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

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Key Words

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

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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 spasmolytic, antidiarrhoeal, anti-inflammatory and antinociceptive drugs.

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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 in-

testinal 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 homeostasis.

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 gas-

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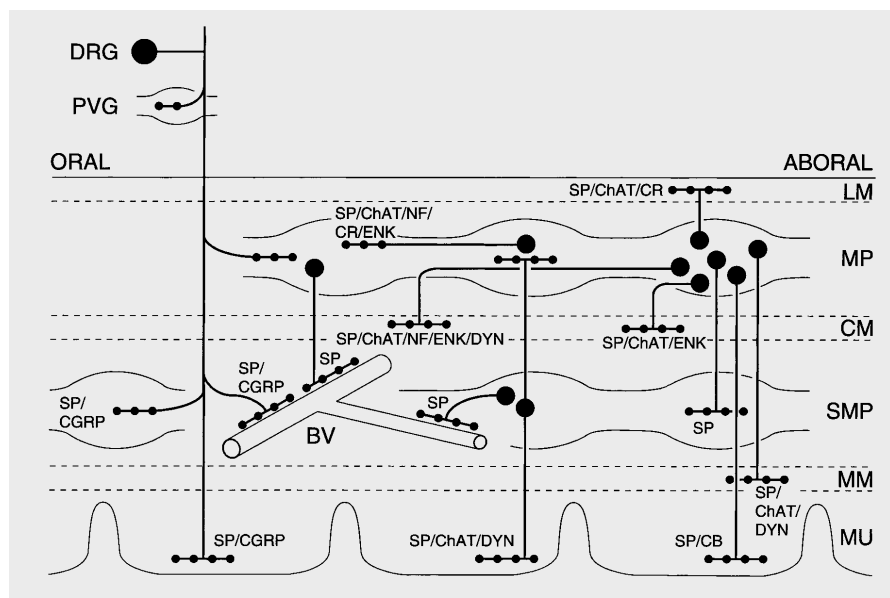
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Fig. 1. Projections of SP-immunoreactive neurons within the guinea pig intestine, with information on the co-existence with other neuropeptides or neuronal markers. BV = Blood vessel; CB = calbindin; ChAT = choline acetyltransferase; CM = circular 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-

tion [1, 9]. Most of the peptidergic afferents in the gastrointestinal system originate from cell bodies in the dorsal root ganglia and reach the gut via sympathetic (splanchnic, colonic and hypogastric) and sacral parasympathetic (pelvic) nerves while passing through prevertebral ganglia and forming collateral synapses with sympathetic ganglion cells [3, 9, 10]. In contrast, vagal afferents emanating from cell bodies in the nodose ganglia make a relatively small contribution to the gastro-intestinal content of CGRP and SP [10–12]. Within the wall of the alimentary canal, the spinal afferents project primarily to submucosal arteries and arterioles where they form a para- and perivascular network of axons [1, 3, 9, 10, 11, 13], although some axons can also be traced to the lamina propria of the 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 extrinsic afferents is CGRP- α , whereas the only form of CGRP in enteric neurons is CGRP- β [15, 16].

As is expected for substances with a vesicular localization, CGRP and tachykinins are released from the gastrointestinal tract if extrinsic afferent or intrinsic enteric neurons are depolarized [3, 4, 9]. The calcium dependency of the release process points to an exocytotic mechanism. 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 acidification 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 CGRP_{8–37}, 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 therapeutic 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 attribute 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.

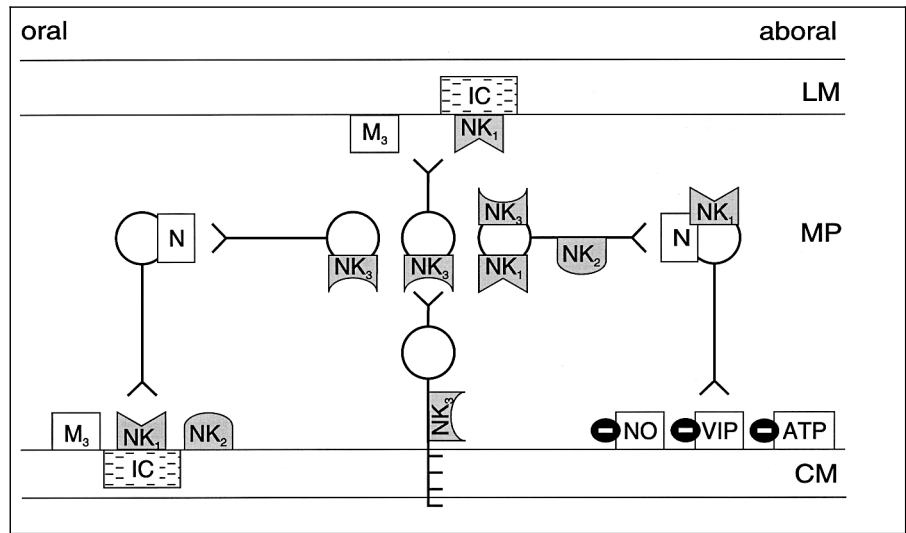
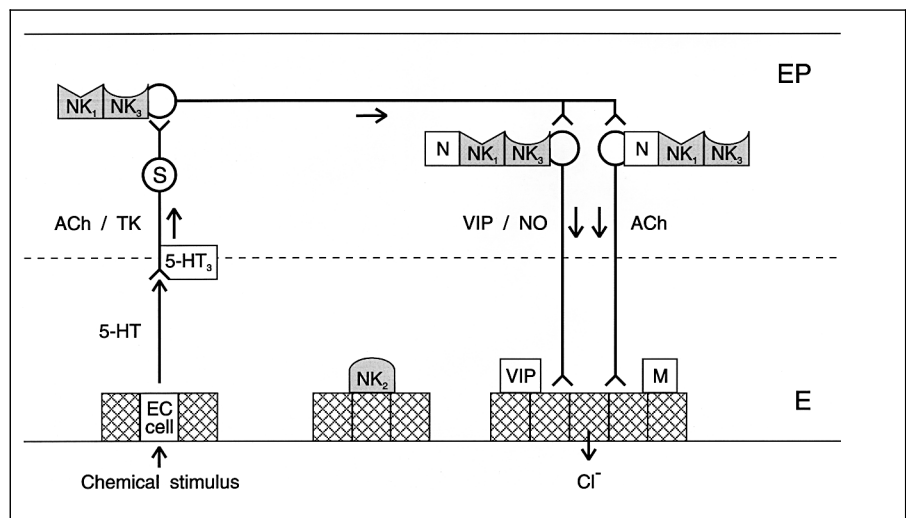


Fig. 3. Implication of tachykinins and tachykinin NK₁, NK₂ and NK₃ receptors in intestinal secretomotor reflexes. Chemical stimuli from the lumen may act on enterochromaffin (EC) cells to release 5-hydroxytryptamine (5-HT) which stimulates enteric sensory neurons (S) of a secretomotor reflex. Circles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



nal 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

Table 1. Neuropeptide changes in gastro-intestinal infection, inflammation and injury

Species and region	Insult or disease	Peptide	Change
Human stomach	Gastro-oesophageal reflux	SP	Decrease [108]
	Non-ulcer dyspepsia	SP	Increase [105]
Rat stomach	Experimental mucosal lesions	CGRP	Decrease [90, 109]
Human ileum	Pouchitis	SP, VIP	Increase [110]
Rat small intestine	Cysteamine-induced ulcers	CGRP, SP	Decrease [109, 111]
	<i>Trichinella spiralis</i>	SP	Increase [112]
	γ -Irradiation	SP	Decrease [45]
	<i>Escherichia coli</i> endotoxin	SP, NKA	Decrease [113]
	<i>Clostridium difficile</i> toxin A	SP	Increase [48]
	<i>Nippostrongylus brasiliensis</i>	SP	Increase [107]
	Mouse small intestine	<i>Trichinella spiralis</i>	SP
Guinea pig small intestine	<i>Schistosoma mansoni</i>	SP	Decrease [114]
	<i>Trichinella spiralis</i>	SP	Decrease [115]
Ferret small intestine	TNBSA ileitis	SP	Decrease [116]
	<i>Trichinella spiralis</i>	SP	Decrease [117]
Human colon	Crohn's disease	SP, CGRP	Decrease [118]
		VIP	Increase [119]
	Ulcerative colitis	SP	Increase [120–122]
Rat colon	TNBSA colitis	SP, CGRP	Decrease [118]
		SP, CGRP	Decrease [40, 41]
	Dextran sulphate colitis	SP	Increase [44]
Rabbit colon	Immune complex colitis	SP, CGRP	Decrease [42, 43]

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 or 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

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].

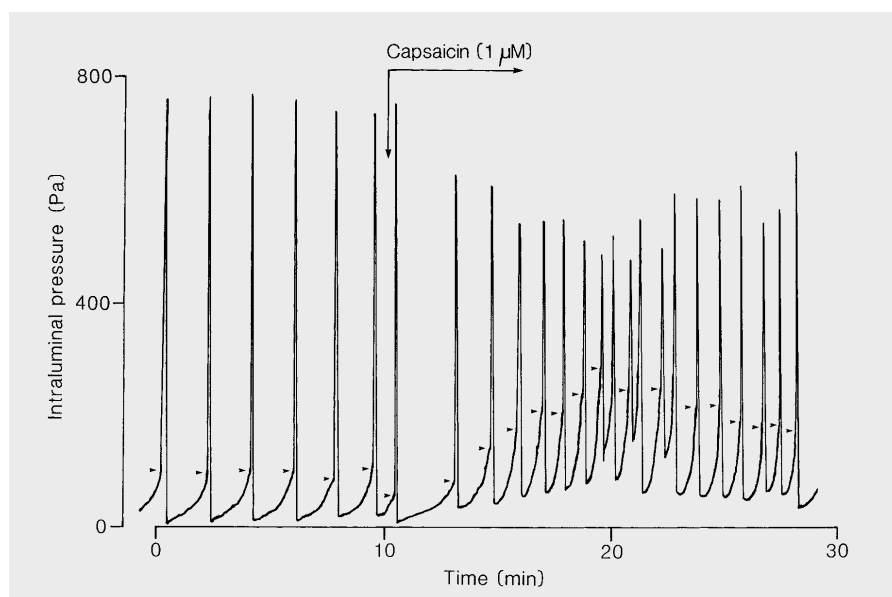


Table 2. Neuropeptide implications in pathological disturbances of gastrointestinal motility

Stimulus or insult	Motor dysfunction	Neuropeptide/receptor implication
Cancer chemotherapy, motion sickness	Emesis (various species)	NK ₁ receptors [3]
Luminal acidification	Relaxation of lower oesophageal sphincter (ferret)	NK ₁ receptors [56]
Intraperitoneal irritation by capsaicin, acetic acid	Inhibition of gastric motility or emptying (peritoneo-gastric reflex in the rat)	NK ₁ and CGRP ₁ receptors [98, 99]
Abdominal surgery	Inhibition of gastro-intestinal transit (intestino-intestinal reflex in the rat)	NK ₁ , CGRP ₁ and VIP receptors [123–125]
Ovalbumin anaphylaxis	Disruption of migrating motor complex in small intestine (rat)	NK ₁ receptors [57]
Castor-oil induced diarrhoea	Giant colonic contractions (rat)	NK ₂ and, partly, NK ₁ receptors [59]
Rectal distension	Inhibition of colonic motility (recto-colonic reflex in the rat)	NK ₁ receptors [103]
Restraint stress	Increased defaecation (rat)	NK ₁ receptors [126, 127]

[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

Stimulus or insult	Dysfunction	Neuropeptide/receptor implication
<i>Trichinella spiralis</i>	Inflammation and lymphocyte proliferation in small intestine (mouse)	NK ₁ receptors [77]
<i>Clostridium difficile</i> toxin A	Granulocyte infiltration, mast cell degranulation, TNF- α release from macrophages, hypersecretion and inflammation in small intestine (rat)	NK ₁ receptors [48, 67]
Delayed-type hypersensitivity to DNBSA (after DNFB exposure)	Mast cell degranulation and plasma leakage in small intestine (mouse)	NK ₁ receptors [75]
TNBSA	Granulocyte infiltration, increase in mucosal permeability and inflammation in colon (rat)	NK ₁ and NK ₂ receptors [78, 95]
Castor oil	Diarrhoea (rat)	NK ₂ and, partly, NK ₁ receptors [59]
Rectal distension	Hypersecretion in colon (rat)	NK ₁ , NK ₂ and NK ₃ receptors [70]

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 1 β [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

Region	Injurious factor	Evidence for peptidergic nerve implication
Rabbit oesophagus	Ethanol	Protection by capsaicin-induced afferent neuron stimulation [128]
Rat stomach	Aspirin, ethanol, indomethacin, others	Protection by capsaicin-induced afferent neuron stimulation via CGRP ₁ and NK ₂ receptors [34, 81, 82, 84]
Rat stomach	Aspirin, ethanol, hydrochloric acid, indomethacin, others	Aggravation by capsaicin-induced afferent neuron ablation [34, 85]
Rat stomach	Acetic acid, ethanol, hydrochloric acid	Delay of ulcer healing by capsaicin-induced afferent neuron ablation [89–91]
Rat small intestine	Dulcerozine, hydrochloric acid	Protection by capsaicin-induced afferent neuron stimulation or NK ₂ receptor agonist [84, 129]
Rat small intestine	Cysteamine, dulcerozine, histamine, hydrochloric acid, indomethacin	Aggravation by capsaicin-induced afferent neuron ablation [129–132]
Rabbit small intestine	Ricin	Aggravation by capsaicin-induced afferent neuron ablation [61]
Rat colon	TNBSA	Protection by capsaicin-induced afferent neuron stimulation or CGRP [83, 133]
Rat colon	Acetic acid, TNBSA	Aggravation by capsaicin-induced afferent neuron ablation [41, 133–136]
Rabbit colon	Immune complex	Aggravation by capsaicin-induced afferent neuron ablation [137]

deduced from the implication of tachykinins in the secretory response of the rat colon to interleukin 1 β [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-

Fig. 5. Schematic diagram of the homeostatic 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.

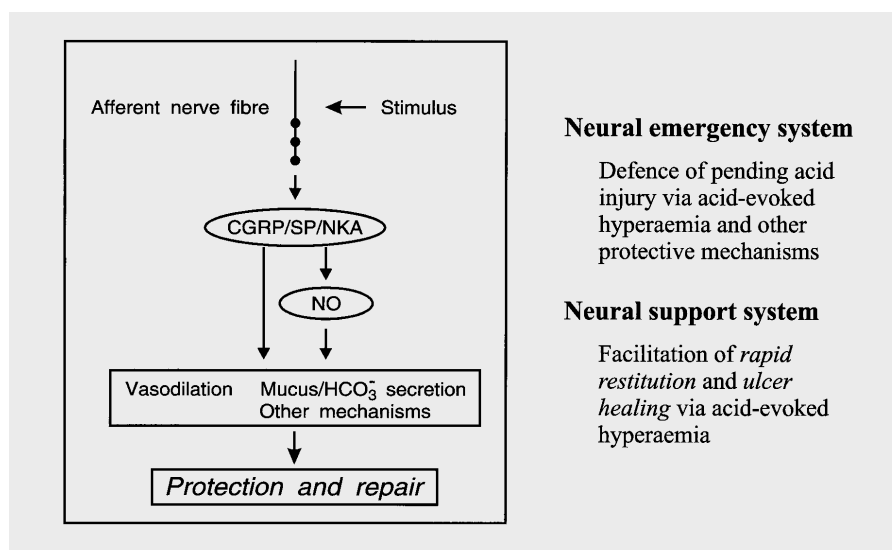


Table 5. Implication of peptidergic neurons in visceral sensitivity and pain

Response indicative of nociception	Sensory stimulus	Neuropeptide/receptor implication
Fall of blood pressure	Intraperitoneal capsaicin	Intrathecal NK ₁ receptors [98]
Fall of blood pressure	Intraperitoneal capsaicin	Sensitization by PGE ₂ via peripheral NK ₁ receptors [98]
Fall of blood pressure	Jejunal distension	Peripheral NK ₂ receptors [106]
Fall of blood pressure	Jejunal distension	Sensitization by <i>Nippostrongylus brasiliensis</i> infection via peripheral NK ₂ receptors [106]
Abdominal muscle contractions	Intraperitoneal acetic acid, CGRP, SP, NK ₂ receptor agonist, PGE ₁ plus PGE ₂	Peripheral NK ₂ and CGRP ₁ receptors [44, 64, 99]
Abdominal muscle contractions	Rectal distension	Peripheral/central NK ₂ receptors [103]
Abdominal muscle contractions	Rectal distension	Sensitization by intraluminal acetic acid or CGRP via peripheral/central CGRP ₁ receptors [100]

tective 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

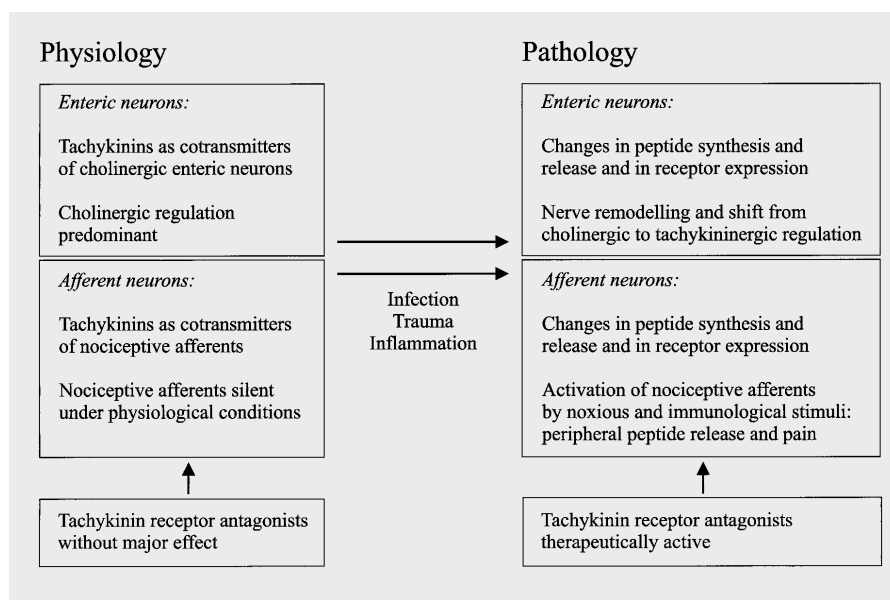


Fig. 6. Schematic diagram of some neuronal and functional changes associated with intestinal inflammation, which highlight tachykinins as possible targets at which to aim novel therapeutic strategies.

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 admin-

istration 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 algescic 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 algescic 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 gastro-intestinal 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 inflam-

mation 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 homeostasis 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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References

- 1 Costa M, Furness JB, Gibbins IL: Chemical coding of enteric neurons. *Prog Brain Res* 1986;68:217-239.
- 2 Nakanishi S: Substance P precursor and kininogen: Their structures, gene organizations, and regulation. *Physiol Rev* 1987;67:1117-1142.
- 3 Holzer P, Holzer-Petsche U: Tachykinins in the gut. I. Expression, release and motor function. *Pharmacol Ther* 1997;73:173-217.
- 4 Holzer P: Calcitonin gene-related peptide; in Walsh JH, Dockray GJ (eds): *Gut Peptides: Biochemistry and Physiology*. New York, Raven Press, 1994, pp 493-523.
- 5 Wimalawansa SJ: Calcitonin gene-related peptide and its receptors: Molecular genetics, physiology, pathophysiology, and therapeutic potentials. *Endocr Rev* 1996;17:533-585.
- 6 Furness JB, Bornstein JC, Murphy R, Pompolo S: Roles of peptides in transmission in the enteric nervous system. *Trends Neurosci* 1992;15:66-71.
- 7 Costa M, Brookes SJH, Steele PA, Gibbins I, Burcher E, Kandiak CJ: Neurochemical classification of myenteric neurons in the guinea-pig ileum. *Neuroscience* 1996;75:949-967.
- 8 Ekblad E, Winther C, Ekman R, Håkanson R, Sundler F: Projections of peptide-containing neurons in rat small intestine. *Neuroscience* 1987;20:169-188.
- 9 Holzer P: Peptidergic sensory neurons in the control of vascular functions: Mechanisms and significance in the cutaneous and splanchnic vascular beds. *Rev Physiol Biochem Pharmacol* 1992;121:49-146.
- 10 Green T, Dockray GJ: Characterization of the peptidergic afferent innervation of the stomach in the rat, mouse and guinea-pig. *Neuroscience* 1988;25:181-193.
- 11 Bäck N, Ahonen M, Häppölä O, Kivilaakso E, Kiviluoto T: Effect of vagotomy on expression of neuropeptides and histamine in rat oxyntic mucosa. *Digest Dis Sci* 1994;39:353-361.
- 12 Suzuki T, Kagoshima M, Shibata M, Inaba N, Onodera S, Yamaura T, Shimada H: Effects of several denervation procedures on distribution of calcitonin gene-related peptide and substance P immunoreactive fibers in rat stomach. *Digest Dis Sci* 1997;42:1242-1254.
- 13 Furness JB, Papka RE, Della NG, Costa M, Eskay RL: Substance P-like immunoreactivity in nerves associated with the vascular system of guinea-pigs. *Neuroscience* 1982;7:447-459.
- 14 Gibbins IL, Furness JB, Costa M: Pathway-specific patterns of the co-existence of substance P, calcitonin gene-related peptide, cholecystokinin and dynorphin in neurons of the dorsal root ganglia of the guinea-pig. *Cell Tissue Res* 1987;248:417-437.
- 15 Sternini C: Enteric and visceral afferent CGRP neurons: Targets of innervation and differential expression patterns. *Ann NY Acad Sci* 1992;657:170-186.
- 16 Muldery PK, Ghatei MA, Spokes RA, Jones PM, Pierson AM, Hamid QA, Kanse S, Amara SG, Burren JM, Legon S, Polak JM, Bloom SR: Differential expression of α -CGRP and β -CGRP by primary sensory neurons and enteric autonomic neurons of the rat. *Neuroscience* 1988;25:195-205.
- 17 Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D: The capsaicin receptor: A heat-activated ion channel in the pain pathway. *Nature* 1997;389:816-824.
- 18 Geppetti P, Tramontana M, Evangelista S, Renzi D, Maggi CA, Fusco BM, Del Bianco E: Differential effect on neuropeptide release of different concentrations of hydrogen ions on afferent and intrinsic neurons of the rat stomach. *Gastroenterology* 1991;101:1505-1511.
- 19 Manela FD, Ren J, Gao J, McGuigan JE, Harty RF: Calcitonin gene-related peptide modulates acid-mediated regulation of somatostatin and gastrin release from rat antrum. *Gastroenterology* 1995;109:701-706.
- 20 Nakanishi S: Mammalian tachykinin receptors. *Annu Rev Neurosci* 1991;14:123-136.
- 21 Maggi CA: The mammalian tachykinin receptors. *Gen Pharmacol* 1995;26:911-944.

- 22 Aiyar N, Rand K, Elshourbagy NA, Zeng ZZ, Adamou JE, Bergsma DJ, Li YA: A cDNA encoding the calcitonin gene-related peptide type 1 receptor. *J Biol Chem* 1996;271:11325-11329.
- 23 Sternini C, Su D, Gamp PD, Bunnett NW: Cellular sites of expression of the neurokinin-1 receptor in the rat gastrointestinal tract. *J Comp Neurol* 1995;358:531-540.
- 24 Grady EF, Baluk P, Böhm S, Gamp PD, Wong H, Payan DG, Ansel J, Portbury AL, Furness JB, McDonald DM, Bunnett NW: Characterization of antisera specific to NK₁, NK₂ and NK₃ neurokinin receptors and their utilization to localize receptors in the rat gastrointestinal tract. *J Neurosci* 1996;16:6975-6986.
- 25 Portbury AL, Furness JB, Young HM, Southwell BR, Vigna SR: Localisation of NK₁ receptor immunoreactivity to neurons and interstitial cells of the guinea-pig gastrointestinal tract. *J Comp Neurol* 1996;367:342-351.
- 26 Vannucchi MG, DeGiorgio R, Fausson-Pellegrini MS: NK₁ receptor expression in the interstitial cells of Cajal and neurons and tachykinin distribution in rat ileum during development. *J Comp Neurol* 1997;383:153-162.
- 27 Portbury AL, Furness JB, Southwell BR, Wong H, Walsh JH, Bunnett NW: Distribution of neurokinin-2 receptors in the guinea-pig gastrointestinal tract. *Cell Tissue Res* 1996;286:281-292.
- 28 Mann PT, Southwell BR, Ding Y-Q, Shigemoto R, Mizuno N, Furness JB: Localisation of neurokinin 3 (NK3) receptor immunoreactivity in the rat gastrointestinal tract. *Cell Tissue Res* 1997;289:1-9.
- 29 Jin JG, Misra S, Grider JR, Makhlof GM: Functional difference between SP and NKA: Relaxation of gastric muscle by SP is mediated by VIP and NO. *Am J Physiol* 1993;264:G678-G685.
- 30 Maggi CA, Patacchini R, Meini S, Giuliani S: Nitric oxide is the mediator of tachykinin NK₃ receptor-induced relaxation in the circular muscle of the guinea-pig ileum. *Eur J Pharmacol* 1993;240:45-50.
- 31 Holzer P: Involvement of nitric oxide in the substance P-induced inhibition of intestinal peristalsis. *Neuroreport* 1997;8:2857-2860.
- 32 Holzer P, Maggi CA: Synergistic role of muscarinic acetylcholine and tachykinin NK-2 receptors in intestinal peristalsis. *Naunyn-Schmiedeberg's Arch Pharmacol* 1994;349:194-201.
- 33 Holzer P, Lippe IT, Heinemann A, Barthó L: Tachykinin NK₁ and NK₂ receptor-mediated control of peristaltic propulsion in the guinea-pig small intestine in vitro. *Neuropharmacology*, in press.
- 34 Holzer P: Neural emergency system in the stomach. *Gastroenterology* 1998;114:823-839.
- 35 Holzer P, Holzer-Petsche U: Tachykinins in the gut. II. Roles in neural excitation, secretion and inflammation. *Pharmacol Ther* 1997;73:219-263.
- 36 Moore BA, Vanner S, Bunnett NW, Sharkey KA: Characterization of neurokinin-1 receptors in the submucosal plexus of guinea pig ileum. *Am J Physiol* 1997;273:G670-G678.
- 37 Figini M, Emanuelli C, Grady EF, Kirkwood K, Payan DG, Ansel J, Gerard C, Geppetti P, Bunnett NW: Substance P and bradykinin stimulate plasma extravasation in the mouse gastrointestinal tract and pancreas. *Am J Physiol* 1997;272:G785-G793.
- 38 Prystowsky JB, Rege RV: Neurogenic inflammation in cholecystitis. *Digest Dis Sci* 1997;42:1489-1494.
- 39 Renzi D, Calabró A, Panerai C, Tramontana M, Evangelista S, Milani S, Surrenti C: Preprotachykinin mRNA expression in the colonic tissue during experimental colitis in rats. *Digestion* 1994;55(suppl 2):36.
- 40 Renzi D, Tramontana M, Panerai C, Surrenti C, Evangelista S: Decrease of calcitonin gene-related peptide, but not vasoactive intestinal polypeptide and substance P, in the TNB-induced experimental colitis in rats. *Neuropeptides* 1992;22:56-57.
- 41 Reinshagen M, Patel A, Sottili M, French S, Sternini C, Eysselein VE: Action of sensory neurons in an experimental rat colitis model of injury and repair. *Am J Physiol* 1996;270:G79-G86.
- 42 Eysselein VE, Reinshagen M, Cominelli F, Sternini C, Davis W, Patel A, Nast CC, Bernstein D, Anderson K, Khan H, Snape WJ: Calcitonin gene-related peptide and substance P decrease in the rabbit colon during colitis: A time study. *Gastroenterology* 1991;101:1211-1219.
- 43 Reinshagen M, Adler G, Eysselein VE: Substance P gene expression in acute experimental colitis. *Regul Pept* 1995;59:53-58.
- 44 Kishimoto S, Kobayashi H, Machino H, Tari A, Kajiyama G, Miyoshi A: High concentrations of substance P as a possible transmission of abdominal pain in rats with chemical induced ulcerative colitis. *Biomed Res* 1994;15(suppl 2):133-140.
- 45 Esposito V, Linaud C, Maubert C, Aigueperse J, Gourmelon P: Modulation of gut substance P after whole-body irradiation: A new pathological feature. *Digest Dis Sci* 1996;41:2070-2077.
- 46 Bost KL, Breeding SA, Pascual DW: Modulation of the mRNAs encoding substance P and its receptor in rat macrophages by LPS. *Reg Immunol* 1992;4:105-112.
- 47 Bost KL: Inducible preprotachykinin mRNA expression in mucosal lymphoid organs following oral immunization with *Salmonella*. *J Neuroimmunol* 1995;62:59-67.
- 48 Castagliuolo I, Keates AC, Qiu BS, Kelly CP, Nikulasson S, Leeman SE, Pothoulakis C: Increased substance P responses in dorsal root ganglia and intestinal macrophages during *Chlostridium difficile* toxin A enteritis in rats. *Proc Natl Acad Sci USA* 1997;94:4788-4793.
- 49 Mantyh CR, Gates TS, Zimmerman RP, Welton ML, Passaro EPJ, Vigna SR, Maggio JE, Kruger L, Mantyh PW: Receptor binding sites for substance P, but not substance K or neuropeptide Y, are expressed in high concentrations by arterioles, venules, and lymph nodules in surgical specimens obtained from patients with ulcerative colitis and Crohn disease. *Proc Natl Acad Sci USA* 1988;85:3235-3239.
- 50 Mantyh CR, Vigna SR, Maggio JE, Mantyh PW, Bollinger RR, Pappas TN: Substance P binding sites on intestinal lymphoid aggregates and blood vessels in inflammatory bowel disease correspond to authentic NK-1 receptors. *Neurosci Lett* 1994;178:255-259.
- 51 Mantyh CR, Vigna SR, Bollinger RR, Mantyh PW, Maggio JE, Pappas TN: Differential expression of substance P receptors in patients with Crohn's disease and ulcerative colitis. *Gastroenterology* 1995;109:850-860.
- 52 Mantyh CR, Maggio JE, Mantyh PW, Vigna SR, Pappas TN: Increased substance P receptor expression by blood vessels and lymphoid aggregates in *Clostridium-difficile*-induced pseudomembranous colitis. *Digest Dis Sci* 1996;41:614-620.
- 53 Renzi D, Calabró A, Panerai C, Tramontana M, Evangelista S, Surrenti C: NK1 and NK2 receptor gene expression during TNB-induced colitis in rats. *Gut* 1996;39(suppl 3):A137.
- 54 Evangelista S, Maggi M, Renzetti AR: Down-regulation of substance P receptors during colitis induced by trinitrobenzene sulfonic acid in rats. *Neuropeptides* 1996;30:425-428.
- 55 Barthó L, Holzer P: The inhibitory modulation of guinea-pig intestinal peristalsis caused by capsaicin involves calcitonin gene-related peptide and nitric oxide. *Naunyn-Schmiedeberg's Arch Pharmacol* 1995;353:102-109.
- 56 Blackshaw LA, Dent J: Lower oesophageal sphincter responses to noxious oesophageal chemical stimuli in the ferret: Involvement of tachykinin receptors. *J Auton Nerv Syst* 1997;66:189-200.
- 57 Fargeas MJ, Fioramonti J, Buéno L: Involvement of capsaicin-sensitive afferent nerves in the intestinal motor alterations induced by intestinal anaphylaxis in rats. *Int Arch Allergy Immunol* 1993;101:190-195.
- 58 Castex N, Fioramonti J, Fargeas MJ, More J, Buéno L: Role of 5-HT₃ receptors and afferent fibers in the effects of mast cell degranulation on colonic motility in rats. *Gastroenterology* 1994;107:976-984.
- 59 Croci T, Landi M, Emonds-Alt X, Le Fur G, Maffrand J-P, Manara L: Role of tachykinins in castor oil diarrhoea in rats. *Br J Pharmacol* 1997;121:375-380.
- 60 Goldhill JM, Shea-Donohue T, Ali N, Pineiro-Carrero VM: Tachykinergic neurotransmission is enhanced in small intestinal circular muscle in a rabbit model of inflammation. *J Pharmacol Exp Ther* 1997;282:1373-1378.
- 61 Shea-Donohue T, Goldhill JM, Montcalm-Mazzilli E, Colleton C, Pineiro-Carrero VM, Sjogren RW: Role of sensory afferents in the myoelectric response to acute enteric inflammation in the rabbit. *Am J Physiol* 1997;273:G447-G455.
- 62 Otsuka M, Yoshioka K: Neurotransmitter functions of mammalian tachykinins. *Physiol Rev* 1993;73:229-308.
- 63 Goldhill JM, Sanders KM, Sjogren R, Shea-Donohue T: Changes in enteric neural regulation of smooth muscle in a rabbit model of small intestinal inflammation. *Am J Physiol* 1995;268:G823-G830.

- 64 Friese N, Diop L, Chevalier E, Angel F, Rivière PJM, Dahl SG: Involvement of prostaglandins and CGRP-dependent sensory afferent in peritoneal irritation-induced visceral pain. *Regul Pept* 1997;70:1-7.
- 65 Tsukamoto M, Sarna SK, Condon RE: A novel motility effect of tachykinins in normal and inflamed colon. *Am J Physiol* 1997;35:G1607-G1614.
- 66 Croci T, Emonds-Alt X, Manara L: SR 48968 selectively prevents faecal excretion following activation of tachykinin NK₂ receptors in rats. *J Pharm Pharmacol* 1994;46:383-385.
- 67 Pothoulakis C, Castagliuolo I, LaMont JT, Jaffer A, O'Keane JC, Snider RM, Leeman SE: CP-96,345, a substance P antagonist, inhibits rat intestinal responses to *Clostridium difficile* toxin A but not cholera toxin. *Proc Natl Acad Sci USA* 1994;91:947-951.
- 68 Castagliuolo I, LaMont JT, Letourneau R, Kelly C, O'Keane JC, Jaffer A, Theoharides TC, Pothoulakis C: Neuronal involvement in the intestinal effects of *Clostridium difficile* toxin A and *Vibrio cholerae* enterotoxin in rat ileum. *Gastroenterology* 1994;107:657-665.
- 69 Eutamene H, Theodorou V, Fioramonti J, Buéno L: Implication of NK₁ and NK₂ receptors in rat colonic hypersecretion induced by interleukin_{1β}: Role of nitric oxide. *Gastroenterology* 1995;109:483-489.
- 70 Eutamene H, Theodorou V, Fioramonti J, Buéno L: Rectal distention-induced colonic net water secretion in rats involves tachykinins, capsaicin sensory, and vagus nerves. *Gastroenterology* 1997;112:1595-1602.
- 71 Maggi CA: The effects of tachykinins on inflammatory and immune cells. *Regul Pept* 1997;70:75-90.
- 72 Stead RH, Tomioka M, Quinonez G, Simon GT, Felten SY, Bienenstock J: Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. *Proc Natl Acad Sci USA* 1987;84:2975-2979.
- 73 Shanahan F, Denburg JA, Fox J, Bienenstock J, Befus D: Mast cell heterogeneity: Effects of neuroenteric peptides on histamine release. *J Immunol* 1985;135:1331-1337.
- 74 Lowman MA, Rees PH, Benyon RC, Church MK: Human mast cell heterogeneity: Histamine release from mast cells dispersed from skin, lung, adenosis, tonsils, and colon in response to IgE-dependent and nonimmunologic stimuli. *J Allergy Clin Immunol* 1988;81:590-597.
- 75 Kraneveld AD, Buckley TL, van Heuven-Nolsen D, van Schaik Y, Koster AS, Nijkamp FP: Delayed-type hypersensitivity-induced increase in vascular permeability in the mouse small intestine: Inhibition by depletion of sensory neuropeptides and NK₁ receptor blockade. *Br J Pharmacol* 1995;114:1483-1489.
- 76 Stanisz A, Scicchitano R, Dazin P, Bienenstock J, Payan DG: Distribution of substance P receptors on murine spleen and Peyer's patch T and B cells. *J Immunol* 1987;139:749-754.
- 77 Kataeva G, Agro A, Stanisz AM: Substance P-mediated intestinal inflammation: Inhibitory effects of CP 96,345 and SMS 201-995. *Neuroimmunomodulation* 1994;1:350-356.
- 78 McCafferty D-M, Sharkey KA, Wallace JL: Beneficial effects of local or systemic lidocaine in experimental colitis. *Am J Physiol* 1994;266:G560-G567.
- 79 Weinstock JV, Blum AM: Release of substance P by granuloma eosinophils in response to secretagogues in murine schistosomiasis mansoni. *Cell Immunol* 1990;125:380-385.
- 80 Metwali A, Blum AM, Ferraris L, Klein JS, Fiocchi C, Weinstock JV: Eosinophils within the healthy or inflamed human intestine produce substance P and vasoactive intestinal peptide. *J Neuroimmunol* 1994;52:69-78.
- 81 Lambrecht N, Burchert M, Respondek M, Müller KM, Peskar BM: Role of calcitonin gene-related peptide and nitric oxide in the gastroprotective effect of capsaicin in the rat. *Gastroenterology* 1993;104:1371-1380.
- 82 Stroff T, Plate S, Seyed Ebrahim J, Ehrlich K-H, Respondek M, Peskar BM: Tachykinin-induced increase in gastric mucosal resistance: Role of primary afferent neurons. CGRP, and NO. *Am J Physiol* 1996;271:G1017-G1027.
- 83 Goso C, Evangelista S, Tramontana M, Manzini S, Blumberg PM, Szallasi A: Topical capsaicin administration protects against trinitrobenzene sulfonic acid-induced colitis in the rat. *Eur J Pharmacol* 1993;249:185-190.
- 84 Evangelista S, Maggi CA, Rovero P, Patacchini R, Giuliani S, Giachetti A: Analogs of neurokinin A(4-10) afford protection against gastroduodenal ulcers in rats. *Peptides* 1990;11:293-297.
- 85 Holzer P, Livingston EH, Guth PH: Sensory neurons signal for an increase in rat gastric mucosal blood flow in the face of pending acid injury. *Gastroenterology* 1991;101:416-423.
- 86 Holzer P, Wachter C, Jocič M, Heinemann A: Vascular bed-dependent roles of the peptide CGRP and nitric oxide in acid-evoked hyperaemia of the rat stomach. *J Physiol (Lond)* 1994;480:575-585.
- 87 Li D-S, Raybould HE, Quintero E, Guth PH: Calcitonin gene-related peptides mediates the gastric hyperemic response to acid back-diffusion. *Gastroenterology* 1992;102:1124-1128.
- 88 Takeuchi K, Matsumoto J, Ueshima K, Okabe S: Role of capsaicin-sensitive afferent neurons in alkaline secretory response to luminal acid in the rat duodenum. *Gastroenterology* 1991;101:954-961.
- 89 Takeuchi K, Ueshima K, Ohuchi T, Okabe S: The role of capsaicin-sensitive sensory neurons in healing of HCl-induced gastric mucosal lesions in rats. *Gastroenterology* 1994;106:1524-1532.
- 90 Tramontana M, Renzi D, Calabro A, Panerai C, Milani S, Surrenti C, Evangelista S: Influence of capsaicin-sensitive afferent fibers on acetic acid-induced chronic gastric ulcers in rats. *Scand J Gastroenterol* 1994;29:406-413.
- 91 Peskar BM, Lambrecht N, Stroff T, Respondek M, Müller K-M: Functional ablation of sensory neurons impairs healing of acute gastric mucosal damage in rats. *Digest Dis Sci* 1995;40:2460-2464.
- 92 Evangelista S, Cianchi G, Zecchi-Orlandini S, Martini M, DeGaudio AR: Protective effects of capsaicin in *E. coli* toxin-induced intestinal damage in rats. *Gastroenterology* 1997;112:A361.
- 93 Karmeli F, Eliakim R, Okon E, Rachmilewitz D: Gastric mucosal damage by ethanol is mediated by substance P and prevented by ketotifen, a mast cell stabilizer. *Gastroenterology* 1991;100:1206-1216.
- 94 Grønbech JE, Lacy ER: Substance P attenuates gastric mucosal hyperemia after stimulation of sensory neurons in the rat stomach. *Gastroenterology* 1994;106:440-449.
- 95 Mazelin L, Theodorou V, Fioramonti J, Buéno L: Involvement of NK1 and NK2 receptors in inflammatory reaction during experimental colitis in rats. *Gastroenterology* 1997;112:A1035.
- 96 Buéno L, Fioramonti J, Delvaux M, Frexinos J: Mediators and pharmacology of visceral sensitivity: From basic to clinical investigations. *Gastroenterology* 1997;112:1714-1743.
- 97 Maggi CA: Tachykinins as peripheral modulators of primary afferent nerves and visceral sensitivity. *Pharmacol Res* 1997;36:153-169.
- 98 Holzer-Petsche U, Rordorf-Nikolić T: Central versus peripheral site of action of the tachykinin NK₁-antagonist RP 67580 in inhibiting chemoreception. *Br J Pharmacol* 1995;115:486-490.
- 99 Julia V, Buéno L: Tachykininergic mediation of viscerosensitive responses to acute inflammation in rats: Role of CGRP. *Am J Physiol* 1997;272:G141-G146.
- 100 Plourde V, St-Pierre S, Quirion R: Calcitonin gene-related peptide in viscerosensitive response to colorectal distension in rats. *Am J Physiol* 1997;273:G191-G196.
- 101 Lew WY, Longhurst JC: Substance P, 5-hydroxytryptamine, and bradykinin stimulate abdominal visceral afferents. *Am J Physiol* 1986;250:R465-R473.
- 102 Barber WD, Burks TF: Brain-gut interactions: Brain stem neuronal response to local gastric effects of substance P. *Am J Physiol* 1987;253:G369-G377.
- 103 Julia V, Morteau O, Buéno L: Involvement of neurokinin 1 and 2 receptors in viscerosensitive response to rectal distension in rats. *Gastroenterology* 1994;107:94-102.
- 104 Urban L, Thompson SWN, Dray A: Modulation of spinal excitability: Co-operation between neurokinin and excitatory amino acid neurotransmitters. *Trends Neurosci* 1994;17:432-438.
- 105 Kaneko H, Mitsuma T, Uchida K, Furusawa A, Morise K: Immunoreactive-somatostatin, substance P, and calcitonin gene-related peptide concentrations of the human gastric mucosa in patients with nonulcer dyspepsia and peptic ulcer disease. *Am J Gastroenterol* 1993;88:898-904.

- 106 McLean PG, Picard C, Garcia-Villar R, Moré J, Fioramonti J, Buéno L: Effects of nematode infection on sensitivity to intestinal distension: Role of tachykinin NK₂ receptor. *Eur J Pharmacol* 1997;337:279–282.
- 107 Masson SD, McKay DM, Stead RH, Agro A, Stanisz A, Perdue MH: *Nippostrongylus brasiliensis* infection evokes neuronal abnormalities and alterations in neurally regulated electrolyte transport in rat jejunum. *Parasitology* 1996;113:173–182.
- 108 Wattchow DA, Jamieson GG, Maddern GJ, Furness JB, Costa M: Distribution of peptide-containing nerve fibers in the gastric musculature of patients undergoing surgery for gastroesophageal reflux. *Ann Surg* 1992;216:153–160.
- 109 Evangelista S, Renzi D, Tramontana M, Surrenti C, Theodorsson E, Maggi CA: Cysteamine induced-duodenal ulcers are associated with a selective depletion in gastric and duodenal calcitonin gene-related peptide-like immunoreactivity in rats. *Regul Pept* 1992;39:19–28.
- 110 Keranen U, Järvinen H, Kiviluoto T, Kivilaakso E, Soinila S: Substance P- and vasoactive intestinal polypeptide-immunoreactive innervation in normal and inflamed pouches after restorative proctocolectomy for ulcerative colitis. *Digest Dis Sci* 1994;41:1658–1664.
- 111 Evangelista S, Renzi D, Mantellini P, Surrenti C, Meli A: Duodenal SP-like immunoreactivity is decreased in experimentally induced duodenal ulcers. *Neurosci Lett* 1990;112:352–355.
- 112 Swain MG, Agro A, Blennerhassett P, Stanisz A, Collins SM: Increased levels of substance P in myenteric plexus of *Trichinella*-infected rats. *Gastroenterology* 1992;102:1913–1919.
- 113 Hellström PM, Al Saffar A, Ljung T, Theodorsson E: Endotoxin actions on myoelectric activity, transit, and neuropeptides in the gut: Role of nitric oxide. *Digest Dis Sci* 1997;42:1640–1651.
- 114 Varilek GW, Weinstock JV, Williams TH, Jew J: Alterations of the intestinal innervation in mice infected with *Schistosoma mansoni*. *J Parasitol* 1991;77:472–478.
- 115 Palmer JM, Koch TR: Altered neuropeptide content and cholinergic enzymatic activity in the inflamed guinea pig jejunum during parasitism. *Neuropeptides* 1995;28:287–297.
- 116 Miller MJ, Sadowska-Krowicka H, Jeng AY, Chotinaruemol S, Wong M, Clark DA, Ho W, Sharkey KA: Substance P levels in experimental ileitis in guinea pigs: Effects of misoprostol. *Am J Physiol* 1993;265:G321–G330.
- 117 Palmer JM, Greenwood B: Regional content of enteric substance P and vasoactive intestinal peptide during intestinal inflammation in the parasitized ferret. *Neuropeptides* 1993;25:95–103.
- 118 Kimura M, Masuda T, Hiwatashi N, Toyota T, Nagura H: Changes in neuropeptide-containing nerves in human colonic mucosa with inflammatory bowel disease. *Pathol Int* 1994;44:624–634.
- 119 Belai A, Boulos PB, Robson T, Burnstock G: Neurochemical coding in the small intestine of patients with Crohn's disease. *Gut* 1997;40:767–774.
- 120 Koch TR, Carney JA, Go VL: Distribution and quantitation of gut neuropeptides in normal intestine and inflammatory bowel diseases. *Digest Dis Sci* 1987;32:369–376.
- 121 Bernstein CN, Robert ME, Eysselein VE: Rectal substance P concentrations are increased in ulcerative colitis but not in Crohn's disease. *Am J Gastroenterol* 1993;88:908–913.
- 122 Keranen U, Kiviluoto T, Järvinen H, Bäck N, Kivilaakso E, Soinila S: Changes in substance P-immunoreactive innervation of human colon associated with ulcerative colitis. *Digest Dis Sci* 1995;40:2250–2258.
- 123 Plourde V, Wong HC, Walsh JH, Raybould HE, Taché Y: CGRP antagonists and capsaicin on celiac ganglia partly prevent postoperative gastric ileus. *Peptides* 1993;14:1225–1229.
- 124 Espat NJ, Cheng G, Kelley MC, Vogel SB, Sninsky CA, Hocking MP: Vasoactive intestinal peptide and substance P receptor antagonists improve postoperative ileus. *J Surg Res* 1995;58:719–723.
- 125 Zittel TT, Lloyd KCK, Rothenhöfer I, Wong H, Walsh JH, Raybould HE: Calcitonin gene-related peptide and spinal afferents partly mediate postoperative colonic ileus in the rat. *Gastroenterology* 1997;112:A1487.
- 126 Ikeda K, Miyata K, Orita A, Kubota H, Yamada T, Tomioka K: RP67580, a neurokinin₁ receptor antagonist, decreased restraint stress-induced defecation in rat. *Neurosci Lett* 1995;198:103–106.
- 127 Castagliuolo I, LaMont JT, Qiu BS, Fleming SM, Bhaskar KR, Nikulasson ST, Kornetsky C, Pothoulakis C: Acute stress causes mucin release from rat colon: Role of corticotropin releasing factor and mast cells. *Am J Physiol* 1996;271:G884–G892.
- 128 Bass BL, Trad KS, Harmon JW, Hakki FZ: Capsaicin-sensitive nerves mediate esophageal mucosal protection. *Surgery* 1991;110:419–426.
- 129 Leung FW: Primary sensory neurons mediate in part the protective mesenteric hyperemia after intraduodenal acidification in rats. *Gastroenterology* 1993;105:1737–1745.
- 130 Maggi CA, Evangelista S, Abelli L, Somma V, Meli A: Capsaicin-sensitive mechanisms and experimentally induced duodenal ulcers in rats. *J Pharm Pharmacol* 1987;39:559–561.
- 131 Takeuchi K, Matsumoto J, Ueshima K, Ohuchi T, Okabe S: Induction of duodenal ulcers in sensory deafferented rats following histamine infusion. *Digestion* 1992;51:203–210.
- 132 Evangelista S, Maggi CA, Meli A: Involvement of capsaicin-sensitive mechanism(s) in the antiulcer defence of intestinal mucosa in rats. *Proc Soc Exp Biol Med* 1987;184:264–266.
- 133 Evangelista S, Tramontana M: Involvement of calcitonin gene-related peptide in rat experimental colitis. *J Physiol (Paris)* 1993;87:277–280.
- 134 Evangelista S, Meli A: Influence of capsaicin-sensitive fibres on experimentally induced colitis in rats. *J Pharm Pharmacol* 1989;41:574–575.
- 135 McCafferty D-M, Wallace JL, Sharkey KA: Effects of chemical sympathectomy and sensory nerve ablation on experimental colitis in the rat. *Am J Physiol* 1997;272:G272–G280.
- 136 Leung FW: Role of capsaicin-sensitive afferent nerves in mucosal injury and injury-induced hyperemia in rat colon. *Am J Physiol* 1992;262:G332–G337.
- 137 Reinshagen M, Patel A, Sottili M, Nast C, Davis W, Mueller K, Eysselein V: Protective function of extrinsic sensory neurons in acute rabbit experimental colitis. *Gastroenterology* 1994;106:1208–1214.