive not only preganglionic [3, 62] but also primary afferent input (fig. 1).

The perturbations of gastro-intestinal motility in inflammatory bowel disease may also depend on neuropeptides, given that SP (table 1) and NK₁ receptors [49–51] are changed in the inflamed gut, inflammatory mediators such as prostaglandins and leukotrienes interact with neurons releasing SP and CGRP [3, 35, 61–64] and inflammation alters the motor effects of tachykinins. Thus, ricin-evoked ileitis in the rabbit causes upregulation of neurogenic contractions that are mediated by tachykinins.
Cancer chemotherapy, neuropeptides in inflammatory bowel disease

**Fig. 4.** Effect of capsaicin, at a concentration that selectively stimulates extrinsic afferents, on peristaltic motor activity of the guinea pig small intestine in vitro. The pressure threshold of peristalsis is marked by arrowheads. Note that the regular course of peristalsis is profoundly disturbed and the pressure threshold significantly enhanced by capsaicin. Data taken from Barthó and Holzer [55].

**Table 2.** Neuropeptide implications in pathological disturbances of gastrointestinal motility

<table>
<thead>
<tr>
<th>Stimulus or insult</th>
<th>Motor dysfunction</th>
<th>Neuropeptide/receptor implication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer chemotherapy, motion sickness</td>
<td>Emesis (various species)</td>
<td>NK₁ receptors [3]</td>
</tr>
<tr>
<td>Luminal acidification</td>
<td>Relaxation of lower oesophageal sphincter (ferret)</td>
<td>NK₁ receptors [56]</td>
</tr>
<tr>
<td>Intraperitoneal irritation by capsaicin, acetic acid</td>
<td>Inhibition of gastric motility or emptying (peritoneo-gastric reflex in the rat)</td>
<td>NK₁ and CGRP₁ receptors [98, 99]</td>
</tr>
<tr>
<td>Abdominal surgery</td>
<td>Inhibition of gastro-intestinal transit (intestino-intestinal reflex in the rat)</td>
<td>NK₁, CGRP₁ and VIP receptors [123–125]</td>
</tr>
<tr>
<td>Ovalbumin anaphylaxis</td>
<td>Disruption of migrating motor complex in small intestine (rat)</td>
<td>NK₁ receptors [57]</td>
</tr>
<tr>
<td>Castor-oil induced diarrhoea</td>
<td>Giant colonic contractions (rat)</td>
<td>NK₂ and partly, NK₁ receptors [59]</td>
</tr>
<tr>
<td>Rectal distension</td>
<td>Inhibition of colonic motility (recto-colonic reflex in the rat)</td>
<td>NK₁ receptors [103]</td>
</tr>
<tr>
<td>Restraint stress</td>
<td>Increased defaecation (rat)</td>
<td>NK₁ receptors [126, 127]</td>
</tr>
</tbody>
</table>

[60], and inflammation induced by γ-irradiation enhances the sensitivity of the rat jejunum to contract in response to SP [45]. Tachykinins can stimulate migrating giant contractions of the colon [65], an action that seems to be of pathophysiological significance since the giant contractions, which in the rat colon are associated with castor-oil-evoked inflammation and diarrhoea, are prevented by an NK₂ receptor antagonist and reduced by an NK₁ receptor antagonist [59]. It would hence seem that tachykinin NK₂ receptor antagonists may prove beneficial in depressing exaggerated motility associated with infection and inflammation, particularly since they are spasmolytic in the rat colon without having constipating activity [59, 66]. Conversely, NK₁ receptor antagonists could be used to interrupt the pathological downregulation of motility associated with gastro-oesophageal reflux of acid, abdominal surgery and peritonitis (table 2).
### Table 3. Neuropeptide implications in intestinal hypersecretion and inflammation

<table>
<thead>
<tr>
<th>Stimulus or insult</th>
<th>Dysfunction</th>
<th>Neuropeptide/receptor implication</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichinella spiralis</em></td>
<td>Inflammation and lymphocyte proliferation in small intestine (mouse)</td>
<td>NK₁ receptors [77]</td>
</tr>
<tr>
<td><em>Clostridium difficile</em> toxin A</td>
<td>Granulocyte infiltration, mast cell degranulation, TNF-α release from macrophages, hypersecretion and inflammation in small intestine (rat)</td>
<td>NK₁ receptors [48, 67]</td>
</tr>
<tr>
<td>Delayed-type hypersensitivity to DNBSA (after DNFB exposure)</td>
<td>Mast cell degranulation and plasma leakage in small intestine (mouse)</td>
<td>NK₁ receptors [75]</td>
</tr>
<tr>
<td>TNBSA</td>
<td>Granulocyte infiltration, increase in mucosal permeability and inflammation in colon (rat)</td>
<td>NK₁ and NK₂ receptors [78, 95]</td>
</tr>
<tr>
<td>Castor oil</td>
<td>Diarrhoea (rat)</td>
<td>NK₂ and, partly, NK₁ receptors [59]</td>
</tr>
<tr>
<td>Rectal distension</td>
<td>Hypersecretion in colon (rat)</td>
<td>NK₁, NK₂ and NK₃ receptors [70]</td>
</tr>
</tbody>
</table>

TNF-α = Tumour necrosis factor α; DNBSA = dinitrobenzene sulphonic acid; DNFB = dinitrofluorobenzene.

**Implications of Neuropeptides in Diarrhoea and Gastro-Intestinal Inflammation**

Pathological changes in gastro-intestinal fluid and electrolyte secretion are frequent manifestations of infection, inflammation and tissue damage. There is increasing evidence which suggests that SP and NKA participate in a variety of hypersecretory and inflammatory reactions of the gut (table 3). Thus, *C. difficile* toxin A causes capsaicin-sensitive extrinsic afferents in the rat small intestine to release tachykinins which via activation of NK₁ receptors stimulate enteric secretomotor neurons [52] and lead to degranulation of mast cells, release of tumour necrosis factor α from macrophages, granulocyte infiltration, hypersecretion, inflammation and necrosis [48, 67, 68]. Tachykinins also take part in the hypersecretory and inflammatory responses associated with anaphylaxis, delayed-type hypersensitivity and *Trichinella spiralis* infection (table 3) but not in the diarrhoea due to cholera toxin [67]. The actions of interleukin 1β [69], TNBSA and castor oil (table 3) to induce hypersecretion and inflammation in the rat colon depend on both NK₁ and NK₂ receptor activation, but the interrelationship between the two tachykinin receptor systems has not yet been delineated in all instances. This is also true for the secretory response to rectal distension which is inhibited by intraperitoneal injection of an NK₁ or NK₂ receptor antagonist or intracerebroventricular administration of an NK₂ or NK₃ receptor antagonist [70].

Important for understanding inflammatory disease is the hypothesis that SP and CGRP are messengers at the interface between the nervous and immune system and that mast cells, lymphocytes, granulocytes and macrophages are under the influence of peptidergic neurons in the gut [71]. Tachykinin-positive nerve fibres lie in close proximity to mucosal mast cells [72], and SP can interact with G proteins in mast cells of the rat intestinal mucosa to cause release of histamine and other factors [73, 74]. Indeed, the mucosal inflammation induced by *C. difficile* toxin A in the rat ileum [67] and the plasma protein leakage evoked by a delayed-type hypersensitivity reaction in the mouse small intestine [75] depend on both tachykinins and mast-cell-derived factors. Other SP-reactive immune cells include lymphocytes from Peyer’s patches of the mouse, whose synthesis of immunoglobulins is stimulated by SP via interaction with specific SP receptors [76]. The ability of an NK₁ receptor antagonist to reduce lymphocyte proliferation and inflammation in the small intestine of *T. spiralis*-infected mice [77] indicates that the SP-lymphocyte axis is of pathophysiological relevance. A regulatory influence of neuropeptides on granulocytes is suggested by the observation that an NK₁ receptor antagonist attenuates the action of *C. difficile* toxin A and TNBSA to stimulate granulocyte infiltration in the rat intestine [67, 78].

The interrelationship between the tachykinin and immune system is of a bidirectional nature as can be
Table 4. Implications of peptidergic neurons in protection of gastro-intestinal mucosa from injury

<table>
<thead>
<tr>
<th>Region</th>
<th>Injurious factor</th>
<th>Evidence for peptidergic nerve implication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit oesophagus</td>
<td>Ethanol</td>
<td>Protection by capsaicin-induced afferent neuron stimulation [128]</td>
</tr>
<tr>
<td>Rat stomach</td>
<td>Aspirin, ethanol, indomethacin, others</td>
<td>Protection by capsaicin-induced afferent neuron stimulation via CGRP₁ and NK₂ receptors [34, 81, 82, 84]</td>
</tr>
<tr>
<td>Rat stomach</td>
<td>Aspirin, ethanol, hydrochloric acid, indomethacin, others</td>
<td>Aggravation by capsaicin-induced afferent neuron ablation [34, 85]</td>
</tr>
<tr>
<td>Rat stomach</td>
<td>Acetic acid, ethanol, hydrochloric acid</td>
<td>Delay of ulcer healing by capsaicin-induced afferent neuron ablation [89–91]</td>
</tr>
<tr>
<td>Rat small intestine</td>
<td>Dulcerozine, hydrochloric acid</td>
<td>Protection by capsaicin-induced afferent neuron stimulation or NK₂ receptor agonist [84, 129]</td>
</tr>
<tr>
<td>Rat small intestine</td>
<td>Cysteamine, dulcerozine, histamine, hydrochloric acid, indomethacin</td>
<td>Aggravation by capsaicin-induced afferent neuron ablation [129–132]</td>
</tr>
<tr>
<td>Rabbit small intestine</td>
<td>Ricin</td>
<td>Aggravation by capsaicin-induced afferent neuron ablation [61]</td>
</tr>
<tr>
<td>Rat colon</td>
<td>TNBSA</td>
<td>Protection by capsaicin-induced afferent neuron stimulation or CGRP [83, 133]</td>
</tr>
<tr>
<td>Rat colon</td>
<td>Acetic acid, TNBSA</td>
<td>Aggravation by capsaicin-induced afferent neuron ablation [41, 133–136]</td>
</tr>
<tr>
<td>Rabbit colon</td>
<td>Immune complex</td>
<td>Aggravation by capsaicin-induced afferent neuron ablation [137]</td>
</tr>
</tbody>
</table>

deduced from the implication of tachykinins in the secretory response of the rat colon to interleukin 1β [69]. It needs in addition to be considered that immune cells are not only targets, at which SP acts to modify immune responses, but under pathological conditions can also be induced to synthesize and release per se tachykinins and other neuropeptides. This is true for rat peritoneal macrophages exposed to bacterial endotoxin [46], mononuclear cells of mice infected with S. dublin [47], eosinophils from intestinal granulomas of schistosoma-infected mice [79] and eosinophils from the mucosa of the inflamed human colon [80].

Implications of Neuropeptides in Gastro-Intestinal Tissue Defence and Repair

The first hint that gastro-intestinal mucosal integrity and repair are under the control of peptidergic neurons was obtained in experimental studies of gastric mucosal injury. Sensory neuron stimulation by intragastric administration of capsaicin protects the gastric mucosa of humans and experimental animals against a variety of injurious factors, whereas ablation of extrinsic afferent neurons with a neurotoxic dose of capsaicin weakens gastric mucosal defence [34]. As summarized in table 4, the mucosa-protective action of peptidergic afferents can be demonstrated along the whole gut from the oesophagus to the colon. In the stomach, sensory neuron-mediated protection of the mucosa involves both CGRP acting via CGRP₁ receptors [81] and tachykinins acting via NK₂ receptors [82]. CGRP also mimics the ability of sensory neuron stimulation to protect the rat colon from the acute and subacute, but not chronic, phase of inflammation and tissue destruction induced by TNBSA [41, 83], and an NK₂ receptor agonist reproduces the protective action of capsaicin in the dulcerozine-threatened duodenum [84]. It appears as if challenge of the gastro-intestinal mucosa with injurious agents stimulates sensory nerve fibres within the gut wall to release CGRP and NKA, which in turn enhance mucosal resistance to injury via formation of nitric oxide, vasodilatation and hyperaemia-independent mechanisms (fig. 5). The pathophysiological potential of this neural emergency system [34] is particularly well portrayed by the protective rise of gastric mucosal blood flow which is elicited when luminal acid enters the mucosa through a disrupted gastric mucosal barrier [85–87]. In addition, afferent neuron stimulation increases duodenal bicarbonate secretion [88] and reinforces a variety of pro-
Table 5. Implication of peptidergic neurons in visceral sensitivity and pain

<table>
<thead>
<tr>
<th>Response indicative of nociception</th>
<th>Sensory stimulus</th>
<th>Neuropeptide/receptor implication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall of blood pressure</td>
<td>Intraperitoneal capsaicin</td>
<td>Intrathecal NK1 receptors [98]</td>
</tr>
<tr>
<td>Fall of blood pressure</td>
<td>Intraperitoneal capsaicin</td>
<td>Sensitization by PGE2 via peripheral NK1 receptors [98]</td>
</tr>
<tr>
<td>Fall of blood pressure</td>
<td>Jejunal distension</td>
<td>Peripheral NK2 receptors [106]</td>
</tr>
<tr>
<td>Fall of blood pressure</td>
<td>Jejunal distension</td>
<td>Sensitization by Nippostrongylus brasiliensis infection via peripheral NK2 receptors [106]</td>
</tr>
<tr>
<td>Abdominal muscle contractions</td>
<td>Intraperitoneal acetic acid, CGRP, SP, NK2 receptor agonist, PGE1 plus PGE2</td>
<td>Peripheral/central NK2 receptors [103]</td>
</tr>
<tr>
<td>Abdominal muscle contractions</td>
<td>Rectal distension</td>
<td>Sensitization by intraluminal acetic acid or CGRP via peripheral/central CGRP1 receptors [100]</td>
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Fig. 5. Schematic diagram of the homoeostatic role of extrinsic afferent neurons in the stomach to protect the mucosa from injury and to facilitate the repair of the wounded mucosa. NO = Nitric oxide.

Neural emergency system
Defence of pending acid injury via acid-evoked hyperaemia and other protective mechanisms

Neural support system
Facilitation of rapid restitution and ulcer healing via acid-evoked hyperaemia

Protective mechanisms other than vasodilatation [34]. These reactions not only strengthen acute defense against pending injury but also facilitate repair of the wounded mucosa [89–91]. Peptidergic neurons thus have a bearing on mucosal homeostasis in the gut (fig. 5), a conjecture that is supported by the finding that mucosal injury may develop as a result of sensory neuropathies or defects in the neuropeptide-operated effectors systems [34].

It must not go unnoticed, however, that mucosal damage caused by bacterial toxins such as C. difficile toxin A [67, 68] or Escherichia coli toxin [92] is rather promoted, not counteracted, by SP released from capsaicin-sensitive extrinsic afferents. This action of SP is analogous to the peptide’s effect to aggravate experimental injury of the rat gastric mucosa through mast cell degranulation [93, 94], an effect by which tachykinins may boost development of certain forms of intestinal tissue destruction such as that evoked by TNBSA in the rat colon [95].

Implications of Neuropeptides in Visceral Sensitivity and Pain
Since SP, NKA and CGRP are transmitter substances of extrinsic afferent neurons innervating the gut, it is obvious to think of an implication of peptidergic neurons
in visceral nociception. A survey of pertinent findings (table 5) supports this hypothesis although the sites of neuropeptide action in the pain pathways remain to be identified. Tachykinins and CGRP released within the gut may, on the one hand, facilitate the excitation of extrinsic afferents and, on the other hand, participate in the central transmission of nociceptive traffic between afferent neurons and second-order neurons in the spinal cord and brainstem [96, 97]. Irritants, immunological and inflammatory mediators release SP and CGRP within the intestinal wall where these peptides may lead to sensitization [98–100] or even excitation [101, 102] of extrinsic afferents. Thus, intraperitoneal administration of acetic acid enhances the formation of prostaglandins which in turn release CGRP from afferent nerve fibres and give rise to abdominal muscle contractions, a reaction indicative of pain [64]. The peripheral injection of CGRP or a selective NK2 receptor agonist mimics the acetic-acid-evoked pain reaction which is inhibited by capsaicin-induced ablation of extrinsic afferents and pretreatment with a CGRP1 or NK2 receptor antagonist (table 5), whereas an NK1 receptor antagonist is ineffective [64, 99]. A similar NK2 receptor selectivity applies to the abdominal muscle contractions triggered by rectal distension [103]. Prior induction of colonic inflammation with acetic acid facilitates the pain reaction to rectal distension; this hypersensitivity is blocked by a CGRP1 receptor antagonist [100].

The precise sites at which NK2 and CGRP1 receptors mediate visceral hypersensitivity and pain are not known. The finding that both intravenous and intrathecal administration of neuropeptide antagonists is effective [100, 103] makes it conceivable that SP, NKA and CGRP facilitate visceral nociception both in the periphery and central nervous system. Since, however, NK2 receptors are absent from the spinal cord of adult mammals [104] and SP, NKA and CGRP are unlikely to penetrate the blood-brain barrier, it would appear that the pain reaction to intraperitoneal injection of these peptides [44, 64, 99] reflects a peripheral action. This algesic action may be of pathophysiological significance, given that the rise of the SP concentration in the colonic wall and blood plasma of rats affected with dextran sulphate-induced colitis is associated with a pain reaction [44] and there is an analogous upregulation of SP in the gastric mucosa of patients suffering from painful non-ulcer dyspepsia [105]. Because extrinsic afferent nerve fibres in the gut themselves do not possess receptors for tachykinins and CGRP, it would seem that neuropeptide-evoked sensitization or excitation of afferents is indirect [97]. The algesic action could be a consequence of peptide-induced changes in muscle tone, which excites mechanosensitive afferents, or the result of other peptide-induced processes in the gastrointestinal tract which ultimately sensitize or excite extrinsic afferents [97]. It is worth noting in this context that the NK2-receptor-mediated hypersensitivity to intestinal distension, which is observed in rats infected with Nippostrongylus brasiliensis, is confined to areas of hypermastocytosis [106].
Conclusions

The neuropeptides SP, NKA and CGRP participate in the physiological regulation of various digestive functions, an implication that is portrayed by the cell-specific expression of the peptides and their receptors in the gut. SP and NKA are cotransmitters of enteric cholinergic neurons which control gastro-intestinal motor activity, secretion of electrolytes and fluid, vascular and immune functions. In additions, tachykinins, and particularly CGRP, are expressed in extrinsic afferent nerve fibres wherefrom they can be released in response to irritant or noxious stimulation of the gut. Gastro-intestinal disorders of various aetiology, particularly those due to infection or inflammation, are related to changes in the peptidergic innervation and neuropeptide receptor expression of the digestive tract (fig. 6). It is hypothesized, therefore, that the contribution of peptidergic neurons to normal gastrointestinal physiology is out of balance in the diseased gut (fig. 6). Thus, nerve remodelling and a shift in the enteric nervous system away from cholinergic to peptidergic regulation takes place in experimental infection and inflammation of the intestine [60, 107]. In accordance with this scheme (fig. 6) it has been observed that antagonists of SP, NKA and CGRP are little active in the normal gut but are able to correct disturbed motility, hypersecretion, tissue homoeostasis and pain associated with certain forms of intestinal anaphylaxis, infection and inflammation. Extrapolation of these experimental findings to disorders of the human digestive system identifies tachykinin and CGRP receptors as novel targets for gastro-enterological therapy. It must not be neglected, however, that neuropeptides are messengers within a multifactorial control system and that manipulation of particular neuropeptide receptors alone may not be therapeutically sufficient.

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