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Tachykinins in the Gut. Part II. Roles in Neural Excitation, Secretion and Inflammation

Peter Holzer and Ulrike Holzer-Petsche*

DEPARTMENT OF EXPERIMENTAL AND CLINICAL PHARMACOLOGY, UNIVERSITY OF GRAZ, UNIVERSITÄTSPLATZ 4, A-8010 GRAZ, AUSTRIA

ABSTRACT. The preprotachykinin-A gene-derived peptides substance (substance P; SP) and neurokinin (NK) A are expressed in intrinsic enteric neurons, which supply all layers of the gut, and extrinsic primary afferent nerve fibers, which innervate primarily the arterial vascular system. The actions of tachykinins on the digestive effector systems are mediated by three different types of tachykinin receptor, termed NK_1, NK_2 and NK_3 receptors. Within the enteric nervous system, SP and NKA are likely to mediate, or comediate, slow synaptic transmission and to modulate neuronal excitability via stimulation of NK_3 and NK_1 receptors. In the intestinal mucosa, tachykinins cause net secretion of fluid and electrolytes, and it appears as if SP and NKA play a messenger role in intramural secretory reflex pathways. Secretory processes in the salivary glands and pancreas are likewise influenced by tachykinins. The gastrointestinal arterial system may be dilated or constricted by tachykinins, whereas constriction and an increase in the vascular permeability are the only effects seen in the venous system. Various gastrointestinal disorders are associated with distinct changes in the tachykinin system, and there is increasing evidence that tachykinins participate in the hypersecretory, vascular and immunological disturbances associated with infection and inflammatory bowel disease. In a therapeutic perspective, it would seem conceivable that tachykinin antagonists could be exploited as antidiarrheal, anti-inflammatory and antinociceptive drugs. PHARMACOL. THER. 73(3): 219–263, 1997. **© 1997 Elsevier Science Inc.**

KEY WORDS. Substance P, neurokinin A, enteric nervous system, primary afferent neurons, intestinal secretion, inflammatory bowel disease.

CONTENTS

*Corresponding author

ABBREVIATIONS. ACh, acetylcholine; CCK, cholecystokinin; CGRP, calcitonin gene-related peptide; EPSP, excitatory postsynaptic potential; GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine; Ig, immunoglobulin; I_{sc}, short circuit current; MP, myenteric plexus; NANC, nonadrenergic noncholinergic; NK, neurokinin; NO, nitric oxide; NPK, neuropeptide K; PPT, preprotachykinin; SMP, submucosal plexus; SP, substance P; TTX, tetrodotoxin; TNBSA, trinitrobenzene sulphonic acid; VIP, vasoactive intestinal polypeptide.

1. INTRODUCTION

The tachykinins are a family of small biologically active peptides whose principal mammalian members are substance P (SP), neurokinin (NK) A and NKB. These peptides are derived from precursor proteins, the preprotachykinins (PPTs), which are encoded by two different PPT genes. PPT-A contains the sequences that encode both SP and NKA, whereas the PPT-B encodes NKB only. As outlined in the companion article (Holzer and Holzer-Petsche, 1997), SP and NKA abound in the digestive system, in which they are primarily expressed in intrinsic enteric and extrinsic primary afferent neurons. In view of their stimulus-induced release and predominant excitatory action on intestinal nerve and muscle, SP and NKA are thought to play a neurotransmitter and/or neuromodulator role, and in this way to regulate various digestive functions. The actions of tachykinins are brought about by specific tachykinin receptors, of which three subtypes (NK_1, NK_2, NK_3) have been identified and characterized by molecular pharmacological techniques (Regoli *et al.*, 1994; Maggi, 1995).

Ever since SP was discovered to occur in the intestine and to contract gastrointestinal smooth muscle (von Euler and Gaddum, 1931), the implication of tachykinins in the regulation of gastrointestinal motility has been the most extensively studied area of visceral tachykinin research. The expression, release, pharmacology and motor functions of SP and NKA in the gut are reviewed in the companion article (Holzer and Holzer-Petsche, 1997). Recent work, however, has provided novel information that extends the physiological and pathophysiological implications of tachykinins, signifying that these peptides may be important regulators of neural excitability, secretory processes and con-

tributory factors in certain secretory and inflammatory disturbances of the gut. The present review attempts to discuss the distribution, actions and physiological roles of tachykinins in the mucosal, glandular and vascular systems of the gut in a comprehensive manner and to review pathological changes in these systems with a perspective for novel strategies in the therapy of secretory and inflammatory disorders.

2. OCCURRENCE OF TACHYKININS IN THE MUCOSAL, GLANDULAR AND VASCULAR SYSTEMS OF THE GUT

2.1. *Overview*

The mammalian gastrointestinal tract contains both SP and NKA and various N-terminal-extended forms of these tachykinins, which all are derived from alternative splicing of the primary transcripts of the PPT-A gene. In contrast, there is little evidence that the PPT-B gene, with its major product NKB, is expressed within the digestive system to any substantial degree. In terms of cellular sources of tachykinins in the gut, some six different systems have been identified: (1) intrinsic enteric neurons of the myenteric plexus (MP); (2) intrinsic enteric neurons of the submucosal plexus (SMP); (3) extrinsic primary afferent nerve fibers; (4) endocrine cells within the gastrointestinal epithelium; (5) blood-derived or resident immune cells; and (6) endothelial cells. The quantitatively most important source of tachykinins in the gut is the enteric nervous system, which has its cell bodies in the MP and SMP (submucous) and supplies all gastrointestinal effector systems. The SP-containing enteric neurons in the guinea-pig intestine have been mapped to such a precision that their projections within and between the nerve plexuses and to the muscular, vascular and epithelial effector systems are well understood.

2.2. *Tachykinins in the Gastrointestinal Mucosa*

SP and NKA are present in the mucosa of the stomach and small and large intestine in a variety of mammalian species, including humans, in which they are expressed by at least five types of cells: (1) nerve fibers originating from the MP; (2) nerve fibers originating from the SMP; (3) extrinsic primary afferent nerve fibers; (4) endocrine cells within the intestinal epithelium; and (5) blood-derived or resident immune cells. The distinct characteristics of the SP-immunoreactive enteric neurons and extrinsic afferent nerve fibres are described in the companion article (Holzer and Holzer-Petsche, 1997). There are considerable quantitative differences in the tissue concentration of tachykinins and in the density of SP/NKA-containing nerve fibers and epithelial cells between different regions of the gut and between species (Otsuka and Yoshioka, 1993). While the mucosa of the human, equine, canine, rat and guinea-pig small intestine receives a dense supply by SP-containing nerve fibers, the innervation of the feline and rabbit intestinal mucosa is comparatively sparse (Brodin *et al.*, 1983; Keast *et al.*, 1985a, 1987; Burns and Cummings, 1993).

The quantitatively most significant source of mucosal SP and NKA is provided by fibers that originate from the ganglionated plexus of the enteric nervous system (Fig. 1). The fibers of these neurons typically supply the lamina propria and may run in immediate proximity to the epithelium of the human, equine, sheep, porcine, canine, marmoset, guinea-pig, rat and murine intestine (Costa *et al.*, 1981; Llewellyn-Smith *et al.*, 1984; Ekblad *et al.*, 1985, 1987, 1988; Keast *et al.*, 1985a; Wathuta, 1986; Daniel *et al.*, 1987; Schmidt *et al.*, 1991; Burns and Cummings, 1993; Kitamura *et al.*, 1993). SP-immunoreactive nerve fibers in the human duodenum come close to the acini of Brunner's glands (Keast *et al.*, 1985a; Bosshard *et al.*, 1989). Whereas myenteric neurons are probably the only intrinsic neural source of SP in the gastric mucosa of dogs and other species (Furness *et al.*, 1991), the SP-containing nerve fibers in the intestinal mucosa of the dog and guinea-pig originate from both the MP and SMP (Costa *et al.*, 1981; Keast *et al.*, 1984; Daniel *et al.*, 1987; Messenger and Furness, 1990; Furness *et al.*, 1991; Messenger, 1993). Of the myenteric neurons that in the guinea-pig small intestine project to the mucosa, 43% have been found to be immunoreactive for SP and calbindin, while 18% are immunoreactive for SP alone (Song *et al.*, 1991). On the basis of morphological and electrophysiological characteristics, these neurons are thought to be intrinsic sensory neurons (Furness *et al.*, 1990; Song *et al.*, 1991). By analogy, SP neurons in the MP of the murine intestine, which may co-express 5-hydroxytryptamine (5-HT) or γ-aminobutyric acid (GABA), but not calretinin, are also considered to be sensory neurons (Sang and Young, 1996).

The SMP is the preeminent source of mucosal enteric nerve fibers, and in the guinea-pig small intestine, it is

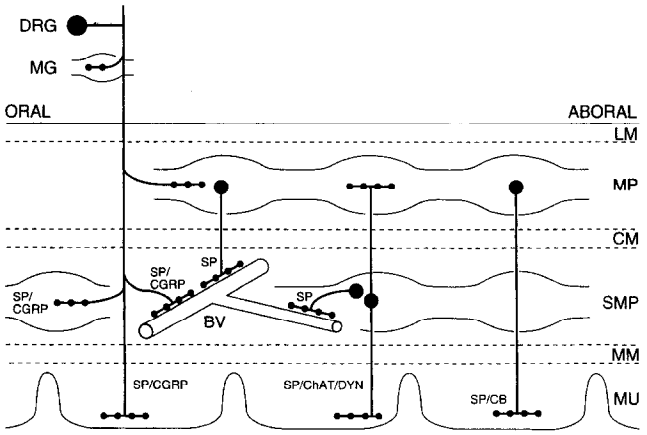

Figure 1. Schematic diagram of known projections of SP-immunoreactive neurons in the vasculature and mucosa of the guinea-pig intestine. The projection of SP-positive neurons from the MP to the submucosal blood vessels is seen only after extrinsic denervation of the gut (Galligan *et al.*, 1990; Jiang and Surprenant, 1992). Where known, the coexistence with other neuropeptides or neuronal markers is indicated. BV, blood vessel; CB, calbindin; CM, circular muscle; DRG, dorsal root ganglion; DYN, dynorphin; LM, longitudinal muscle; MG, mesenteric prevertebral ganglion; MM, muscularis mucosae; MU, mucosa.

about 20% of the submucosal neurons with mucosal projections that contain SP (Song *et al.*, 1992). The SP-immunoreactive neurons of the SMP issue mainly circumferential projections of intermediate length, with no obvious polarity in the longitudinal axis of the gut (Song *et al.*, 1992), a finding that is also true for the myenteric SP axons running to the mucosa (Song *et al.*, 1991). The submucosal SP neurons co-express choline acetyltransferase and send processes not only to the mucosa, but also to the MP (Furness *et al.*, 1984; Steele and Costa, 1990; Brookes *et al.*, 1991). In synopsis with their functional and morphological properties, these submucosal SP neurons are considered to be intrinsic sensory neurons (Bornstein and Furness, 1988; Bornstein *et al.*, 1989; Song *et al.*, 1992).

Although most SP/NKA-immunoreactivity in the gut is derived from intrinsic enteric neurons, there is immunohistochemical evidence that extrinsic afferent nerve fibres also make a small, but distinct, contribution (Fig. 1). The fibers of extrinsic afferent neurons in the gastrointestinal mucosa differ from those of intrinsic enteric neurons, not only in terms of their origin, but also with regard to their targets of projection, chemical coding (Costa *et al.*, 1986) and sensitivity to capsaicin (Holzer, 1991). The majority of SP/NKA-positive afferent nerve fibers in the gastrointestinal tract originates from dorsal root ganglia and reaches 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 (Lindh *et al.*, 1983, 1988; Matthews and Cuello, 1984; Sharkey *et al.*, 1984; Ekblad *et al.*, 1987, 1988; Su *et al.*, 1987; Green and Dockray, 1988). Although these spinal SP/NKA-containing afferents, which typically co-express calcitonin gene-related peptide (CGRP), project predominantly to the vascular system in the submucosa and mucosa, they also supply the lamina propria of the gastrointestinal mucosa and come close to epithelial cells (Costa *et al.*, 1981; Furness *et al.*, 1982; Lee *et al.*, 1985; Uddman *et al.*, 1986; Ekblad *et al.*, 1987, 1988; Gibbins *et al.*, 1987; Su *et al.*, 1987; Green and Dockray, 1988; Kirchgessner *et al.*, 1988; Lindh *et al.*, 1988; Chéry-Croze *et al.*, 1988; Ichikawa *et al.*, 1991; Domoto *et al.*, 1992; Sternini *et al.*, 1992; Isaacs *et al.*, 1995). In addition, the gut is also innervated by vagal afferents, which have their cell bodies in the nodose and jugular ganglia, but there is little evidence that vagal afferent nerve fibers contribute significantly to the SP content of the gastrointestinal mucosa (Green and Dockray, 1988).

The endocrine cells, which in the gastrointestinal mucosa were first recognized to express SP, are a population of 5-HT-containing enterochromaffin cells that occur throughout the gastrointestinal tract of humans (Heitz *et al.*, 1976; Sundler *et al.*, 1977; Sjölund *et al.*, 1983). Further analysis has shown that there are at least two subpopulations of SP-containing enterochromaffin cells, which in the mouse small intestine differ with regard to certain developmental characteristics (Aiken *et al.*, 1994). In addition, some epithelial cells, which in the human colon express SP, may represent a separate population of endocrine cells that are distinct from enterochromaffin cells (Sokolski and Lechago, 1984). SP-containing endocrine cells occur not only in the human and murine gastrointestinal tract, but have also been found in the gastrointestinal mucosa of species such as rabbit, dog and sheep; however, they seem to be absent from the rat, guinea-pig, feline and porcine intestine (Calingasan *et al.*, 1984; Grönstad *et al.*, 1985; Keast *et al.*, 1985a, 1987; Wathuta, 1986; Schmidt *et al.*, 1991).

A further source of SP in the lamina propria of the gastrointestinal mucosa is blood-derived or resident immune cells. Eosinophils isolated from the inflamed human large intestine (Metwali *et al.*, 1994) or intestinal granulomas of *Schistosoma*-infected mice (Weinstock and Blum, 1990) express mRNA for SP and contain the authentic peptide.

2.3. *Tachykinins in the Vascular System of the Gut*

It has long been recognized that the arterial bed of the gastrointestinal tract receives a relatively dense supply of SP-containing nerve fibers. A synopsis of the available data indicates that tachykinins in the vascular system of the gut may be derived from as many as four different cellular sources: (1) extrinsic primary afferent neurons; (2) intrinsic enteric neurons; (3) endothelial cells; and (4) blood cells.

The quantitatively most important source of SP and NKA in the wall of gastrointestinal arteries and veins is afferent nerve fibers, which have their cell bodies in dorsal root ganglia (Fig. 1) and reach the digestive system by running in the sympathetic and sacral parasympathetic nerves, passing through prevertebral ganglia and following the mesenteric arteries towards the wall of the gut (Costa *et al.*, 1981; Furness *et al.*, 1982; Barja *et al.*, 1983; Sharkey *et al.*, 1984; Ekblad *et al.*, 1987, 1988; Su *et al.*, 1987; Green and Dockray, 1988; Lindh *et al.*, 1988). Although there is functional evidence that vagal afferents supply the gastric vascular system (Thiefin *et al.*, 1990), it does not seem that vagal afferent nerve fibers contribute significantly to the SP content of the rat stomach (Green and Dockray, 1988).

SP-containing spinal afferents form a particularly dense plexus of para- and perivascular axons around the superior mesenteric artery of the guinea-pig and rat, whereas the mesenteric and portal veins contain relatively few SP-positive nerve fibers (Barja and Mathison, 1982; Furness *et al.*, 1982; Barja *et al.*, 1983). The SP-immunoreactive axons run primarily in the connective tissue (tunica adventitia) surrounding the vessels and at the border between adventitia and media (muscle layer) (Barja and Mathison, 1982; Furness *et al.*, 1982; Barja *et al.*, 1983). The interlacing network of fibers is very dense in the main arteries and becomes loose towards the precapillary arterioles (Furness *et al.*, 1982; Barja *et al.*, 1983; Uddman *et al.*, 1986). SP-positive afferent nerve fibers within the gastrointestinal wall supply predominantly submucosal and mucosal blood vessels, especially arterioles (Costa *et al.*, 1981; Furness *et al.*, 1982; Minagawa *et al.*, 1984; Papka *et al.*, 1984; Sharkey *et al.*, 1984; Gibbins *et al.*, 1985; Fehér and Burnstock, 1986;

Uddman *et al.*, 1986; Ekblad *et al.*, 1987, 1988; Su *et al.*, 1987; Galligan *et al.*, 1988; Green and Dockray, 1988; Domoto *et al.*, 1992; Sternini *et al.*, 1992). In addition, nerve fibers containing SP and CGRP come also close to the central lacteal lymphatics in the canine intestinal villi (Ichikawa *et al.*, 1991).

Compared with the extrinsic SP-containing nerve fibers, enteric tachykininergic neurons issue only sparse, if any, projections to blood vessels within the gastrointestinal wall. There is both morphological and functional evidence that cholinergic and noncholinergic neurons of the SMP supply arterioles in the submucosa of the guinea-pig ileum and colon (Furness *et al.*, 1984; Furness and Costa, 1987; Bornstein and Furness, 1988; Galligan *et al.*, 1990; Neild *et al.*, 1990; Brookes *et al.*, 1991; Vanner and Surprenant, 1991; Jiang and Surprenant, 1992). While in the guinea-pig colon SP-containing submucosal neurons do project to submucosal arterioles (Vanner and Surprenant, 1991), there is little evidence that SP or NKA is expressed in periarteriolar projections of submucosal neurons in the guinea-pig small intestine. However, 2 months after extrinsic denervation of the guinea-pig small intestine nerve fibers that contain SP, but not CGRP, are found to supply the submucosal arterioles (Galligan *et al.*, 1988, 1990; Jiang and Surprenant, 1992). As these newly appearing periarteriolar SP fibers are insensitive to the neurotoxic action of capsaicin, but are eliminated by ablation of the MP, it has been concluded that the source of the reinnervation to submucosal arterioles is the MP (Galligan *et al.*, 1990; Jiang and Surprenant, 1992). In the intact small intestine, only very few of these SP-positive/CGRP-negative nerve fibers can be visualized around submucosal blood vessels (Galligan *et al.*, 1990).

Further potential sources of tachykinins in the vascular system of the gut are endothelial (Loesch and Burnstock, 1988) and blood-derived immune cells, but their actual contribution, if any, has not been assessed yet.

2.4. *Tachykinins in the Salivary Glands*

SP and NKA are present in the different salivary glands of mammals, but there are considerable quantitative differences in the density of SP/NKA-containing nerve fibers and the tissue concentration of tachykinins between different glands and between species (Otsuka and Yoshioka, 1993). While the salivary glands of rats contain a comparatively high number of SP-containing nerve fibers, the salivary glands of humans seem to receive a rather sparse innervation by SP-immunoreactive nerve fibers, which are primarily located around blood vessels and large salivary ducts (Ekström *et al.*, 1988a; Hauser-Kronberger *et al.*, 1992; Konttinen *et al.*, 1992). These perivascular and periductular fibers, which also contain CGRP and NKA, represent, by all means, axons of capsaicin-sensitive primary afferent neurons having their cell bodies in the trigeminal and cervical C_3 and C_4 dorsal root ganglia (Sharkey and Templeton, 1984; Ekström *et al.*, 1988a, 1989; Virta *et al.*, 1991; Forsgren *et al.*, 1992; Konttinen *et al.*, 1992; Tornwall *et al.*, 1994; Salo *et al.*, 1995). The afferent peptide-containing nerve fibers are very sparse in the acinar tissue of rat salivary glands (Ekström *et al.*, 1988a; Salo *et al.*, 1995).

The major source of SP and NKA in the rat salivary glands is represented by postganglionic parasympathetic efferent neurons (Goedert *et al.*, 1982; Sharkey and Templeton, 1984; Ekström *et al.*, 1984, 1988a). Some 90% of the SP contained in the rat parotid gland is derived from the auriculotemporal nerve, while a similar percentage of the SP content of the rat submaxillary gland is provided by the chorda tympani (Ekström *et al.*, 1984, 1988a). These parasympathetic nerve fibers, which are most probably cholinergic, and in the rat and guinea-pig contain SP, NKA and vasoactive intestinal polypeptide (VIP), but not CGRP as co-transmitters, are mainly distributed around the secretory acini and small glandular ducts (al Hadithi *et al.*, 1988; Ekström *et al.*, 1988a, 1989; Gibbins 1990; Virta *et al.*, 1991, 1992; Forsgren *et al.*, 1992; Tornwall *et al.*, 1994). The supply of the ferret salivary glands by parasympathetic efferent and primary afferent peptide-containing nerve fibers appears to be similar to that in the rat, but the overall density of SP-immunoreactive axons is relatively moderate (Tobin *et al.*, 1990). Additional minor sources of SP in the rat salivary glands may be nerve fibers from the facial nerve and local parasympathetic ganglia within the glands (Goedert *et al.*, 1982; Ayer-LeLievre and Seiger, 1984; Sharkey and Templeton, 1984; Ekström *et al.*, 1988a; Forsgren *et al.*, 1992). In contrast, sympathetic nerve fibers originating from the superior cervical ganglia do not contribute to the SP content of salivary glands (Ekström *et al.*, 1984; Tornwall *et al.*, 1994).

2.5. *Tachykinins in the Hepatobiliary System*

As in the gut, the tachykinins present in the hepatobiliary system are derived from two principal sources, intrinsic neurons and extrinsic primary afferent nerve fibers. As is outlined in the companion article (Holzer and Holzer-Petsche, 1997), SP has been localized to cell bodies in both ganglionated nerve plexuses, which are analogous to the intrinsic nerve plexuses of the gut and which supply all layers of the gallbladder and bile duct. The fibers of extrinsic neurons co-express CGRP and are most abundant around blood vessels of the human, canine, rat and guinea-pig gallbladder (Goehler *et al.*, 1988; Mawe and Gershon, 1989; Sternini *et al.*, 1992; De Giorgio *et al.*, 1995; Goehler and Sternini, 1996). Experiments involving extrinsic nerve section and capsaicin pretreatment have revealed that the CGRP/SP-positive fibres represent axons of spinal afferent neurons (Maggi *et al.*, 1989; Goehler and Sternini, 1996). Some enterochromaffin cells that contain SP have been identified in the rabbit bile duct (Heitz *et al.*, 1977), but are absent from the human gallbladder (De Giorgio *et al.*, 1995).

SP-containing axons also extend into the liver, in which a number of nerve fibers has been localized to the portal vein, the hepatic arteries and veins, while relatively few fibers are seen in the sinusoids, the intrahepatic bile ducts, the hepatic lobules and the interlobular connective tissue of the human, guinea-pig and rat liver (Sasaki *et al.*, 1984;

Goehler *et al.*, 1988; Ueno *et al.*, 1991; Inoue *et al.*, 1992; el Salhy *et al.*, 1993; Goehler and Sternini, 1996). It appears as if the SP-positive fibers associated with the hepatic artery and bile ducts of the rat stem from the greater splanchnic nerves, whereas those found in the portal and hepatic veins are derived from both the vagal and greater splanchnic nerves (Inoue *et al.*, 1992; Goehler and Sternini, 1996).

2.6. *Tachykinins in the Pancreas*

As in the hepatobiliary system, the tachykinins present in the pancreas are derived from both intrinsic neurons and extrinsic primary afferent nerve fibers. In the pancreas of humans and other mammalian species, SP has been localized to cell bodies and fibers of intrapancreatic ganglia, which are considered to be extensions of the enteric nerve plexuses in the gut (Kirchgessner and Gershon, 1990; Huchtebrock *et al.*, 1991; Kirchgessner and Pintar, 1991; Kirchgessner *et al.*, 1992a; Ekblad *et al.*, 1994). More prominent in most species, though, are intrapancreatic nerve fibers that co-express CGRP and SP/NKA and that are of extrinsic primary afferent origin. In the rat pancreas, these extrinsic fibers originate from the thoracolumbar T6-L2 dorsal root ganglia and are depleted of SP and CGRP following exposure to a neurotoxic dose of capsaicin (Sharkey *et al.*, 1984; Su *et al.*, 1987; Karlsson *et al.*, 1992, 1994). In humans, sheep, dogs, cats, guinea-pigs and rats, extrinsic SP fibers form a plexus around pancreatic blood vessels, intrapancreatic ganglion cells and islets of Langerhans (Larsson and Rehfeld, 1979; Sharkey *et al.*, 1984; Wathuta, 1986; Su *et al.*, 1987; Huchtebrock *et al.*, 1991; Kirchgessner and Pintar, 1991; Büchler *et al.*, 1992; De Giorgio *et al.*, 1992, 1993). In addition, some fibers project into the connective tissue between the pancreatic lobules, whereas virtually no SP-immunoreactive fibers are associated with the pancreatic ducts (Sharkey *et al.*, 1984). Endocrine cells in the adult rat pancreas do not contain SP, although the rat insulinoma cell line RINm5F, which resembles developing pancreatic endocrine cells, expresses the PPT-A gene (McGregor *et al.*, 1995).

3. EFFECTS OF TACHYKININS ON ENTERIC NEURONS

3.1. *Overview*

SP, NKA and related peptides excite most enteric neurons in the guinea-pig gut. The typically slow depolarization that is evoked by tachykinins derives primarily from a decrease in the membrane conductance for K^+, but opening of Cl^- and cation channels also do contribute. Increased excitability, frequent discharge of action potentials and release of a variety of enteric neurotransmitters, notably acetylcholine (ACh), are immediate consequences of these membrane actions of tachykinins. The depolarizing effect of SP and NKA on enteric neurons in the guinea-pig gut seems to be predominantly due to activation of NK_3 receptors. Several lines of evidence indicate that tachykinins play an important physiological role as mediators, or comediators, of slow synaptic transmission in the enteric nervous system and as modulators of the excitability of enteric neurons.

3.2. *Membrane Actions of Tachykinins on Enteric Neurons*

The effects of mammalian tachykinins on enteric neurons have been studied extensively in the guinea-pig gut. The common observation made with extracellular and intracellular recording techniques is that SP, NKA and NKB excite the majority of neurons with a time course that, in most cases, is very similar to that of the slow excitatory postsynaptic potential (EPSP). When applied by perfusion, iontophoresis or pressure ejection, SP, NKA and NKB depolarize 70–100% of the myenteric neurons of the guinea-pig stomach and intestine (Johnson *et al.*, 1981; Galligan *et al.*, 1987; Hanani *et al.*, 1988; Wade and Wood, 1988; Schemann and Kayser, 1991). The vast majority of neurons in the SMP of the guinea-pig ileum and cecum is also depolarized by SP, NKA or NKB (Mihara *et al.*, 1985; Surprenant *et al.*, 1987; Akasu and Tokimasa, 1989; Mihara and Nishi, 1994). Typically, the depolarization is slow in onset and of long duration (20–180 sec). Blockade of axonal conduction with tetrodotoxin (TTX) or synaptic transmitter release does not affect the depolarization, which indicates that the tachykinins act directly on the soma to cause excitation (Katayama *et al.*, 1979; Wade and Wood, 1988; Schemann and Kayser, 1991; Morita and Katayama, 1992). While excitation is the prevailing action, it should not go unnoticed that SP may hyperpolarize a minority of myenteric neurons in the guinea-pig ileum (Katayama *et al.*, 1979; Johnson *et al.*, 1981; Hanani and Burnstock, 1985).

Although SP, NKA and NKB excite enteric neurons in the guinea-pig gut with comparable potency and efficacy (Galligan *et al.*, 1987; Akasu and Tokimasa, 1989; Schemann and Kayser, 1991), it has been demonstrated by cross-desensitization experiments and the use of receptor-selective agonists that the responsible tachykinin receptors are primarily of the NK_3 type. This conjecture is supported by the high potency and efficacy of senktide to cause depolarization, whereas NK_1 and NK_2 receptor-selective agonists are virtually inactive (Hanani *et al.*, 1988; Schemann and Kayser, 1991; Bertrand and Galligan, 1994, 1995). The identification of the enteric tachykinin receptors as NK_3 receptors receives further indirect support from the observation that none of the peptidic tachykinin antagonists, which express preferential affinity for NK_1 and NK_2 receptors, is able to suppress tachykinin-induced depolarization of myenteric neurons in the guinea-pig and rat gut (Nemeth *et al.*, 1983; Galligan *et al.*, 1987; Wade and Wood, 1988; Willard, 1990; Schemann and Kayser, 1991). The ability of the newly available antagonists for NK_3 receptors to block tachykinin-evoked excitation of enteric neurons has not been tested yet.

Despite the pharmacological predominance of NK_3 receptors on enteric neurons, it is important to realize that myenteric and submucosal neurons of the guinea-pig gut

bear, in addition, NK_1 receptors (Portbury *et al.*, 1996), whose membrane actions and functional implications are still unknown. It remains also to be examined which tachykinin receptors are responsible for the tachykinin-induced depolarization of rat myenteric neurons (Brookes *et al.*, 1988; Willard, 1990), given that NK_1 receptors are abundantly expressed in the enteric nerve plexuses of the rat (Sternini *et al.*, 1995).

Tachykinin-evoked depolarization of enteric neurons is associated with an increase in neuronal excitability and frequent discharge of action potentials. The major ionic mechanism that in most enteric neurons, underlies the excitatory action of tachykinins is a reduction of the membrane K^+ conductance, which in turn increases input resistance and causes depolarization. Detailed analysis shows that, on the one hand, tachykinins inactivate the voltage-independent resting K^+ conductance and, on the other hand, prevent activation of voltage-sensitive Ca^{2+}-dependent K^+ channels (Katayama *et al.*, 1979; Mihara *et al.*, 1985; Surprenant *et al.*, 1987; Hanani *et al.*, 1988; Wade and Wood, 1988; Akasu and Tokimasa, 1989; Schemann and Kayser, 1991; Morita and Katayama, 1992; Shen and Surprenant, 1993; Bertrand and Galligan, 1994; Mihara and Nishi, 1994). Inhibition of the Ca^{2+}-dependent K^+ conductance suppresses the slow after-hyperpolarization that follows an action potential in AH/Type II enteric neurons (Akasu and Tokimasa, 1989; Morita and Katayama, 1992).

A reduction of membrane conductance is not the only mode of action, though, as tachykinin-induced depolarization of a certain proportion of myenteric neurons is associated with a decrease in membrane resistance (Galligan *et al.*, 1987; Schemann and Kayser, 1991; Bertrand and Galligan, 1994, 1995). The rise of membrane conductance that is seen in myenteric neurons of the guinea-pig stomach is thought to reflect opening of nonselective cation channels (Schemann and Kayser, 1991). A similar increase in membrane conductance that is carried by a predominantly Na^+-selective current has been observed in submucosal neurons of the guinea-pig ileum under conditions in which the resting and Ca^{2+}-dependent K^+ conductances are inactivated (Shen and Surprenant, 1993). Conversely, the rise of membrane conductance that senktide evokes in myenteric neurons of the guinea-pig ileum (Bertrand and Galligan, 1994, 1995) and SP induces in submucosal neurons of the guinea-pig cecum (Mihara and Nishi, 1994) results from an increase in the membrane conductance for Cl^-. Taking all pertinent data together, it is obvious, therefore, that tachykinins influence multiple ionic conductances in enteric neurons and that net excitation reflects a combination of inactivation of the K^+ conductance and activation of Cl^- and cation channels.

3.3. *Tachykinin-evoked Release of Enteric Neurotransmitters*

In view of the ability of tachykinins to depolarize enteric neurons, it is not unexpected to see that tachykinin receptor agonists influence the release of a variety of neurotransmitters and messenger molecules in the gastrointestinal tract (Table 1). Congruent with the participation of cholinergic neurons in many of the gastrointestinal actions of tachykinins is the ability of SP and NKA to release ACh from myenteric neurons in all regions of the mammalian gut (Table 1), whereas in the guinea-pig SMP, ACh release in response to SP has not been seen (Yau *et al.*, 1990). The effect of tachykinins on myenteric cholinergic neurons has been studied most extensively in the guinea-pig small intestine and like the depolarizing action, is predominantly mediated by NK_3 receptors (Fosbraey *et al.*, 1984; Featherstone *et al.*, 1986; Kilbinger *et al.*, 1986; Fox and Morton, 1991; Guard and Watson, 1991; Yau *et al.*, 1992; Emonds-Alt *et al.*, 1994). There is evidence, though, that stimulation of NK_1 receptors also causes some release of ACh (Guard and Watson, 1991), which is in keeping with the presence of NK_1 receptor immunoreactivity on myenteric and submucosal enteric neurons (Portbury *et al.*, 1996).

The action of tachykinins, particularly of NK_3 receptor agonists, to enhance *spontaneous release* of ACh is prevented by ω-conotoxin or removal of external Ca^{2+} and requires nerve conduction via TTX-sensitive Na^+ channels (Table 1). As has been found with other stimuli, the tachykinin-evoked release of ACh from the MP of the guinea-pig small intestine is under the inhibitory control of noradrenaline acting via α_2-adrenoceptors, opioid peptides acting via μ and κ receptors and galanin. Particularly worth noting are the findings that tachykinin-induced release of ACh from the guinea-pig MP is inhibited by mepacrine, indomethacin and nordihydroguaiaretic acid (Table 1), which indicates that neural stimulation by tachykinins depends on activation of phospholipase A_2, formation of cyclooxygenase products (prostaglandins) and formation of lipoxygenase products (leukotrienes). This NK_3 receptor-mediated effect is analogous to the effect of SP to enhance the generation of 6-keto-prostaglandin $F_{1\alpha}$ in the vascularly perfused ileum of the dog, an action that, however, is brought about by stimulation of NK_1 receptors (Parrish *et al.*, 1994).

Further, though indirect, evidence for a NK_1 receptor-induced formation of prostaglandins has been obtained in the isolated antrum of the canine stomach (Mayer *et al.*, 1990). The *electrically evoked release* of ACh from this preparation is inhibited by SP methyl ester, an effect that is prevented by indomethacin and VIP immunoneutralization. Dissection of the sequence of events has shown that NK_1 receptor stimulation first releases VIP, which in turn increases the synthesis of prostaglandins, and in this way, attenuates the output of ACh (Mayer *et al.*, 1990). Tachykinin-induced inhibition of the electrically stimulated release of ACh can also be observed in the MP of the guinea-pig small intestine (Table 1). Although it has not been tested yet whether prostaglandins are involved, it has been demonstrated with the use of receptor-selective agonists and antagonists that, as in the canine stomach, it is NK_1 receptors that mediate the inhibitory effect of tachykinins on the electrically stimulated output of ACh from the guinea-pig MP (Kilbinger *et al.*, 1986; Löffler *et al.*, 1994).

TABLE 1. Effects of Tachykinins on the Release of Neurotransmitters and Hormones in the Gut *In Vitro*

Tissue	Neurotransmitter or hormone	Tachykinin to evoke release	Inhibition of evoked release	Reference
Strips of guinea-pig gastric corpus	VIP	SP, NKB, SP methyl ester or senktide	TTX or L-NNA	Jin *et al.*, 1993
	NO		TTX, L-NNA or VIP-ANT	
Strips of canine gastric antrum	[^{3}H]-ACh	SP, NKA or NKB	Ca^{2+} removal, TTX, enkephalin or somatostatin	Koelbel *et al.*, 1988a,b
Guinea-pig small intestine MP-longitudinal muscle layer	[^{3}H]-ACh	SP	TTX	Yau and Youther, 1982; Teitelbaum *et al.*, 1984; Tanaka and Taniyama, 1985; Vizi and Barthó, 1985; Kilbinger *et al.*, 1986; Takeuchi *et al.*, 1991b; Ramirez *et al.*, 1994
			Ca^{2+} removal	Tanaka and Taniyama, 1985; Vizi and Barthó, 1985; Yau *et al.*, 1986c
			Mepacrine or indomethacin	Takeuchi *et al.*, 1991b
			Bicuculline	Tanaka and Taniyama, 1985
			Galanin	Yau *et al.*, 1986a
			Noradrenaline	Vizi and Barthó, 1985
			Enkephalin	Vizi and Barthó, 1985; Yau *et al.*, 1986b
		Eledoisin > kassinin > SP	Ca^{2+} removal	Fosbraey *et al.*, 1984; Featherstone *et al.*, 1986; Kilbinger *et al.*, 1986
		NKB or senktide	Ca^{2+} removal	Yau *et al.*, 1992; Emonds-Alt *et al.*, 1994; Ramirez *et al.*, 1994
			ω-Conotoxin or diltiazem	Yau *et al.*, 1992
			TTX	Guard and Watson, 1991; Yau *et al.*, 1992
			Mepacrine or NDHGA	Yau *et al.*, 1991
			μ/κ Opiate receptor agonists	Fox and Morton, 1991
		SP methyl ester	TTX	Guard and Watson, 1991
	[^{3}H]-GABA	SP	Ca^{2+} removal or TTX	Tanaka and Taniyama, 1985
Myenteric ganglia of the guinea-pig small intestine	[^{3}H]-ACh	SP		Yau *et al.*, 1989
Guinea-pig small intestine myenteric varicosities	[^{3}H]-ACh	SP		Yau *et al.*, 1985
Cultures of submucosal neurons from canine ileum	Neurotensin	SP	Somatostatin	Barber *et al.*, 1989
Strips of rabbit colon	[^{3}H]-ACh	SP, NKA or NKB	Ca^{2+} removal or TTX	Koelbel *et al.*, 1989
Vascularly perfused stomach of rainbow trout	[^{3}H]-5-HT	SP	TTX	Holmgren *et al.*, 1985
Vascularly perfused canine pancreas-duodenum	Somatostatin insulin, glucagon	SP		Hermansen, 1980
Vascularly perfused rat small intestine	Neurotensin	SP		Herrmann *et al.*, 1992
Vascularly perfused canine small intestine	6-keto-$PGF_{1\alpha}$	SP, NKA or NK_1 receptor agonists	CP-96,345 or Ca^{2+} removal	Parrish *et al.*, 1994

ANT, antagonist; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; L-NNA, N^G-nitro-L-arginine NDHGA nordihydroguaiaretic acid.

The inhibitory effect of somatostatin on SP-evoked ACh release in the canine gastric antrum (Koelbel *et al.*, 1988b) is not seen in the guinea-pig MP (Teitelbaum *et al.*, 1984; Yau *et al.*, 1986b; Kowal *et al.*, 1989). Conversely, there are also divergent reports concerning the influence of SP on somatostatin release (Table 1). While the output of somatostatin from the vascularly perfused stomach of the rat is reduced by tachykinins acting through presumably NK_2 re-

ceptors (Kwok *et al.*, 1985, 1988; McIntosh *et al.*, 1987), somatostatin release into the portal blood of the rat *in vivo* (Saito and Saito, 1980) and from the vascularly perfused pancreas-duodenum of the dog *in vitro* (Hermansen, 1980) is enhanced by SP.

Other enteric neurotransmitters that are released by SP include neurotensin, GABA, VIP and nitric oxide (NO) (Table 1). GABA has been implicated in the tachykinin's ability to stimulate cholinergic neurons in the guinea-pig MP, given that SP-evoked release of ACh is attenuated by bicuculline (Tanaka and Taniyama, 1985). Activation of NK_1 and NK_3 receptors in the guinea-pig stomach augments the release of VIP and NO, which reflects the ability of tachykinins to stimulate inhibitory enteric motor neurons (Jin *et al.*, 1993). Intraarterial administration of SP to the cat *in vivo* causes release of VIP into the blood, an effect that is reduced by hexamethonium, enkephalin and sympathetic nerve stimulation (Brunsson *et al.*, 1995). While there is also indirect evidence for a NK_1 receptor-mediated release of VIP from the isolated canine stomach (Mayer *et al.*, 1990), SP, NK_1 and NK_2 agonists fail to enhance the release of VIP in the vascularly perfused ileum of the dog (Watson *et al.*, 1994).

3.4. *Physiological Implications of Tachykinins as Mediators of the Slow Excitatory Postsynaptic Potential*

SP and NKA are major candidates for being comediators of the slow EPSP, which in the myenteric and submucosal neurons of the guinea-pig gut is elicited by enteric fiber tract stimulation. A strong line of evidence comes from the ability of tachykinins to mimic the slow EPSP with respect to time course of depolarization, increase in membrane excitability, change in membrane conductance and underlying ionic mechanisms (Morita *et al.*, 1980; Johnson *et al.*, 1981; Surprenant, 1984; Surprenant *et al.*, 1987; Tamura and Wood, 1989; Willard, 1990; Shen and Surprenant, 1993; Bertrand and Galligan, 1994, 1995; Mihara and Nishi, 1994). This mimicry is particularly evident in myenteric neurons of the guinea-pig ileum in which not only the decrease in K^+ conductance, but also the increase in Cl^- conductance, associated with the slow EPSP is reproduced by the NK_3 receptor agonist senktide (Bertrand and Galligan, 1994, 1995). Both the slow EPSP and the senktide-induced depolarization are mediated by pertussis toxin-insensitive G-proteins that activate phospholipase C and a protein kinase C pathway (Bertrand and Galligan, 1995). Conversely, the slow EPSP in submucosal neurons of the guinea-pig cecum is solely the result of a decrease in K^+ conductance, and in this respect, is more closely mimicked by NKA than by SP, which, in addition, increases a Cl^- conductance (Mihara and Nishi, 1994). Furthermore, only those somata in the SMP of the guinea-pig small intestine that show a slow EPSP respond to SP with a depolarization of similar time course (Surprenant, 1984).

Further support for the hypothesis that SP and NKA participate in slow synaptic transmission has come from nerve lesion experiments, showing that the slow EPSP persists when lesions to the MP of the guinea-pig small intestine are made in such a way that the only immunohistochemically identified neurons whose fibers stay intact are those containing tachykinins (Bornstein *et al.*, 1984). Conclusive pharmacological evidence for a mediator role of SP and NKA in the slow EPSP is sparse, however, because effective antagonists for the tachykinin receptors situated on enteric neurons in the guinea-pig gut have not been available until very recently. It nevertheless has been demonstrated that the slow depolarization brought about by SP and other tachykinin receptor agonists undergoes desensitization, and that in neurons desensitized to SP, the slow EPSP is reduced (Katayama *et al.*, 1979; Morita *et al.*, 1980; Johnson *et al.*, 1981; Surprenant, 1984; Mihara *et al.*, 1985, 1987; Schemann and Kayser, 1991). Whether this proves a mediator role of tachykinins in the slow EPSP has been questioned because in some studies, SP desensitization of enteric neurons has been found to lack specificity (Surprenant, 1984; Willard, 1990). This limitation, however, is unlikely to apply to the ability of SP immunoneutralization to inhibit the slow EPSP in cultured myenteric neurons from the rat small intestine (Willard, 1990). Furthermore, chymotrypsin, which degrades SP and in this way reduces SP-evoked depolarization, has been found to depress the slow EPSP in the guinea-pig MP (Morita *et al.*, 1980; Johnson *et al.*, 1981). In summary, therefore, SP and NKA meet many criteria for being mediators of the slow EPSP in the guinea-pig and rat enteric nervous system. Since this function is expectedly mediated by NK_3 receptors, it will be important to examine whether the slow EPSP is blunted by the newly available NK_3 antagonists.

The proposed role of tachykinins as comediators of slow excitation ascribes SP and NKA an important function in the control of the excitability of enteric neurons. Although in the guinea-pig stomach only Type I and Type II gastric neurons are depolarized by tachykinins, while Type III gastric neurons are unresponsive (Schemann and Kayser, 1991), it is important to realize that the two major types of electrophysiologically defined enteric neurons in the guinea-pig ileum, S/Type I and AH/Type II neurons, are similarly sensitive to SP (Morita *et al.*, 1980; Bornstein *et al.*, 1984; Surprenant *et al.*, 1987). As AH/Type II neurons are considered to be intrinsic sensory neurons, while S/Type I neurons represent interneurons and motor neurons (Smith *et al.*, 1990; Song *et al.*, 1991), and tachykinins are present in both enteric sensory neurons and interneurons (Brookes *et al.*, 1991; Song *et al.*, 1991, 1992), it can be envisaged that tachykinins subserve transmission at multiple sites within the enteric nerve plexuses. Simultaneous intracellular recording from pairs of neurons within the same myenteric ganglion suggests that tachykinins and ACh acting via muscarinic receptors are likely to comediate transmission from AH/Type II neurons to other myenteric neurons (Kunze *et al.*, 1993).

The reported ability of tachykinins to alter the release of a variety of enteric neurotransmitter and messenger mole-

cules (Table 1) is in keeping with the potentially important position of SP and NKA in enteric neuroneuronal communication. The tachykinin-induced release of ACh, GABA, VIP and NO illustrates that both excitatory and inhibitory neural pathways are being activated. The implications of altered transmitter release extend to all gastrointestinal effector systems and are being discussed together with the actions and physiological roles of tachykinins in gastrointestinal motility, secretion and circulation.

Apart from comediating the slow EPSP, SP and NKA may play an important role in modulating neurotransmission in the enteric nervous system. Tachykinin-induced inactivation of the resting K^+ conductance increases the excitability of neurons for a prolonged period of time, and in this way, facilitates excitatory neurotransmission and hinders inhibitory neurotransmission. This neuromodulatory function has still been little explored, but may turn out to be of similar relevance as the transmitter function.

4. TACHYKININS AND SALIVARY SECRETION

4.1. *Overview*

Although not directly pertinent to the gastrointestinal tract, the action and role of tachykinins in the secretion of saliva is dealt with here both because salivary secretion is a component of digestive activity and the ability of tachykinins to stimulate salivation is very prominent in some mammals. The sialogogic action of SP in dogs and rats was discovered before SP was isolated (Vogler *et al.*, 1963; Lembeck and Starke, 1968), and the rat salivation response was used by Chang and Leeman (1970) as a bioassay to purify and isolate SP. The information that is now available indicates that in the rat and ferret, SP and NKA are co-transmitters of postganglionic parasympathetic efferent neurons and synergize with ACh and VIP to cause fluid secretion from the salivary glands. The effects of tachykinins on salivary secretion in the rat are mediated by NK_1 receptors.

4.2. *Tachykinin Effects on Salivary Secretion*

In the dog, mink, ferret, rat and guinea-pig, SP is potent in stimulating salivation, whereas in humans, cat, rabbit, mouse and hamster, it is virtually inactive (Vogler *et al.*, 1963; Lembeck and Starke, 1968; Pernow, 1983; Larsson *et al.*, 1986; Fazekas *et al.*, 1987; Ekström *et al.*, 1988c; Iwabuchi *et al.*, 1989; Tobin and Ekström, 1992). Administration of SP to dogs and rats is followed by a quick and dose-dependent discharge of saliva, an effect that soon becomes tachyphylactic both *in vivo* and *in vitro* (Vogler *et al.*, 1963; Lembeck and Starke, 1968; Pernow, 1983; Sugiya and Putney, 1988; Soltoff *et al.*, 1989; Yoshimura and Nezu, 1991). The principal effect of SP and NKA is to enhance the flow of salivary fluid, which in the rat and ferret results in the discharge of watery saliva poor in protein (Ekström, 1987; Ekström and Tobin, 1989; Ekström *et al.*, 1988b; Tobin and Ekström, 1992). The secretion of fluid is stimulated in all salivary glands of the rat, but the submandibular glands are most sensitive to SP, whereas the parotid, and particularly the sublingual, glands are less responsive (Ekström *et al.*, 1983).

Apart from secretion of fluid, which has been studied extensively in the parotid, submandibular and submaxillary glands of the rat (Yu *et al.*, 1983; Bobyock *et al.*, 1986; Ekström and Olgart, 1986; Ekström *et al.*, 1987; Iwabuchi *et al.*, 1986; Murray *et al.*, 1987), there are also changes in the output of the salivary components. The secretion of K^+ and Cl^- ions is stimulated, as is the discharge of proteins, glycoproteins, proteolytic enzymes, kallikrein, amylase and mucin (Rudich and Butcher, 1976; Friedman and Selinger, 1978; Liang and Cascieri, 1979; Brown and Hanley, 1981; Gallacher, 1983; Fleming *et al.*, 1984; Bobyock *et al.*, 1986; Duner-Engström *et al.*, 1986b; Ekström and Olgart, 1986; Iwabuchi *et al.*, 1986; Dreux *et al.*, 1987; Ekström *et al.*, 1987; Culp *et al.*, 1991; Damas and Bourdon, 1994).

Saliva is formed by a two-stage process, with the secretion of a primary fluid by the acinar cells, followed by various ionic modifications in the salivary ducts (Valdez and Turner, 1991). The sialogogic effect of SP and NKA in the salivary glands of those species that are sensitive to tachykinins derives from a predominant action on the secretory structures in the acini. In contrast, tachykinins are virtually devoid of a secretory action on the intralobular (granular) ducts in the rat submandibular glands, most probably because no tachykinin receptors are expressed in these structures (Fleming *et al.*, 1984; Dehaye and Turner, 1991; Valdez and Turner, 1991; Dinudom *et al.*, 1993). This circumstance is likely to explain why the main action of tachykinins is to cause a flow of watery fluid rather than to produce fully processed saliva.

4.3. *Tachykinin Receptors and Receptor Mechanisms in Salivary Secretion*

There is unanimous agreement that the secretory action of tachykinins in the rat salivary glands is mediated by NK_1 receptors. High levels of mRNA for NK_1, but not NK_2 and NK_3, receptors are expressed in the salivary glands of rats (Takeda and Krause, 1989; Tsuchida *et al.*, 1990; Hershey *et al.*, 1991). Numerous tachykinin binding sites are present in the acini of the rat salivary glands, as shown by binding and autoradiographic studies. Displacement studies with nonselective and selective tachykinin receptor agonists and a selective NK_1 antagonist have shown that the binding sites are NK_1 in nature (Liang and Cascieri, 1981; Lee *et al.*, 1983; Buck and Burcher, 1985; Lew *et al.*, 1990; Snider *et al.*, 1991; Wei and Lee, 1992; Goll *et al.*, 1994), which is consistent with the pharmacological characterization of the tachykinin receptors on the salivary glands. Thus, SP is more potent than NKA and NKB in stimulating salivary secretion in the rat, although the most potent tachykinin is neuropeptide K (NPK; Holzer-Petsche *et al.*, 1985; Maggi *et al.*, 1985; Ekström *et al.*, 1987; Murray *et al.*, 1987; Iwabuchi *et al.*, 1989; Takeda and Krause, 1989; Wagner *et al.*, 1991). However, the use of selective tachykinin receptor agonists has confirmed that the actions of tachykinins on rat sali-

vary glands are exclusively mediated by NK_1 receptors, an inference that is convincingly supported by the use of tachykinin-receptor selective antagonists (Giuliani *et al.*, 1988; Arkle *et al.*, 1989; Maggi *et al.*, 1991; Rollandy *et al.*, 1991; Snider *et al.*, 1991; Wagner *et al.*, 1991; Guillemain *et al.*, 1992; Rouissi *et al.*, 1993; Jung *et al.*, 1994).

The NK_1 receptors mediating salivary secretion are coupled to phospholipase C by way of GTP-binding proteins (Taylor *et al.*, 1986). As a consequence of receptor activation, polyphosphoinositides are broken down to generate inositol trisphosphate and diacylglycerol, to cause intracellular calcium mobilization, to stimulate protein kinase C, to activate calcium-dependent potassium and chloride channels, and to promote calcium influx (Hanley *et al.*, 1980; Gallacher, 1983; Putney, 1983; Aub and Putney, 1985; Fleming *et al.*, 1987; Merritt and Rink, 1987; Sugiya and Putney, 1988; Arkle *et al.*, 1989; Soltoff *et al.*, 1989; Guillemain *et al.*, 1992; Wei and Lee, 1992; Soltoff and Toker, 1995). Consistently with this scheme, the effects of tachykinins are blunted by removal of extracellular calcium (Brown and Hanley, 1981; Katoh *et al.*, 1983; Dreux *et al.*, 1987). Some authors hold that additional transduction mechanisms participate in the action of tachykinins on salivary secretion but adenylate cyclase does not seem to play a role (Lee *et al.*, 1983; Arkle *et al.*, 1989).

4.4. *Physiological Roles of Tachykinins in Salivary Secretion*

There is multiple evidence that tachykinins (SP and NKA) participate in the parasympathetic regulation of salivary secretion in concert with a variety of other sialogogic messengers. The release of SP and NKA, as well as VIP, from rat parotid glands is enhanced after auriculo-temporal nerve stimulation, as is the release of SP, VIP and CGRP from the ferret submandibular gland following chorda-lingual nerve stimulation (Ekström, 1987; Tobin *et al.*, 1991). The stimulus-induced release of SP into the venous effluent from the ferret submandibular gland, like the secretory response, is abolished by hexamethonium, whereas atropine augments the stimulus-evoked release of the peptide (Tobin *et al.*, 1991). Indirect evidence for a release of SP and VIP in the salivary glands comes from the observation that the tissue content of SP is considerably reduced after prolonged stimulation of the parasympathetic, but not sympathetic, nerves supplying the salivary glands (Ekström *et al.*, 1985; Sugisawa and Takai, 1991). The secretion of saliva, which also decreases with prolonged nerve stimulation, can be restored by infusion of a subthreshold dose of SP (Sugisawa and Takai, 1991). In humans, SP seems to be released into the salivary fluid, with the result that the concentrations of SP in the saliva are considerably higher than those in blood plasma (Nicolodi and Del Bianco, 1990; Parris *et al.*, 1990; Takeyama *et al.*, 1990; Pikula *et al.*, 1992).

The use of receptor-nonselective tachykinin antagonists has proven that SP and NKA participate in the salivary response to parasympathetic nerve stimulation, particularly in the atropine-resistant component of the secretory response. Tachykinin antagonists cause some reduction of the overall secretory response to parasympathetic nerve stimulation in the ferret and rat, whereas the secretory response, which is left after atropine pretreatment, is practically abolished by tachykinin antagonists (Gallacher, 1983; Ekström *et al.*, 1987, 1988b; Sugisawa and Takai, 1991; Tobin *et al.*, 1991). Further consistent with a transmitter role of tachykinins is the observation that parasympathetic denervation leads to supersensitivity of the salivary glands, not only to ACh, but also to SP (Ekström *et al.*, 1983, 1989; Asking and Emmelin, 1989; Thesleff, 1989). The salivation evoked by sympathetic nerve stimulation in the rat remains unaltered by antagonism of tachykinin receptors (Sugisawa and Takai, 1991).

There is a number of other findings that support, or are consistent with, the notion that SP and NKA are messengers of nonadrenergic noncholinergic (NANC) parasympathetic nerve fibers in the salivary glands. SP mimics the membrane potential and secretory responses to NANC nerve stimulation in the rat parotid glands and appears to act directly on the secretory structures, since antagonists of muscarinic acetylcholine receptors and adrenoceptors do not change the sialogogic effects of SP and NKA (Ekström *et al.*, 1983, 1987; Gallacher, 1983; Fleming *et al.*, 1984; Giuliani *et al.*, 1988; Takeda and Krause, 1989; Thesleff, 1989; Wagner *et al.*, 1991; Wei and Lee, 1992). The findings that the salivary response to SP may be attenuated and that to NKB blocked by atropine (Yu *et al.*, 1983; Murray *et al.*, 1987; Thesleff, 1989; Ueha *et al.*, 1989; Sugisawa and Takai, 1991; Wei and Lee, 1992) does not necessarily mean that tachykinins act presynaptically on cholinergic neurons, but could also be explained by synergism of ACh and tachykinins to stimulate salivary secretion. This inference is in keeping with the ability of parasympathetic nerve stimulation to enhance the sialogogic effects of SP and NKA in the rat and ferret (Ekström and Olgart, 1986; Ekström *et al.*, 1988c; Thesleff, 1989). Many authors have noted that VIP is likewise able to facilitate the secretory responses to SP and NKA in the rat, mink and ferret (Ekström and Olgart, 1986; Ekström, 1987; Ekström *et al.*, 1987, 1988c; Bobyock and Chernick, 1989; Tobin *et al.*, 1991; Tobin and Ekström, 1992; Turner and Camden, 1992; Goll *et al.*, 1994; Iwabuchi and Masuhara, 1994). Synergism in secretory stimulation also exists between tachykinins and CGRP and other activators of adenylate cyclase (Dreux *et al.*, 1987; Ekström, 1987; Poat *et al.*, 1987; Ekström *et al.*, 1988a; Salo *et al.*, 1995). Although the mechanism of these synergistic interactions that seem to take place at a postreceptor level distal to the second messenger production (Dreux *et al.*, 1987; Poat *et al.*, 1987) is not understood, it is important to realize that tachykinins do participate in the multimessenger control of salivary secretion.

While tachykinins released from parasympathetic nerve fibers contribute to the neural regulation of salivation, SP and NKA released from perivascular afferent nerve fibers

do not seem to be of significance for salivary gland function (Ekström *et al.*, 1989). Although stimulation of afferent nerve fibers with capsaicin causes some salivation in humans and rats (Gallacher, 1983; Duner-Engström *et al.*, 1986a), ablation of these fibers with a neurotoxic dose of capsaicin does not alter the salivary responses to parasympathetic nerve stimulation and to exogenous ACh and SP (Ekström *et al.*, 1989).

5. TACHYKININS AND GASTRIC SECRETION

5.1. *Overview*

Tachykinins, which in the gastric mucosa are present in both nerve fibers and enterochromaffin cells, are able to influence the gastric secretion of acid, bicarbonate and pepsinogen in certain mammalian species. The effects are often weak and variable, though, and ill defined with regard to the receptors under operation, and there is no conclusive evidence that tachykinins participate in the physiological regulation of gastric secretory processes. From the available information, it may at best be envisaged that gastric secretion is under the modulatory influence of tachykinins.

5.2. *Tachykinin Effects on Exocrine Gastric Secretion*

5.2.1. Fluid and acid secretion into the stomach. Systemic administration of the nonmammalian tachykinins physalaemin and eledoisin stimulates the basal gastric output of fluid and acid in a primitive vertebrate, the codfish, whereas SP is comparatively little active (Holstein and Cederberg, 1986). In the rat stomach, neither SP nor NK_2 or NK_3 receptor-selective agonists are able to influence the basal output of fluid and acid (Yokotani and Fujiwara, 1985; Coruzzi *et al.*, 1991; Zanelli *et al.*, 1992), which is consistent with the inability of SP to affect basal acid production in dispersed rat parietal cells (Schepp *et al.*, 1990). SP likewise fails to alter basal acid secretion in gastric fistula dogs (Konturek *et al.*, 1981; Modlin *et al.*, 1981; Martensson *et al.*, 1984), whereas in dogs fitted with a Pavlov pouch, basal acid output has been found to decrease in response to SP (Ogoshi *et al.*, 1984). To the contrary, basal gastric acid secretion in gastric fistula cats seems to be facilitated by SP, but not by NK_2 receptor-selective agonists (Coruzzi *et al.*, 1991).

The effects of tachykinins on stimulated gastric acid secretion are diverse and difficult to explain in a coherent manner. In the rat, acid secretion induced by bethanechol is left unaltered by SP (Yokotani and Fujiwara, 1985), which is in keeping with the failure of SP to influence ACh-induced acid production in dispersed rat parietal cells (Schepp *et al.*, 1990). In contrast, acid output from the rat stomach evoked by vagal nerve stimulation is reduced by SP via an action that is independent of noradrenaline acting via α/β-adrenoceptors, histamine acting via H_1 receptors and prostaglandins (Yokotani and Fujiwara, 1985). The secretory response of the rat isolated gastric corpus to histamine seems to be enhanced by SP and NK_2 receptor-selective agonists (Coruzzi *et al.*, 1991), a finding that contrasts with the effect of SP and NKA to reduce histamine-stimulated acid production in dispersed rat parietal cells (Schepp *et al.*, 1990). As SP and NKA also attenuate the secretory effect of adenylate cyclase activation in a histamine H_2 receptor-independent fashion, it has been proposed that tachykinins can act directly on rat parietal cells to modulate acid production at an intracellular step distal to adenylate cyclase (Schepp *et al.*, 1990).

While these data imply the presence of SP receptors on parietal cells of the rat stomach, parietal cells isolated from the canine stomach appear to lack SP receptors, as shown by binding studies (Vigna *et al.*, 1989). If so, the finding that stimulated acid secretion in gastric fistula dogs may be inhibited by SP depending on the nature of the secretory stimulus, points to a site of action of SP on secretory pathway components other than parietal cells. There is consensus that pentagastrin-stimulated acid output in the dog is inhibited by SP, as is the acid secretion evoked by peptone (Konturek *et al.*, 1981; Martensson *et al.*, 1984; Ogoshi *et al.*, 1984; Korshak *et al.*, 1992). In contrast, the acid-secretory effect of histamine in gastric fistula dogs seems to be enhanced by SP (Barashkova *et al.*, 1987; Liashchenko *et al.*, 1992), whereas the secretory effect of the histamine H_2 receptor agonist dimaprit and of pentagastrin in gastric fistula cats remains unchanged by SP and NK_2 receptor-selective agonists (Coruzzi *et al.*, 1991). All that can be concluded from the divergent actions of tachykinins on gastric acid secretion is that the responses depend on the species under study, the experimental conditions, the secretory status of the stomach and the stimuli used to enhance acid output. The effects of SP on gastric hormone release, which are dealt with in Section 5.3, do not help much in clarifying this issue.

There is at present no evidence that SP and NKA participate in the physiological regulation of gastric acid secretion, but this possibility has not been rigorously tested yet with potent and selective tachykinin antagonists. Such tests would be worthwhile in view of the observation that less SP appears to be released into the gastric juice of humans as the pH of the gastric juice increases, whereas the plasma levels of SP appear to be independent of gastric acidity (Mueller *et al.*, 1991). The plasma levels of SP in the dog rise in response to food ingestion or bombesin administration, and these changes in plasma SP are reduced by atropine or propranolol (Jaffe *et al.*, 1982; Ferrara *et al.*, 1987). It has been ruled out, however, that SP acts as an enterogastrone that is released by duodenal acidification and causes feedback inhibition of stimulated gastric acid secretion (Martensson *et al.*, 1984). Although not directly pertinent to the roles of tachykinins within the gut, it is worth noticing here that tachykinins in the CNS might play a role in the inhibitory control of gastric acid secretion. Intracerebroventricularly injected NK_3 and NK_2, but not NK_1, receptor-selective agonists inhibit both basal gastric acid secretion in the rat and the acid output induced by histamine, whereas the secretory responses to bethanechol and pentagastrin remain unchanged (Improta and Broccardo,

1990, 1991). Conversely, intrathecal administration of SP to rats inhibits acid output evoked by vagal nerve stimulation, presumably by activation of the sympatho-adrenomedullary system (Okuma and Osumi, 1991)

5.2.2. Pepsinogen secretion into the stomach. Tachykinins are able to pepsinogen secretion, and this action may have been established early in vertebrate evolution because the nonmammalian tachykinins physalaemin and eledoisin, and to a much lesser extent SP, are potent stimulants of pepsinogen secretion from the codfish stomach (Holstein and Cederberg, 1986). SP, NKA and NKB activate chief cells of the canine and guinea-pig stomach, as demonstrated by an increase in the intracellular calcium ion concentration and by the secretion of pepsinogen (Vigna *et al.*, 1989; Fiorucci and McArthur, 1990; Kitsukawa *et al.*, 1996). Similarly, physalaemin has been reported to release pepsinogen from dispersed gastric glands of the rabbit (Kasbekar *et al.*, 1983). The tachykinin receptors expressed by canine and guinea-pig chief cells have been characterized as being of the NK_1 type (Vigna *et al.*, 1989; Kitsukawa *et al.*, 1996).

5.2.3. Mucus and bicarbonate secretion into the stomach. Mucous cells isolated from the canine stomach do not display any binding sites for SP (Vigna *et al.*, 1989), but it has not been examined yet whether or not tachykinins influence mucus secretion in the mammalian stomach. Likewise, the effects of tachykinins on the gastric bicarbonate output remain to be studied, as there is only one report to show that bicarbonate secretion in cats pretreated with hexamethonium and guanethidine may be enhanced by SP in an atropine-sensitive manner (Fändriks and Delbro, 1983).

5.3. *Tachykinin Effects on Endocrine Gastric Secretion*

Neither in rats nor in dogs does SP affect the basal secretion of gastrin, and the peptone-evoked increase in circulating gastrin likewise is not affected by SP (Konturek *et al.*, 1981; Modlin *et al.*, 1981; Martensson *et al.*, 1984; McIntosh *et al.*, 1987). Similarly, the release of histamine from rat enterochromaffin-like cells remains unaltered by SP, NKA and NKB (Sandor *et al.*, 1996). To the contrary, there is clear evidence that SP inhibits the release of somatostatin from the rat isolated stomach. Both the basal release of somatostatin and the somatostatin release evoked by gastric inhibitory peptide or isoproterenol are attenuated by SP and NKA (Chiba *et al.*, 1980; Kwok *et al.*, 1985, 1988). From the relative potency of SP, NKA, NKB and nonmammalian peptides, it appears that tachykinins reduce somatostatin release by way of NK_2 receptor stimulation (Kwok *et al.*, 1988). The inhibitory action of SP on somatostatin release remains unaltered by atropine, hexamethonium, naloxone, pyrilamine and cimetidine (Kwok *et al.*, 1985; McIntosh *et al.*, 1987). It thus seems as if SP acts directly on gastric D cells to reduce the liberation of somatostatin (Kwok *et al.*, 1985). The significance of this tachykinin action for gastric secretory processes is not understood, which is also true for the ability of SP to stimulate the expression of somatostatin mRNA in the gastric antrum of rats pretreated with a neurotoxic dose of capsaicin, but not in intact animals (Dimaline *et al.*, 1994).

6. TACHYKININS AND INTESTINAL SECRETION

6.1. *Overview*

There is mounting evidence that tachykinins influence the secretory activity of the small and large intestine and can switch its function from net absorption to net secretion of fluid and electrolytes. The bottom line of the pertinent studies is that tachykinins are released from intrinsic sensory neurons and play a messenger role in intramural secretory reflex pathways. All three tachykinin receptors are present in the intestinal mucosa, but show a differential distribution to submucosal neurons and epithelial cells. There is also increasing evidence that tachykinins participate in the hypersecretory states associated with intestinal infection and inflammation. The upcoming information demonstrates that the significance of tachykinins in intestinal secretion has hitherto been underestimated and predicts that their physiological and pathophysiological roles will soon be appreciated in a more comprehensive manner, and may become targets of therapeutic intervention in certain bowel diseases.

6.2. *Secretory Effects of Tachykinins in the Intestine*

6.2.1. Receptors and mediator systems involved in the *in vivo* secretory effects of tachykinins.

6.2.1.1. *Small Intestine.* Administration of SP into the lumen of the canine jejunum causes a net secretion of chloride and reduces the absorption of water and sodium, whereas the handling of potassium is left unchanged (McFadden *et al.*, 1986a). Systemic administration of SP is more effective than intraluminal application of the peptide and induces a net secretion of water, sodium, potassium and chloride into the small intestine of dogs, cats and ferrets (Zinner *et al.*, 1985; McFadden *et al.*, 1986b; Greenwood *et al.*, 1990; Brunsson *et al.*, 1995). The secretory effect of SP in the ferret is reduced by atropine (Greenwood *et al.*, 1990), and that evoked in the cat is associated with a release of VIP into the blood (Brunsson *et al.*, 1995). Since both the SP-evoked secretion and VIP release are blocked by TTX and hexamethonium, it would appear that SP stimulates an enteric reflex arc that results in the activation of secretomotor VIP neurons (Brunsson *et al.*, 1995). Histamine acting via histamine H_1 receptors and diclofenac-sensitive prostaglandin formation have been ruled out to contribute to the *in vivo* secretory effect of SP in the feline small intestine (Brunsson *et al.*, 1995). Apart from fluid and electrolyte secretion, SP is also able to elicit mucus secretion from goblet cells in the rat duodenum (Laporte *et al.*, 1993).

6.2.1.2. *Large intestine.* Secretory effects of tachykinins in the colon have been studied in the rat only. Systemic administration of tachykinins increases fecal water content,

an action that seems to be mediated by NK_2 receptors, since NK_2 receptor-selective agonists are considerably more potent in this respect than NK_3 agonists and SP and since the effect of NK_2 agonists is inhibited by the NK_2 antagonist SR-48,968 (Croci *et al.*, 1994). Infusion of NK_1 and NK_2 agonists into the rat colon reverses water absorption to a net secretion of fluid, whereas senktide is inactive (Eutamene *et al.*, 1995). The secretory responses to NK_1 and NK_2 agonists are suppressed by TTX and blockade of NO synthesis. Further analysis of the secretory effect with NK_1 and NK_2 agonists and antagonists has led to the proposal that in the rat colon, tachykinins evoke secretion by a sequential activation of NK_1 and NK_2 receptors and the formation of NO in supposedly different components of an enteric reflex arc (Eutamene *et al.*, 1995).

6.2.2. Receptors and mediator systems involved in the *in vitro* secretory effects of tachykinins

6.2.2.1. *Receptors and neurons involved in the effects of tachykinins*

6.2.2.1.1. Small intestine. Experiments with vascularly perfused segments from the rabbit ileum (Couse *et al.*, 1988; Yeo *et al.*, 1989) have shown that intraarterial infusion of SP elicits secretion of water, sodium and chloride into the lumen, an action that occurs independently of changes in the intestinal microcirculation (Yeo *et al.*, 1989). Most *in vitro* studies have been made with mucosa/submucosa sheets of the small intestine, which have been stripped of the external muscle layer and thus, no longer contain the MP. The removal of these neurons entails a considerable distortion of the mucosal innervation, given that a significant number of mucosal nerve fibers originates from myenteric neurons (Song *et al.*, 1991), and does not allow appreciation of the full impact of the enteric nervous system on intestinal secretory processes.

Application of SP and other tachykinins to the serosal, but not mucosal, surface of mucosa/submucosa preparations of the rat, murine, guinea-pig and porcine small intestine increases the short circuit current (I_{sc}), an index of electrogenic anion secretion (Walling *et al.*, 1977; Kachur *et al.*, 1982; Keast *et al.*, 1985b; Cox and Cuthbert, 1989; Brown *et al.*, 1992; Parsons *et al.*, 1992; Wang *et al.*, 1995). The change in I_{sc} is accompanied by a net secretion of sodium, chloride and bicarbonate (Walling *et al.*, 1977; Perdue *et al.*, 1987; Brown *et al.*, 1992). Data obtained in the mucosa of the guinea-pig and porcine small intestine indicate that the I_{sc} response to tachykinins is, to a large extent, mediated by enteric nerves, although a direct action of the peptides on the epithelium is also evident (Kachur *et al.*, 1982; Keast *et al.*, 1985b; Perdue *et al.*, 1987; Mathison and Davison, 1989; Brown *et al.*, 1992; Parsons *et al.*, 1992; Reddix and Cooke, 1992). The relative degree to which neurons are involved varies with the tachykinin receptor agonists and with the region of the intestine under study (Keast *et al.*, 1985b; Perdue *et al.*, 1987; Mathison and Davison, 1989; Reddix and Cooke, 1992). Since TTX blocks the SP-induced rise of I_{sc} to a larger extent than atropine or hyoscine, it can be inferred that the nerve-mediated response to SP in the mucosa of the guinea-pig small intestine depends on both cholinergic and noncholinergic secretomotor neurons (Keast *et al.*, 1985b; Perdue *et al.*, 1987; Reddix and Cooke, 1992). Cholinergic interneurons are not implicated, however, as blockade of ganglionic transmission does not affect the ability of SP to enhance I_{sc} in the guinea-pig ileum (Reddix and Cooke, 1992).

Although the receptors that mediate the tachykinin-induced rise of I_{sc} in the mucosa of the guinea-pig small intestine have not been thoroughly elucidated yet, it would appear that NK_1 receptors play a major role. Indirect evidence suggests that the nerve-mediated and direct epithelial actions of SP are brought about by different types of tachykinin receptors (Keast *et al.*, 1985b; Mathison and Davison, 1989). The study of Reddix and Cooke (1992), however, implies that both the neural and epithelial tachykinin receptors are of the NK_1 type because only NK_1, but hardly NK_2 and NK_3, agonists are able to enhance I_{sc} and because NK_1, but not NK_2, antagonists inhibit both the nerve-mediated and nonneural component of the I_{sc} response to SP. The implication of NK_1 receptors in the nerve-mediated I_{sc} effect of tachykinins is consistent with the presence of NK_1 receptors on secretomotor neurons in the MP and SMP of the guinea-pig small intestine (Burcher and Bornstein, 1988; Portbury *et al.*, 1996). As much as 42% of all submucosal neurons in the guinea-pig ileum are estimated to bear NK_1 receptors. From the chemical coding of these neurons, it would appear that NK_1 receptors are preferentially expressed by neuropeptide Y-containing (cholinergic) secretomotor neurons, whereas VIP-immunoreactive (noncholinergic) secretomotor neurons are NK_1 receptor-negative (Portbury *et al.*, 1996). NK_1 receptors are also responsible for the SP-evoked rise of I_{sc} in the mucosa of the murine ileum, which is mediated in part by enteric nerves, since the response to SP is mimicked by the NK_1 agonist SP methyl ester and abolished by the NK_1 antagonist CP-96,345 (Wang *et al.*, 1995). Furthermore, NK_1 receptors have been proposed to be responsible for the increase in I_{sc}, which in the jejunal mucosa of pigs, is brought about by serosal application of SP, NKA, NKB and tachykinin receptor-selective agonists (Parsons *et al.*, 1992). This conclusion and the implication of cholinergic secretomotor neurons in the I_{sc} effect of SP in the porcine jejunum are in keeping with the autoradiographic presence of SP binding sites in the mucosa and SMP of the porcine ileum (Parsons *et al.*, 1992).

6.2.2.1.2. Large intestine. As in the small intestine, the stimulant effects of SP and NKA on I_{sc} in the mucosa of the guinea-pig large intestine involve both cholinergic and noncholinergic secretomotor neurons (Kuwahara and Cooke, 1990). The I_{sc} response to SP and NKA is preferentially mediated by NK_1 receptors, as deduced from the rank order of agonist potencies (Kuwahara and Cooke, 1990). This inference is in keeping with the presence of SP (NK_1), but not NK_2 (NKA), binding sites in the mucosa (Burcher *et al.*, 1986) and with the expression of immunoreactive NK_1 receptors on neuropeptide Y-containing (cholinergic)

secretomotor neurons in the MP and SMP of the guinea-pig colon (Portbury *et al.*, 1996). The preponderance of NK_1 receptors differentiates the guinea-pig colon from the rat colon, in which all three tachykinin receptors act to increase I_{sc} (Cox *et al.*, 1993). The I_{sc} response to the NK_3 agonist senktide is virtually abolished by TTX, whereas the effects of NKA, which activates both NK_1 and NK_2 receptors, and SP are reduced, but not blocked, by TTX and the responses to a NK_2 agonist and neuropeptide γ are not significantly altered (Tien *et al.*, 1991; Burleigh and Yull, 1992; Cox *et al.*, 1993). It follows that in the rat colonic mucosa, NK_3 receptors predominate on submucosal neurons, NK_2 receptors exist primarily on epithelial cells, whereas NK_1 receptors are found on both neurons and epithelial cells (Cox *et al.*, 1993), which is in keeping with the presence of NK_1 receptor immunoreactivity on submucosal neurons of the rat intestine (Sternini *et al.*, 1995). Consistent with the diverse location of tachykinin receptors is the ability of SP to enhance I_{sc} even after removal of the SMP, as has been observed in the mucosa of the rat and canine colon (Rangachari *et al.*, 1990; Goerg *et al.*, 1992). The response to SP and NKA in intact mucosal preparations is reduced by TTX, but not atropine or hexamethonium (Rangachari *et al.*, 1990; Tien *et al.*, 1991). This and the observation that VIP immunoneutralization and a VIP antagonist reduce the NKA-induced rise of I_{sc} (Tien *et al.*, 1991) indicate that noncholinergic secretomotor neurons releasing VIP are responsible for the nerve-mediated rise of I_{sc} in response to tachykinin receptor stimulation.

The issue of the identity and location of the tachykinin receptors controlling secretory processes has been further delineated in the mucosa of the canine colon, in which tachykinins enhance I_{sc} in preparations with intact SMP, as well as in functionally nerve-free epithelial sheets (Rangachari *et al.*, 1990). With the use of receptor-selective agonists and antagonists, it has been possible to demonstrate that the nerve-mediated effects of tachykinins on I_{sc} involve both NK_1 and NK_3 receptors, whereas the nonneural effects are mediated by NK_1 receptors alone and NK_2 receptors are functionally absent from the mucosa of the canine colon (Crowther *et al.*, 1994). These functional data have to be seen in context with the observations that mucosal crypts of the canine colon express mRNA for both NK_1 and NK_2, but not NK_3, receptors, whereas in the SMP mRNA for NK_1 and NK_3, but not NK_2, receptors is found (Khan *et al.*, 1995). The autoradiographic demonstration of SP-preferring binding sites on submucosal neurons and epithelial cells of the canine intestinal mucosa (Mantyh, P. W. *et al.*, 1988) is in line with the functional presence of NK_1 receptors on these structures (Crowther *et al.*, 1994).

6.2.2.2. *Nonneural mediators of the secretory effects of tachykinins.*

6.2.2.2.1. Small intestine. In addition to nerves, mediator systems, such as histamine, prostaglandins, NO and adenosine, may also contribute to the secretory effects of tachykinins in the intestinal mucosa, either in their own right or in cooperation with nerves. It is important to realize, though, that the issue is complicated by regional and species differences. Histamine does play a role in the mouse, as the SP-induced, NK_1 receptor-mediated rise of I_{sc} in the mucosa of the murine ileum is significantly reduced by a combination of histamine H_1 and H_2 antagonists and blunted in mucosae taken from genetically mast cell-deficient mice (Wang *et al.*, 1995). Because the I_{sc} response to SP in mast cell-deficient animals remains unaltered by histamine antagonism, but is inhibited by TTX, while in normal mice histamine antagonism reduces the TTX-resistant part of the SP effect, it would appear that in the ileal mucosa of the mouse, enteric nerves and histamine-releasing mast cells represent two separate secretory pathways that are stimulated by SP. In contrast, the effect of SP to increase I_{sc} in the isolated mucosa of the guinea-pig small intestine remains unaltered by histamine H_1 antagonists and cyclooxygenase inhibitors (Kachur *et al.*, 1982; Keast *et al.*, 1985b; Reddix and Cooke, 1992). Prostaglandins, however, may play a role in the tachykinin-evoked rise of I_{sc} in the porcine ileum (Parsons *et al.*, 1992). Whether the secretory action of SP in the *ex vivo* perfused canine ileum is related to the release of 6-keto-prostaglandin $F_{1\alpha}$, which is seen in this tissue (Parrish *et al.*, 1994), remains to be determined.

6.2.2.2.2. Large intestine. The NKA-evoked rise of I_{sc} in the mucosa of the rat and canine colon is left unaffected by histamine H_1 receptor antagonists, whereas in the guinea-pig colonic mucosa, pyrilamine has been found to reduce the I_{sc} response to SP (Kuwahara and Cooke, 1990; Rangachari *et al.*, 1990; Tien *et al.*, 1991). The role of histamine released by SP may be to stimulate cholinergic interneurons, as deduced from the nonadditivity of the inhibitory effects of pyrilamine and mecamylamine (Kuwahara and Cooke, 1990). Prostaglandins may play a role in the tachykinin-evoked increase in I_{sc} in the mucosa of the guinea-pig and canine colon, and in the guinea-pig colonic mucosa, adenosine has also been implicated in the I_{sc} response to SP (Kuwahara and Cooke, 1990; Rangachari *et al.*, 1990). A role of NO, proposed to be a mediator of tachykinin-evoked secretion in the rat colon *in vivo* (Eutamene *et al.*, 1995), has not been tested yet *in vitro*.

6.3. RECEPTOR TRANSDUCTION MECHANISMS OF THE SECRETORY ACTIONS OF TACHYKININS

The stimulant action of SP on I_{sc} in the mucosa of the mammalian small intestine takes place without any change in the tissue levels of cyclic AMP and cyclic GMP, but is critically dependent on calcium influx, because it is inhibited by removal of external calcium or addition of verapamil (Walling *et al.*, 1977; Kachur *et al.*, 1982; Cox and Cuthbert, 1989). The secretory effect of SP in the canine ileum *in vivo* and in the rabbit ileum *in vitro* is likewise blocked by verapamil (Zinner *et al.*, 1985; Couse *et al.*, 1988). Experiments with isolated chicken enterocytes indicate that SP can act directly on epithelial cells to increase the intracel-

lular calcium ion concentration, a response that is also suppressed by removal of external calcium (Chang *et al.*, 1986). Further important factors are sodium and chloride, as removal of external sodium or chloride or addition of chloride channel blockers prevents the SP-induced rise of I_{sc} in the mucosa of the mammalian small and large intestine (Kachur *et al.*, 1982; Kuwahara and Cooke, 1990; Rangachari *et al.*, 1990; Cox *et al.*, 1993). Further analysis has demonstrated that the I_{sc} response to SP involves a variety of transepithelial ion transport and exchange mechanisms (Brown *et al.*, 1992). The inhibitory effects of furosemide and bumetanide attribute the sodium/potassium/chloride cotransport mechanism an important role in the tachykinin-evoked rise of I_{sc} (Kuwahara and Cooke, 1990; Tien *et al.*, 1991; Brown *et al.*, 1992). In contrast, amiloride-sensitive sodium/proton exchange in isolated chicken enterocytes is blocked by SP (Chang *et al.*, 1986).

6.4. *Physiological Implications of Tachykinins in Intestinal Secretion*

Morphological and functional evidence suggests that the myenteric and submucosal SP neurons, which project into the mucosa of the guinea-pig small intestine, are intrinsic sensory neurons (Bornstein and Furness, 1988; Bornstein *et al.*, 1989; Furness *et al.*, 1990; Song *et al.*, 1991, 1992; Kirchgessner *et al.*, 1992b). It has been proposed that stimulation of these neurons releases SP and NKA, not only within the enteric ganglia to activate secretomotor pathways, but also within the mucosa to directly influence epithelial function (Cooke and Reddix, 1994). A role of endogenous tachykinins in intestinal secretion indeed can be envisaged from the ability of SP desensitization, SP immunoneutralization and tachykinin antagonists to inhibit the rise of I_{sc} caused by electrical field stimulation of the guinea-pig jejunal, ileal and colonic mucosa (Keast *et al.*, 1985b; Perdue *et al.*, 1987; Kuwahara and Cooke, 1990; Reddix and Cooke, 1992). Specifically, the electrically evoked increase in I_{sc} is very closely mimicked by the action of SP and inhibited by NK_1 receptor antagonists (Perdue *et al.*, 1987; Reddix and Cooke, 1992). The nerve-mediated I_{sc} responses to neurotensin and pituitary adenylate cyclase-activating peptide in the mucosa of the guinea-pig and rat small intestine also depend on SP, as they are blunted by SP desensitization (Kachur *et al.*, 1982; Cox, 1992).

The conjecture that tachykinins are transmitters of intrinsic sensory neurons, which when released stimulate secretomotor pathways (Fig. 2), is in keeping with the proposal that SP and NKA are candidate mediators of the slow EPSP in submucosal neurons (Surprenant *et al.*, 1987; Akasu and Tokimasa, 1989; Bornstein *et al.*, 1989; Mihara and Nishi, 1994). This view is further corroborated by the observation that tachykinin-containing nerve terminals surround NK_1 receptor-bearing secretomotor neurons in the guinea-pig intestine (Portbury *et al.*, 1996). Since in this species NK_1 receptors are preferentially located on cholinergic secretomotor neurons (Portbury *et al.*, 1996), it comes as no surprise that the tachykinin-evoked rise of I_{sc} in the mucosa of the guinea-pig and porcine small and large intestine involves cholinergic (and noncholinergic) secretomotor neurons, whereas the nerve-mediated I_{sc} responses to tachykinins in the rat and canine colonic mucosa depend primarily on noncholinergic VIP secretomotor neurons (Keast *et al.*, 1985b; Perdue *et al.*, 1987; Kuwahara and Cooke, 1990; Rangachari *et al.*, 1990; Tien *et al.*, 1991; Brown *et al.*, 1992; Reddix and Cooke, 1992). If tachykinins are considered to be messengers of intrinsic sensory neurons being part of secretory reflex pathways, it is likely that elucidation of their physiological role in mucosa/submucosa preparations is potentially difficult because the myenteric sensory neurons (Song *et al.*, 1991) have been disrupted and electrical field stimulation may preferentially stimulate submucosal secretomotor neurons, unless a broad range of stimulation parameters is tested. In view of these limitations, it has to be borne in mind that the inability of SP desensitization or tachykinin antagonists to blunt electrically evoked I_{sc} responses in the porcine jejunum, rabbit ileum, rat and human colon (Hubel, 1984; Hubel *et al.*, 1987; Hildebrand and Brown, 1990; Burleigh and Yull, 1992) does not necessarily rule out a physiological role of tachykinins in intact secretory reflex arcs. Tachykinins, though, do not seem to participate in the I_{sc} responses to distension of the rat rectal mucosa (Schulzke *et al.*, 1995).

Strong support for the concept that SP and NKA are messengers of intrinsic sensory neurons that are part of enteric secretomotor reflex arcs (Fig. 2) comes from *in vivo* studies in the extrinsically denervated feline small intestine. Serosal application of 0.1 M HCl activates an intra-

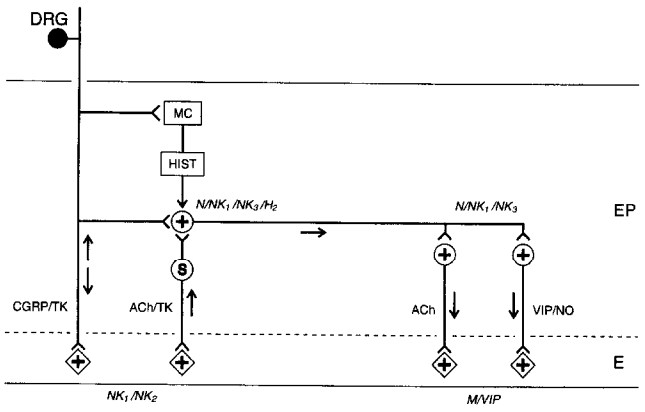

Figure 2. Schematic diagram of tachykinin neurons and tachykinin receptors that have been proposed to participate in secretomotor pathways of the mammalian gut. Neuronal somata are depicted by circles, epithelial cells by diamonds. Receptors are set in *italics*. Note that the direct secretory effects of tachykinins on epithelial cells are mediated by NK_1 receptors in the guinea-pig and dog, and by both NK_1 and NK_2 receptors in the rat. The tachykinin receptors on enteric neurons participating in secretory control are of the NK_1 type in the guinea-pig and of both the NK_1 and NK_3 type in the rat and dog. Histamine (HIST) released from mast cells (MC) activates enteric neurons via histamine H_2 receptors. +, excitation; DRG, dorsal root ganglion; E, epithelium; EP, enteric nerve plexuses; M, muscarinic acetylcholine receptor; N, nicotinic acetylcholine receptor; S, enteric sensory neuron; TK, tachykinin.

mural reflex that results in net secretion of fluid into the jejunal lumen (Brunsson *et al.*, 1990). This secretory reflex is accompanied by a release of NKA and VIP into the blood. Since the release of VIP and fluid secretion is inhibited by hexamethonium, while the release of NKA remains unaltered, it has been concluded that HCl stimulates sensory tachykinin neurons, which in turn activate cholinergic interneurons and VIP secretomotor neurons (Brunsson *et al.*, 1990). This concept has been substantiated by the observation that intraarterial administration of SP itself induces the same VIP-mediated secretory reflex in the feline small intestine (Brunsson *et al.*, 1995). There is also evidence that chemical and mechanical stimuli applied to the lumen of the guinea-pig small intestine activate sensory tachykinin neurons (Fig. 3), a process in which 5-HT released from mucosal endocrine cells plays an important role (Kirchgessner *et al.*, 1992b; Li and Owyang, 1996).

Apart from secretomotor neurons, mediator systems, such as histamine, prostaglandins and NO, may also contribute to the secretory effects of tachykinins in the intestinal mucosa (Fig. 2), but their physiological implication has not been firmly established yet. A potential role of histamine released from mast cells can be envisaged from the juxtaposition of mast cells and SP-containing nerve fibers in the gastrointestinal mucosa (Stead *et al.*, 1987). Histamine has been found to contribute to the stimulant I_{sc} effects of tachykinins in the murine small intestine and guinea-pig colon (Kuwahara and Cooke, 1990; Wang *et al.*, 1995), while in other regions or species, no evidence for a participation of this autacoid in the secretory responses to tachykinins has been obtained. Although not systematically examined, it has to be borne in mind that there are species differences in the responsiveness of intestinal mast cells to SP. While mast cells from the rat intestinal mucosa are responsive to SP, mast cells isolated from the human colon are not (Shanahan *et al.*, 1985; Lowman *et al.*, 1988; Rees *et al.*, 1988; Marshall *et al.*, 1994).

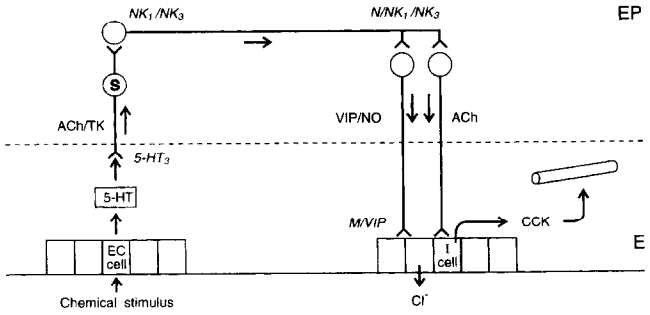

Figure 3. Schematic diagram of secretomotor reflexes that are triggered by chemical stimuli that act on enterochromaffin cells to release 5-HT. This amine, in turn, stimulates enteric sensory neurons that release tachykinins and thereby initiates reflexes that cause secretion of anions into the lumen (Kirchgessner *et al.*, 1992b) or of CCK-releasing peptide and, further on, CCK into the bloodstream (Li and Owyang, 1996). Neuronal somata are depicted by circles, receptors are set in *italics*. E, epithelium; EC cell, enterochromaffin cell; EP, enteric nerve plexuses; I cell, CCK-secreting cell; M, muscarinic acetylcholine receptor; N, nicotinic acetylcholine receptor; S, enteric sensory neuron; TK, tachykinin.

Another group of SP-containing nerve fibers that could influence intestinal secretion are those of extrinsic afferent neurons (Fig. 2). Stimulation of these fibers by capsaicin is known to cause a local release of SP and NKA, as has been summarized in the companion article (Holzer and Holzer-Petsche, 1997), and to depolarize submucosal neurons in the guinea-pig ileum and to produce a chloride-dependent increase in I_{sc} in the guinea-pig ileal and rat colonic mucosa (Yarrow *et al.*, 1991; Vanner and MacNaughton, 1995). An implication of tachykinins has directly been envisaged from the observation that the capsaicin-evoked increase in I_{sc} in the rat colonic mucosa is suppressed by desensitization of the tissue to SP (Yarrow *et al.*, 1991). CGRP, which is co-released from extrinsic afferent nerve fibers, has been found to synergize with SP and NKB to raise I_{sc} in the guinea-pig jejunal mucosa (Mathison and Davison, 1989). Tachykinins, in addition, may have a bearing on the secretion of bicarbonate, which in the rat and feline duodenum, is induced by acidification of the lumen. The acid-induced output of bicarbonate involves capsaicin-sensitive extrinsic afferents and is associated with a release of SP and NKA into the duodenal lumen (Takeuchi *et al.*, 1991a; Hamlet *et al.*, 1992; Smedfors *et al.*, 1994). Indirect evidence suggests that tachykinins may also participate in the effect of sensory nerve stimulation to facilitate mucin output in the rat duodenum (Laporte *et al.*, 1993).

Whether tachykinin-containing endocrine cells in the intestinal mucosa contribute to secretory regulation is not known. It is worth considering in this context that enterochromaffin cells can serve as mucosal detectors of chemical and mechanical stimuli in the intestinal lumen (Fig. 3) and, by releasing 5-HT, activate intrinsic SP/calbindin-immunoreactive sensory neurons and thereby initiate secretory reflexes (Kirchgessner *et al.*, 1992b; Cooke and Reddix, 1994). A similar reflex pathway appears to be responsible for the peptone-evoked release of cholecystokinin (CCK)-releasing peptide in the rat duodenum (Li and Owyang, 1996). The peptone-induced release of CCK-releasing peptide is abolished by antagonism of 5-HT_2 and 5-HT_3 or NK_1 receptors, which together with other observations suggests that peptone stimulates enteric sensory neurons via mucosal 5-HT release (Fig. 3). The tachykininergic sensory neurons transmit to cholinergic interneurons and cholinergic secretomotor neurons, which, in turn, activate epithelial cells to secrete CCK-releasing peptide. This type of enteric circuitry may play an important role in the postprandial release of CCK (Li and Owyang, 1996).

7. TACHYKININS AND HEPATOBILIARY SECRETION

7.1. *Overview*

SP and NKA reduce basal and hormone-stimulated bile output, but the physiological significance of this anticholeretic action is unknown.

7.2. *Anticholeretic Effects of Tachykinins*

SP attenuates basal and hormone-stimulated bile output, an effect that has been studied only in the dog. Under basal conditions, the overall flow of bile and the output of bile acids, sodium, potassium, chloride, bicarbonate and amylase is reduced in response to SP (Starke *et al.*, 1968; Holm *et al.*, 1978; Magnusson, 1984). When choleresis is induced by CCK, infusion of SP dose-dependently attenuates the ability of the hormone to stimulate the output of bile, bile acids and electrolytes (Holm *et al.*, 1978; Magnusson, 1984). A similar anticholeretic effect of SP is seen when bile output is stimulated by VIP (Holm *et al.*, 1978), whereas the secretin-induced choleresis is less affected by SP (Magnusson, 1984). The characteristics of the anticholeretic action of SP in terms of changes in bile acid and electrolyte concentrations have been taken to infer that SP influences the canalicular bile acid-independent secretion by a direct action on canine hepatocytes (Holm *et al.*, 1978; Magnusson, 1984). Consistent with this proposition is the observation that in cultures of rat hepatocytes, SP fails to alter the uptake and release of bile acids (Shimizu *et al.*, 1987). However, the precise site, mechanism and relevance of SP's anticholeretic action are not known, and the type of tachykinin receptors mediating this action remains to be identified.

8. TACHYKININS AND PANCREATIC SECRETION

8.1. *Overview*

Tachykinins have been found to facilitate exocrine secretion from pancreatic acini, an action that in the guinea-pig, seems to be brought about by NK_1 receptors. The physiological role of this action and of the influence of tachykinins on endocrine pancreatic secretion has not been elucidated yet.

8.2. *Effects of Tachykinins on Exocrine Pancreatic Secretion*

The overall response of the exocrine pancreas to tachykinins is a weak increase in secretory activity, as compared with the responses to secretin or CCK (Konturek *et al.*, 1981). SP, NKA and NKB all enhance the basal output of pancreatic juice, amylase and bicarbonate in dogs, as does SP in rats (Starke *et al.*, 1968; Thulin and Holm, 1977; Konturek *et al.*, 1981; Katoh *et al.*, 1984; Pawlik *et al.*, 1992). A more detailed analysis has revealed that the two main secretory epithelia of the exocrine pancreas, the duct and acinar cells, may respond to tachykinins with opposite reactions. In isolated duct cells from the rat pancreas, SP fails to alter the membrane potential, but is able to inhibit basal fluid secretion, as well as fluid secretion stimulated by secretin, bombesin or forskolin, which suggests that SP inhibits secretion by a site of action distal to the generation of cyclic AMP (Ashton *et al.*, 1990; Pahl and Novak, 1993). In contrast, acinar cells of the guinea-pig, rat and murine pancreas are stimulated by tachykinins to secrete amylase and calcium (Jensen and Gardner, 1979; Sjödin *et al.*, 1980, 1984; Jensen *et al.*, 1984; Katoh *et al.*, 1984; Singh, 1985; Krims and Pandol, 1988; Pawlik *et al.*, 1992). Since the ability of SP to augment exocrine secretion from the rat, but not canine, pancreas is left unaltered by atropine (Katoh *et al.*, 1984; Pawlik *et al.*, 1992) and the rat pancreatic acinar AR42J cell line expresses NK_1 receptor mRNA, displays a high density of SP binding sites and releases amylase in response to SP (Womack *et al.*, 1985; Ihara and Nakanishi, 1990), it would seem that in the rat pancreas, SP acts directly on pancreatic acinar cells to elicit exocrine secretion. The same appears to be true for the SP-evoked release of amylase and calcium from guinea-pig pancreatic acinar cells, because these cells are endowed with many binding sites for SP (Jensen and Gardner, 1979; Sjödin *et al.*, 1980, 1984, 1994; Jensen *et al.*, 1984; Krims and Pandol, 1988; Sjödin and Gylfe, 1992). CP-96,345 displaces SP binding to guinea-pig pancreatic acinar cells and inhibits the amylase output induced by SP, which indicates that tachykinin-stimulated exocrine secretion in the guinea-pig pancreas is mediated by NK_1 receptors (Sjödin and Gylfe, 1992).

The transduction mechanisms underlying SP-stimulated pancreatic exocrine secretion have been studied in guinea-pig and rat AR42J pancreatic acinar cells. Although the cellular levels of cyclic GMP rise in response to SP, it appears as if the primary transduction mechanism operated by SP is the formation of inositol trisphosphate and diacylglycerol (Jensen and Gardner, 1979; Sjödin *et al.*, 1980; Vesely, 1985; Horstman *et al.*, 1988; Song *et al.*, 1988; Gallacher *et al.*, 1990; Sjödin *et al.*, 1991). The SP-evoked increase in the intracellular concentration of calcium ions is blocked by drugs that prevent activation of protein kinase C by diacylglycerol (Womack *et al.*, 1985; Horstman *et al.*, 1988; Krims and Pandol, 1988; Song *et al.*, 1988; Gallacher *et al.*, 1990; Sjödin *et al.*, 1991, 1994; Sjödin and Gylfe, 1992). In its initial phase, the rise of the intracellular calcium ion concentration is independent of extracellular calcium, while the following sustained phase of the response requires influx of calcium (Krims and Pandol, 1988; Horstman *et al.*, 1988; Gallacher *et al.*, 1990; Sjödin *et al.*, 1991). Thus, the sequence of events appears to be intracellular calcium mobilization by inositol trisphosphate, diacylglycerol-initiated opening of calcium and sodium channels, cell membrane depolarization, and release of amylase (Krims and Pandol, 1988; Song *et al.*, 1988; Gallacher *et al.*, 1990).

The observation that tachykinins can exert divergent effects on duct and acinar cells of the exocrine pancreas is likely to explain why the secretory effects of CCK (preferentially mediated by acinar cells) and secretin (primarily brought about by duct cells) may be differently affected by tachykinins. Whereas the secretin-evoked secretion of juice and bicarbonate is reduced by tachykinins, the CCK-induced output of juice and amylase from the rat pancreas and from murine and guinea-pig pancreatic acini is facilitated by SP (Katoh *et al.*, 1984; Sjödin *et al.*, 1984; Pawlik *et al.*, 1992). The synergism between CCK and SP may be due to the ability of CCK to prevent endocytosis of SP receptors, as

observed in guinea-pig pancreatic acinar cells (Sjödin, 1992). While the secretory response of the isolated blood-perfused canine pancreas to CCK is also enhanced and that to secretin inhibited by SP (Iwatsuki *et al.*, 1986), there are discrepant reports concerning the effects of tachykinins on the hormone-stimulated exocrine pancreas of dogs *in vivo*. While in conscious dogs the secretory responses to secretin, the CCK-related peptide caerulein, feeding and duodenal acidification are inhibited by SP (Konturek *et al.*, 1981), SP, NKA and NKB augment the secretin-induced pancreatic exocrine secretion and hyperemia in anesthetized dogs (Pawlik *et al.*, 1992).

8.3. *Effects of Tachykinins on Endocrine Pancreatic Secretion*

SP and the NKA-related nonmammalian tachykinin kassinin have been found to influence the pancreatic release of insulin, glucagon and somatostatin and pancreatic polypeptide. Both in the dog *in vivo* and in the perfused canine pancreas *in vitro*, SP is potent in stimulating the release of insulin, glucagon and somatostatin, whereas plasma levels of pancreatic polypeptide remain unchanged (Kaneto *et al.*, 1978; Hermansen, 1980; Modlin *et al.*, 1981; Chiba *et al.*, 1985). In the presence of high glucose levels, the SP-evoked release of insulin and somatostatin is enhanced, while that of glucagon is reduced (Hermansen, 1980). In conscious calves, SP has been found to release pancreatic polypeptide, a response that is abolished by high glucose levels, whereas the release of insulin remains unchanged and the output of glucagon is elevated only if SP is infused together with ACh (Edwards and Bloom, 1994). The prevailing action of tachykinins in the endocrine pancreas of the rat and mouse *in vivo* and *in vitro* seems to be inhibition of basal, glucose- and arginine-stimulated release of insulin, effects that are associated with hyperglycemia (Brown and Vale, 1976; Efendić *et al.*, 1977; Lundquist *et al.*, 1979; Gullner *et al.*, 1982; Chiba *et al.*, 1985). The situation is obscured, however, by the findings that the release of insulin from isolated cultured rat pancreatic islets is stimulated by SP and the plasma level of insulin in the rat is increased by kassinin (Gullner *et al.*, 1982; Fu and Sun, 1989). Divergent reports also exist with regard to glucagon, whose secretion in the rat may be left unaltered by SP, reduced by SP or increased by high doses of SP and kassinin (Brown and Vale, 1976; Efendić *et al.*, 1977; Gullner *et al.*, 1982; Chiba *et al.*, 1985). A similar discrepancy relates to the release of somatostatin from the rat pancreas, which has been found to be enhanced or unchanged by SP (Saito and Saito, 1980; Chiba *et al.*, 1985).

8.4. *Physiological Implications of Tachykinins in the Pancreas*

The physiological implications of tachykinins in the pancreas are largely unknown. Ablation of primary afferent neurons in the mouse with a neurotoxic dose of capsaicin has been reported to reduce the 2-deoxy-D-glucose-evoked release of glucagon and related hyperglycemia, to amplify the glucose-evoked release of insulin and to enhance glucose elimination (Karlsson *et al.*, 1992, 1994). In agreement with the ability of SP to inhibit insulin secretion in the mouse (Lundquist *et al.*, 1979), the data obtained with capsaicin pretreatment imply that SP released from afferent nerve endings within the pancreas may have an inhibitory influence on insulin secretion and may reduce glucose tolerance in the mouse (Karlsson *et al.*, 1994).

9. TACHYKININS AND GASTROINTESTINAL CIRCULATION

9.1. *Overview*

Since its discovery, SP has been known to be a vasoactive peptide causing vasodilatation and hypotension. In the gastrointestinal tract, both vasodilatation and vasoconstriction may be induced by tachykinins, and the actions of these peptides are critically dependent on the vascular bed and species under study. The arterial system of the human, porcine, canine, rabbit and guinea-pig gut is dilated by tachykinins via activation of NK_1 receptors, whereas arterial dilatation is absent in the rat stomach. The venous system of the rat, rabbit and canine gut is constricted by tachykinins, an action in which NK_3 receptors may be important. SP and NKA, in addition, may increase vascular permeability in the splanchnic venous system. Although the physiological implications of tachykinins in the vascular system of the gut have not been fully elucidated yet, their spectrum of actions points to a pathophysiological role in gastrointestinal inflammation.

9.2. *Vasodilator Effects of Tachykinins*

9.2.1. Effects on mesenteric arterial blood flow. The dense innervation of mesenteric arteries by SP-containing nerve fibers has invited many studies into the vascular effects of SP and related peptides on the superior mesenteric artery. Intravenous or close arterial administration of SP increases blood flow through the superior mesenteric artery of the dog and pig (Hallberg and Pernow, 1975; Melchiorri *et al.*, 1977; Schrauwen and Houvenaghel, 1980; Rózsa *et al.*, 1984, 1985; Prokopiw and McDonald, 1994). This effect signifies vascular dilatation, which is also seen in the celiac and hepatic artery of the dog (Withrington, 1992; Prokopiw and McDonald, 1994). The potency rank order of SP, NKA and NKB is indicative of NK_1 receptors mediating the dilator action of tachykinins in the canine mesenteric circulation (Prokopiw and McDonald, 1994). These *in vivo* studies have been extended by *in vitro* work showing that the tachykinin-evoked dilatation of precontracted mesenteric arteries is brought about by NK_1 receptors on endothelial cells and involves formation of NO as vasodilator messenger. Thus, the SP-induced relaxation of isolated mesenteric arteries from the rabbit depends on the presence of the endothelium and, as judged by the relative potencies of SP, NKA and NKB, is mediated by NK_1 receptors (Zawadzki *et al.*, 1981; Stewart-Lee and Burnstock, 1989).

Similar results have been obtained in the precontracted mesenteric arterial bed of the guinea-pig, in which relaxation is caused by selective NK_1 agonists, whereas NK_2 and NK_3 agonists are virtually inactive (Berthiaume *et al.*, 1995). The SP-evoked dilatation of the guinea-pig superior mesenteric artery is blocked by the NK_1 receptor antagonist FK-888, and the dilator responses to both SP and a NK_1 agonist depend on the formation of NO and the presence of endothelium (Bolton and Clapp, 1986; Berthiaume *et al.*, 1995; Matsuda *et al.*, 1995). Human mesenteric artery preparations likewise are dilated by SP (Törnebrandt *et al.*, 1987), while isolated bovine mesenteric arteries are only weakly relaxed by this tachykinin (Axelsson *et al.*, 1989). In contrast, neither SP, NKA and NKB nor selective NK_1, NK_2 and NK_3 agonists are able to dilate the precontracted mesenteric arterial bed of the rat (Barja *et al.*, 1983; Kawasaki *et al.*, 1988; D'Orléans-Juste *et al.*, 1991; Claing *et al.*, 1992; Li and Duckles, 1992). This *in vitro* observation is in keeping with the inability of SP to increase mesenteric arterial blood flow *in vivo* (Prokopiw and McDonald, 1994). One study with isolated rings of rat mesenteric arteries has shown, however, that senktide relaxes the precontracted vascular smooth muscle (Mizuta *et al.*, 1995). Since the dilator response to senktide is inhibited by the NK_3 antagonist SR-142,801 and a blocker of NO synthase, it would appear that some NK_3 receptors are situated on the endothelium of the rat mesenteric arterial bed (Mizuta *et al.*, 1995).

9.2.2. Effects on gastrointestinal blood flow. When compared with the effects on the mesenteric arterial system, it is not unexpected to see that the actions of tachykinins on blood flow within the gut wall are also subject to species differences. Intravenous or intraarterial administration of SP to the dog increases blood flow in the stomach and small intestine (Burcher *et al.*, 1977; Yeo *et al.*, 1984; Ito *et al.*, 1993). The gastric hyperemia in response to tachykinins is attenuated by atropine, but remains unaltered by vagotomy or splanchnicotomy (Ito *et al.*, 1993). A comparison of the potencies of SP, NKA and NKB indicates that the vasodilator responses to tachykinins in the canine stomach are brought about by activation of NK_1 receptors (Ito *et al.*, 1993). In keeping with this inference, submucosal arterioles and venules of the canine and human gastrointestinal tract express a low concentration of SP (NK_1) binding sites, which are found on both the endothelium and vascular smooth muscle (Gates *et al.*, 1988; Mantyh, P. W. *et al.*, 1988). Some NKA (NK_2) binding sites are also present on the endothelium and smooth muscle of arterioles and venules in the canine, but not human, gastrointestinal tract, whereas autoradiographically demonstrable NKB (NK_3) binding sites are completely absent (Gates *et al.*, 1988; Mantyh, P. W. *et al.*, 1988). Intraluminal application of SP to the feline jejunum enhances blood flow preferentially in the mucosa and submucosa, whereas muscular blood flow is augmented by high doses of SP only (Yeo *et al.*, 1982; Grönstad *et al.*, 1986). TTX, lidocaine, hexamethonium, atropine, α/β-adrenoceptor blockers, histamine H_1/H_2 antagonists and indomethacin fail to influence the vasodilator effect of SP (Grönstad *et al.*, 1986). Similarly, close arterial infusion of SP, NKA, NKB or NPK to the feline colon causes hyperemia that is largely independent of nerve conduction and cholinergic transmission (Hellström *et al.*, 1991). Since NKA is the most potent peptide, it is conceivable that the hyperemic effect of tachykinins in the feline colon is mediated by NK_2 receptors (Hellström *et al.*, 1991).

The action of tachykinins on submucosal arterioles has been studied in the guinea-pig gut. Precontracted arterioles of the small intestine and colon are relaxed by SP (Galligan *et al.*, 1990; Vanner and Surprenant, 1991). The SP-evoked arteriolar dilatation in the small intestine is left unchanged by TTX, blockade of muscarinic acetylcholine receptors, extrinsic denervation of the intestine or pretreatment with a neurotoxic dose of capsaicin (Galligan *et al.*, 1990; Jiang and Surprenant, 1992; Vanner, 1993). As CP-96,345 prevents SP from dilating submucosal arterioles in the guinea-pig ileum, it follows that the tachykinin's action is brought about by NK_1 receptors (Vanner, 1994). This inference is consistent with the presence of a small number of NK_1 binding sites on large, but not small, blood vessels of the guinea-pig ileum (Burcher and Bornstein, 1988).

Compared with the gastrointestinal circulation of other species, the vascular systems of the rat stomach and rabbit esophagus are notable exceptions, inasmuch as they do not dilate in response to SP and NKA (Yokotani and Fujiwara, 1985; Holzer and Guth, 1991; Katori *et al.*, 1993; Grønbech and Lacy, 1994; Holzer *et al.*, 1994; McKie *et al.*, 1994). To the contrary, SP has been found to reduce rat gastric mucosal blood flow stimulated by bethanechol, vagal nerve stimulation, capsaicin-evoked sensory nerve stimulation or acid challenge of the mucosa (Yokotani and Fujiwara, 1985; Grønbech and Lacy, 1994; Heinemann *et al.*, 1996). The inhibitory influence of SP on the capsaicin-induced hyperemia is prevented by ketotifen and aprotinin and thus, seems to involve release of mast cell proteases (Grønbech and Lacy, 1994) that degrade CGRP, the vasodilator peptide released by capsaicin (Li *et al.*, 1991).

9.2.3. Effects on blood flow in the salivary glands. In view of their perivascular location, it is conceivable that SP-releasing afferent nerve fibers influence the circulation of the salivary glands. In agreement with this conjecture, SP is able to increase blood flow in the salivary glands of the rat and ferret (Larsson and Olgart, 1989; Ueha *et al.*, 1989; Tobin *et al.*, 1991), and does so even in those species in which it fails to evoke salivation. Thus, the peptide causes an atropine-resistant increase in blood flow through the rabbit submandibular gland and relaxes precontracted arteries isolated from the human submandibular gland (Larsson *et al.*, 1986; Fazekas *et al.*, 1987). The hyperemia, which in the ferret submandibular glands is evoked by parasympathetic nerve stimulation remains unaltered by antagonism of tachykinin receptors (Tobin *et al.*, 1991).

9.3. *Vasoconstrictor Effects of Tachykinins*

With the exception of the rat stomach, vasoconstrictor effects of tachykinins in the gut appear to be restricted to the mesenteric and portal veins. In the *in vivo* rat stomach, though, SP has been found to constrict collecting venules of the mucosa in a leukotriene C_4-independent manner (Katori *et al.*, 1993), which is consistent with the ability of NKA and related analogs to enhance resistance in the vascular bed of the gastric mucosa (Heinemann *et al.*, 1996; Stroff *et al.*, 1996). Further analysis of the gastric vasoconstrictor effect of NKA *in vivo* indicates that it is mediated by NK_1 receptors (Heinemann *et al.*, 1996). Tachykinin-evoked constriction *in vitro* is seen in the perfused rat mesenteric venous bed in which only NKB and selective NK_3 agonists are active, whereas SP, NKA and selective NK_1 and NK_2 agonists are devoid of any effect (D'Orléans-Juste *et al.*, 1991; Claing *et al.*, 1992). It is obvious, therefore, that intravascular tachykinins constrict rat mesenteric veins by activation of NK_3 receptors, an inference that is further supported by the ability of an NK_3 antagonist to inhibit the constrictor response to a NK_3 agonist (D'Orléans-Juste *et al.*, 1991; Claing *et al.*, 1992). The presence of NK_3 receptors in mesenteric veins extends to the rat portal vein, which is most potently contracted by NKB, whereas NKA and SP are less active and which, for this reason, has been used as a NK_3 receptor bioassay (Mathison, 1983; Mastrangelo *et al.*, 1987; Regoli *et al.*, 1994). The *in vitro* constrictor action of tachykinins on the gastrointestinal venous system is complemented by the *in vivo* ability of SP to reduce blood flow in rat mesenteric venules and in the canine portal vein (Zimmerman *et al.*, 1991; Withrington, 1992). Isolated mesenteric veins of the rabbit likewise are contracted by SP (Regoli *et al.*, 1984), whereas the mesenteric venous bed of the guinea-pig appears to be insensitive to NK_1, NK_2 and NK_3 agonists (Berthiaume *et al.*, 1995).

9.4. *Effects of Tachykinins on Vascular Permeability*

A prominent action of SP in many vascular beds outside the digestive system is a marked increase in venular permeability, a reaction that leads to the extravasation of macromolecules, leukocytes and fluid (Holzer, 1988, 1992). The influence of tachykinins on vascular permeability in the digestive system, however, has been investigated less thoroughly, and the tachykinin receptors controlling venular permeability in the gut require further characterization. One laboratory holds that while plasma protein leakage in the esophagus, anal mucosa, biliary system and mesenteric vasculature of the rat is markedly enhanced by intravenously administered SP, vascular permeability in the stomach and small and large intestine is not appreciably altered (Saria *et al.*, 1983; Lundberg *et al.*, 1984). Although the inactivity of intravenous SP in the rat stomach and intestine has been confirmed by Lördal *et al.* (1996), another study shows that SP does increase vascular permeability in the rat stomach, duodenum, ileum and pancreas (Nicolau *et al.*, 1993). Further experiments with SP, NKA, NKB, NK_2 and NK_3 receptor-selective agonists and NK_1/NK_2 receptor-selective antagonists suggest that all three tachykinin receptors control vascular permeability in the rat gut in a regionally distinct manner (Nicolau *et al.*, 1993; Lördal *et al.*, 1996). The action of SP to evoke plasma protein extravasation in the duodenum and pancreas is mediated by NK_1 receptors, while that in the ileum seems to result from stimulation of NK_3 receptors, and both NK_2 and NK_3 receptors might be responsible for the extravasation response in the stomach (Nicolau *et al.*, 1993). The conclusion that NK_2 and NK_3 receptors play a role in tachykinin-enhanced vascular permeability of the rat gut is corroborated by the findings that intravenous NKA induces plasma protein extravasation in the stomach, duodenum, jejunum, caecum and colon, but has no effect in the ileum whereas NKB is active in the stomach only (Lördal *et al.*, 1996).

SP and NKA likewise are able to increase protein leakage in the small intestine of the mouse, an action that is predominantly brought about by stimulation of NK_1 receptors, as it is inhibited by RP-67,580 or FK-888 (Kraneveld *et al.*, 1995). Analogous experiments with frog mesenteric venular capillaries indicate that the SP-evoked increase in venular permeability is brought about by activation of NK_1 receptors that are blocked by CP-96,345 and subsequent formation of NO (Nguyen *et al.*, 1995).

The extravasation that SP causes in the esophagus, biliary system, anal mucosa and mesenteric vasculature remains unaltered by pretreatment of rats with a neurotoxic dose of capsaicin or by blockade of histamine H_1/H_2 receptors (Saria *et al.*, 1983; Lundberg *et al.*, 1984). Histamine, therefore, does not seem to contribute to the ability of SP to enhance vascular permeability, although in the rat mesentery, a close proximity of SP-containing nerve fibers and mast cells has been noted (Skofitsch *et al.*, 1985; Crivellato *et al.*, 1991). In contrast, the NK_1 receptor-mediated increase in vascular permeability that SP causes in the rat salivary glands does involve release of histamine acting via histamine H_1 receptors (Fazekas *et al.*, 1992; Damas, 1995).

It remains to be determined whether the increase in vascular permeability has a bearing on the action of SP to enhance adhesion of leukocytes to the endothelium of rat mesenteric venules (Zimmerman *et al.*, 1991) and to promote granulocyte infiltration in experimental inflammation of the rat ileum and colon (McCafferty *et al.*, 1994; Pothoulakis *et al.*, 1994).

9.5. *Physiological Roles of Tachykinins on the Vascular System of the Gut*

Despite the dense innervation of the rat superior mesenteric artery by SP-containing nerve fibers, there is little evidence that tachykinins are mediators of the NANC dilatation of this vessel, which is induced by perivascular nerve stimulation once sympathetic neurotransmission has been blocked. While CGRP is clearly involved (Holzer, 1992), NANC vasodilatation in the rat and guinea-pig superior mesenteric artery is left unchanged by NK_1 receptor antago-

nists, although SP is released from rat mesenteric arteries and veins in response to afferent nerve stimulation with capsaicin (Manzini *et al.*, 1991; Claing *et al.*, 1992; Matsuda *et al.*, 1995). In contrast, the electrically evoked NANC constriction of rat mesenteric veins is reduced by the NK_1 antagonist CP-96,345, but not by an NK_3 antagonist (Claing *et al.*, 1992), which contrasts with the NK_3 receptor-mediated constriction caused by intravascularly applied tachykinins (D'Orléans-Juste *et al.*, 1991). In explaining this discrepancy, Claing *et al.* (1992) proposed that in the rat mesenteric veins, NK_3 receptors are located on the endothelium, while NK_1 receptors are present on the muscle. If so, intravascularly applied agonists would easily reach the endothelial NK_3, but not the muscular NK_1 receptors, whereas the reverse would be true for SP and NKA released from perivascular nerve fibers.

The absence of a dilator role of tachykinins in the rat mesenteric arteries *in vitro* is complemented by similar negative data obtained *in vivo*. Thus, SP immunoneutralization fails to affect the reflex mesenteric hyperemia that is induced by intrajejunal administration of bile-oleate (Rózsa and Jacobson, 1989). In contrast, the constrictor and dilator responses of the rat superior mesenteric artery to intestinal warming are reduced by an antiserum to SP (Rózsa *et al.*, 1988), but the role that SP plays in these complex vascular reflexes is not understood yet. Capsaicin-induced stimulation of perivascular nerve fibers in the rat stomach does release SP (Kwok and McIntosh, 1990), yet there is no evidence for tachykinins contributing to the gastric hyperemia induced by capsaicin or acid challenge of the mucosa, since tachykinin antagonists fail to prevent these nerve-mediated vasodilator responses (Matsumoto *et al.*, 1991; Holzer *et al.*, 1994; Heinemann *et al.*, 1996; Stroff *et al.*, 1996). The NK_1 antagonist CP-96,345 is similarly ineffective in blocking the sensory nerve-mediated hyperemia, which in the rabbit esophagus, is caused by deoxycholate (McKie *et al.*, 1994). There is information, however, that in the dog, tachykinins participate in nerve-mediated dilatation of the upper gastrointestinal tract. Thus, combined administration of antibodies to SP, VIP and CCK suppresses the mesenteric vasodilator effect that is induced by sensory nerve stimulation with capsaicin, the remaining vasodilatation being abolished by atropine (Rózsa *et al.*, 1985). Similarly, the NANC vasodilatation induced by splanchnic nerve stimulation in the canine stomach is reduced by a tachykinin antagonist (Ito *et al.*, 1993).

There is good evidence that SP is a functional transmitter of extrinsic afferent nerve fibers in submucosal arterioles of the guinea-pig small intestine. Stimulation of these fibers by capsaicin induces a nerve-mediated noncholinergic dilatation of precontracted arterioles, which in part is inhibited by CP-96,345 and a CGRP antagonist, while combination of the two antagonists abolishes the dilator response (Vanner, 1993, 1994). In addition, SP appears to be a vasodilator transmitter of intrinsic submucosal neurons innervating the submucosal arterioles of the guinea-pig colon, since a tachykinin antagonist attenuates the vasodilator response to electrical stimulation of the SMP (Vanner and Surprenant, 1991). In the guinea-pig small intestine, a similar role of enteric SP becomes apparent only after chronic extrinsic denervation of the gut (Galligan *et al.*, 1990; Jiang and Surprenant, 1992). While in the normal small intestine nerve-mediated arteriolar dilatation is exclusively cholinergic (Neild *et al.*, 1990), nerve-mediated relaxation of arterioles in the extrinsically denervated intestine is partially resistant to blockade of cholinergic transmission, the residual response being abolished by a tachykinin antagonist (Galligan *et al.*, 1990; Jiang and Surprenant, 1992). The appearance of tachykininergic vasodilatation is associated with the appearance of perivascular SP-containing nerve fibers that originate from the MP (Galligan *et al.*, 1990; Jiang and Surprenant, 1992). In the cat, it seems conceivable that the hyperemia that in the rectoanal region, is induced by mechanical stimulation of the anus or distension of the rectum depends on NKA and SP, which are released into the bloodstream by these maneuvres (Hellström *et al.*, 1991).

There is circumstantial evidence to infer that tachykinins may be mediators of the increase in vascular permeability, which in the esophagus, stomach, duodenum, biliary system, rectoanal mucosa and mesenteric vasculature of the rat is induced by sensory nerve stimulation with capsaicin or electrical stimulation of the vagus and splanchnic nerves (Saria *et al.*, 1983; Lundberg *et al.*, 1984; Lördal *et al.*, 1996). This inference, though, is based solely on the remarkable parallelism of the extravasation responses to SP or NKA and nerve stimulation, SP or NKA being appreciably active in only those gastrointestinal tissues in which nerve stimulation by electrical impulses or intravenous capsaicin is also active.

10. PATHOPHYSIOLOGICAL IMPLICATIONS OF TACHYKININS IN THE GUT AND THERAPEUTIC PERSPECTIVES

10.1. *Overview*

Gastrointestinal disorders of various etiology, particularly those due to infection or inflammation, are associated with changes in the tachykininergic innervation of the gut, and inflammatory bowel disease goes along with a marked upregulation of NK_1 receptors on intestinal blood vessels and lymphoid structures. Some of these alterations are reproduced in experimental models of gastrointestinal disease, and there is mounting evidence that imbalanced function of distinct tachykinin systems is a causative factor for secretory, vascular and immunological disturbances related to intestinal anaphylaxis, infection and inflammation. Possible implications of tachykinins in pathological changes of gastrointestinal motility are reviewed in the companion article (Holzer and Holzer-Petsche, 1997). In a therapeutic perspective, it would seem conceivable that tachykinin antagonists could be employed as spasmolytic, antidiarrheal, anti-inflammatory and antinociceptive drugs. It must not be neglected, however, that tachykinins are messengers

within a multifactorial control system and that manipulation of the tachykinin system alone may not be therapeutically sufficient.

10.2. *Pathological Changes in Tachykinin and Tachykinin Receptor Expression*

10.2.1. Changes associated with gastrointestinal disease in humans. A number of gastrointestinal disorders has been found to go along with changes in the tissue level of tachykinins or in the density of the tachykininergic innervation of the gut (Table 2). In Hirschsprung's disease, SP is depleted from the aganglionic segment of the colon (Larsson *et al.*, 1983; Taguchi *et al.*, 1983; Tsuto *et al.*, 1985; Lolova *et al.*, 1986; Hamada *et al.*, 1987; Johanson *et al.*, 1991), and whole-mount analysis has revealed that it is the enteric SP-containing neurons that are lost, whereas the mucosal and perivascular SP-positive fibers, which are of extrinsic primary afferent origin, are preserved (Tam and Boyd, 1991). A similar loss of SP is seen in the megacolon of patients suffering from myotonic dystrophy (Yoshida *et al.*, 1988) and in congenital aganglionosis affecting the small and large intestine of the rat and mouse (Kaufman *et al.*, 1985; Nagahama *et al.*, 1985).

While the depletion of SP in aganglionosis is due to the loss or absence of enteric neurons, it is much less clear why there are quantitative changes in the tachykinin system in various other diseases. As is listed in Table 2, SP is virtually absent in the esophagus of patients with achalasia secondary to gastric cancer and is significantly decreased in the stomach of gastroesophageal reflux patients, in the pylorus with hypertrophic stenosis, and in the colon of chronically constipated humans. Conversely, increases in the SP concentration have been found in the duodenum of celiac sprue patients (Domschke *et al.*, 1989) and in the rectal mucosa of diabetic patients (Lysy *et al.*, 1993), whereas the levels of SP in the duodenal mucosa of patients with chronic pancreatitis and secondary diabetes mellitus are significantly reduced (Domschke *et al.*, 1988).

Particular interest has been aroused by the changes that the tachykinin system undergoes in inflammatory bowel disease (Table 2). While the tachykininergic innervation of the colonic mucosa affected by Crohn's disease either remains unchanged or is reduced, most studies have found the SP concentration to increase in ulcerative colitis (Koch *et al.*, 1987; Goldin *et al.*, 1989; Bernstein *et al.*, 1993; Kimura *et al.*, 1994; Keranen *et al.*, 1995). However, one study fails to see any change (Surrenti *et al.*, 1993) and another holds that the SP level decreases in ulcerative colitis (Kimura *et al.*, 1994). As in the majority of ulcerative colitis cases, the expression of SP is up-regulated in the gastric mucosa of patients suffering from nonulcer dyspepsia with abdominal pain (Kaneko *et al.*, 1993) and in the duodenum and pancreas of patients with chronic pancreatitis (Domschke *et al.*, 1988; Büchler *et al.*, 1992). These variable alterations in tachykinin expression are difficult to explain in a coherent manner because it is not known whether they are primary or secondary to the disease and whether they reflect changes in the transcriptional, translational or metabolic fate of PPT and SP or reflect altered nerve activity and release of the peptide.

More important from a functional point of view are the observations that the expression of NK_1 receptor binding sites by blood vessels and lymphoid aggregates is markedly up-regulated in pseudomembranous colitis induced by *Clostridium difficile* infection (Mantyh *et al.*, 1996) and in inflammatory bowel disease (Mantyh, C. R. *et al.*, 1988, 1994, 1995). In Crohn's disease, SP receptors are ectopically expressed on lymphoid aggregates, small blood vessels and myenteric neurons in both active, pathologically positive surgical specimens, and in quiescent, pathologically negative samples of the small and large intestine (Mantyh *et al.*, 1995). In contrast, SP receptor up-regulation in ulcerative colitis is confined to blood vessels and lymphoid aggregates of active, pathologically positive specimens of the colon (Mantyh *et al.*, 1995). These findings may have both etiologic and diagnostic potential in inflammatory bowel disease.

10.2.2. Changes associated with experimental models of gastrointestinal disease. Several studies have attempted to reproduce some of the disease-related changes in the gastrointestinal tachykinin system and thus, to establish experimental models with which to study the mechanisms behind these changes. Not surprisingly, most studies have been concerned with the effects of experimentally induced infection and inflammation. Following infection of rats with larvae of *Trichinella spiralis*, the expression of SP is up-regulated in the inflamed jejunum, but not in the unaffected ileum (Swain *et al.*, 1992). The increase in the SP concentration is virtually absent in capsaicin-pretreated rats, which attributes to extrinsic primary afferent nerve fibers an important role in the response to infection. An involvement of T lymphocytes is envisaged from the failure of *Trichinella spiralis* infection to up-regulate SP in congenitally athymic rats (Swain *et al.*, 1992), and it seems as if interleukin-1β released from these immune cells is an important mediator of the infection-induced tachykinin expression (Hurst *et al.*, 1993). Accordingly, exogenous interleukin-1β increases SP immunoreactivity in the MP of the rat jejunum, and the increase in the jejunal SP level seen in *Trichinella spiralis*-infected rats is prevented by an interleukin-1 receptor antagonist (Hurst *et al.*, 1993). However, the effect of interleukin-1β to enhance SP immunoreactivity takes place in neurons of the MP (Hurst *et al.*, 1993), which suggests that capsaicin-sensitive afferent nerve fibers participate in the cascade of events initiated by *Trichinella spiralis* infection, but are not the tachykinin system that is up-regulated.

An increase of SP immunoreactivity is also seen in the small intestine of mice infected with *Trichinella spiralis*, whereas in the guinea-pig jejunum and ferret small and large intestine, SP immunoreactivity decreases after *Trichinella* infection (Agro and Stanisz, 1993; Palmer and Greenwood, 1993; Palmer and Koch, 1995), much as the

TABLE 2. Pathological Changes in the Tissue Level/Density of PPT mRNA, Tachykinins and Tachykinin Receptors in the Gastrointestinal Tract

Species and tissue	Experimental injury or disease	PPT, peptide or receptor	Change	Reference
Human esophagus	Achalasia secondary to gastric cancer	SP	Loss	Fredens *et al.*, 1989
Human stomach	Gastroesophageal reflux	SP	Decrease	Wattchow *et al.*, 1992
	Nonulcer dyspepsia pain	SP	Increase	Kaneko *et al.*, 1993
Rat stomach	Streptozotocin-induced diabetes	SP	Decrease	Ballman and Conlon, 1985; Willars *et al.*, 1989
			Increase	Karakida *et al.*, 1991
Human pylorus	Hypertrophic pyloric stenosis	SP	Decrease	Wattchow *et al.*, 1987
Human pancreas	Chronic pancreatitis	SP	Increase	Büchler *et al.*, 1992
Feline pancreas	Pancreatitis due to partial duct obstruction	SP	Small increase	De Giorgio *et al.*, 1993
Human duodenum	Advanced chronic pancreatitis	SP	Increase	Domschke *et al.*, 1988
	Celiac sprue	SP	Small increase	Domschke *et al.*, 1989
Rat duodenum	Cysteamine ulcer	SP	Decrease	Evangelista *et al.*, 1990b
	Streptozotocin-induced diabetes	SP	Decrease	Ballmann and Conlon, 1985
Rat jejunum	*Trichinella spiralis* infection	SP	Increase	Swain *et al.*, 1992
	Interleukin-1β treatment	SP	Increase	Hurst *et al.*, 1993
Guinea-pig jejunum	*Trichinella spiralis* infection	SP	Decrease	Palmer and Koch, 1995
Guinea-pig small intestine	TNBSA-induced ileitis	SP	Decrease	Miller *et al.*, 1993a,b
Rat small intestine	Streptozotocin-induced diabetes	SP	Decrease	Ballmann and Conlon, 1985; Willars *et al.*, 1989; Karakida *et al.*, 1991
		SP	Late increase	Belai *et al.*, 1991
	Alloxan-induced diabetes	SP	Decrease	Di Giulio *et al.*, 1989
	Experimental atrophy of exocrine pancreas	SP	Decrease	Ballmann *et al.*, 1985
Murine small intestine	*Trichinella spiralis* infection	SP	Increase	Agro and Stanisz, 1993
	Spontaneous obesity and diabetes	SP, NKA	Decrease	Baily *et al.*, 1986
Lymphoid tissue of murine small intestine	*Salmonella dublin* infection	β-PPT mRNA	Increase	Bost, 1995
Ferret small and large intestine	*Trichinella spiralis* infection	SP	Decrease	Palmer and Greenwood, 1993
Rat ileum and colon	Congenital aganglionosis	SP	Decrease	Nagahama *et al.*, 1985
Murine ileum and colon	*Schistosoma mansoni* infection	SP	Decrease	Varilek *et al.*, 1991
Murine colon	*Trypanosoma cruzi* infection	SP	Decrease	Almeida *et al.*, 1977
	Congential aganglionosis with megacolon	SP	Decrease	Kaufman *et al.*, 1985
Rat colon	TNBSA-induced colitis	SP	Decrease	Sharkey, 1992
		β-PPT mRNA	Increase	Renzi *et al.*, 1994
	Dextran sulphate-induced colitis	SP	Increase	Kishimoto *et al.*, 1994
	Formalin injury	SP	Decrease	Miampamba *et al.*, 1992
	Streptozotocin-induced diabetes	SP	Late increase	Belai *et al.*, 1991
Rabbit colon	Immune complex/formalin-induced colitis	SP	Decrease	Eysselein *et al.*, 1991; Reinshagen *et al.*, 1994, 1995
Human appendix	Neurogenic appendicopathy	SP	Increase	Höfler *et al.*, 1983
Human colon	Crohn's diesease	SP	Decrease	Kimura *et al.*, 1994
		NK_1R	Increase	Mantyh, C. R. *et al*, 1988, 1994, 1995
	Ulcerative colitis	SP	Increase	Koch *et al.*, 1987; Goldin *et al.*, 1989; Bernstein *et al.*, 1993; Keranen *et al.*, 1995
		SP	Decrease	Kimura *et al.*, 1994
		NK_1R	Increase	Mantyh, C. R. *et al.*, 1988, 1994, 1995
	Pseudomembranous colitis due to C. *difficile* infection	NK_1R	Increase	Mantyh *et al.*, 1996
	Chronic constipation	SP	Decrease	Goldin *et al.*, 1989
	Diabetes	SP	Increase	Lysy *et al.*, 1993
	Hirschsprung's disease	SP	Decrease	Larsson *et al.*, 1983; Taguchi *et al.*, 1983; Tsuto *et al.*, 1985; Lolova *et al.*, 1986; Hamada *et al.*, 1987; Johanson *et al.*, 1991; Tam and Boyd, 1991
	Megacolon in myotonic dystrophy	SP	Decrease	Yoshida *et al.*, 1988

NK_1R, NK_1 receptor.

SP concentration declines in the small and large intestine of mice infected with *Trypanosoma cruzi* or *Schistosoma mansoni* (Almeida *et al.*, 1977; Varilek *et al.*, 1991). Cholera toxin does not affect the tachykinin system in the rat jejunum (Sjöqvist *et al.*, 1993), but infection with *Salmonella dublin* leads to an increase in β-PPT-A mRNA expression in various lymphoid organs associated with the gut, including Peyer's patches, mesenteric lymph nodes and spleens (Bost, 1995). Further analysis indicates that induction of β-PPT-A and γ-PPT-A mRNA in mononuclear leucocytes contributes to the overall rise of tachykinin expression in lymphoid organs of *Salmonella dublin*-infected mice (Bost, 1995).

Some of the alterations of the tachykinin system in inflammatory bowel disease have been reproduced in experimental models of ileitis and colitis (Table 2), but the results are too variable to allow any generalized conclusion. Experimental ileitis in the rat caused by trinitrobenzene sulphonic acid (TNBSA) is associated with a decline of the ileal level of SP, which is confined to the mucosa and submucosa and particularly marked in the perivascular extrinsic afferent nerve fibers (Miller *et al.*, 1993a,b). The colonic SP concentration in TNBSA-induced colitis is either left unaltered or decreased, although the expression of β-PPT-A mRNA in myenteric neurons is increased (Renzi *et al.*, 1992, 1994; Sharkey, 1992). A rise of SP immunoreactivity is seen in myenteric neurons of the rat colon following dextran sulphate-induced colitis, and this up-regulation of SP is accompanied by increases in the tissue and blood plasma levels of the peptide (Kishimoto *et al.*, 1994). Conversely, formalin-induced colitis reduces SP immunoreactivity in the rat colon, but increases it in the spinal cord (Miampamba *et al.*, 1992). The formalin-immune complex model of colitis in the rabbit is associated with an early reduction of SP immunoreactivity in the colonic muscle layer and in the dorsal root ganglia projecting to the colon (Eysselein *et al.*, 1991; Reinshagen *et al.*, 1994, 1995). These changes peak around 2 days after the induction of colitis and, since they are not accompanied by any alteration of β-PPT-A mRNA expression in the colon and dorsal root ganglia, seem to reflect inflammation-induced release of SP from both intrinsic and extrinsic afferent nerve fibers (Reinshagen *et al.*, 1995). The increase in the density of intrapancreatic SP/CGRP-positive nerve fibers in cats suffering from experimental pancreatitis due to partial duct ligation (De Giorgio *et al.*, 1993) reflects another instance of inflammation-induced peptide up-regulation in afferent neurons.

Other experimental perturbations of the tachykinin system include alterations of SP, but not NKA, immunoreactivity in response to cysteamine-induced ulceration of the rat duodenum (Evangelista *et al.*, 1990b, 1992) and alterations of SP expression in experimental diabetes (Table 2). While tachykinin levels in the pancreas stay rather normal in spontaneously obese-diabetic mice and in rats fed with a copper-deficient diet to induce atrophy of the exocrine pancreas, it is interesting to note that the levels of SP and NKA in the wall of the small intestine are significantly reduced in these forms of pancreatic pathology (Ballmann *et al.*, 1985; Bailey *et al.*, 1986). The neuropathy associated with alloxan- or streptozotocin-induced diabetes seems also to extend to the tachykinin system in the rat gastrointestinal tract (Table 2). The majority of studies holds that the expression of SP in the stomach and small intestine is decreased in experimental diabetes (Ballmann and Conlon, 1985; Di Giulio *et al.*, 1989; Willars *et al.*, 1989; Karakida *et al.*, 1991), but there is no consistency, since a rise of SP immunoreactivity in the stomach, ileum and colon, or no change at all, have also been reported (Buchan, 1990; Belai *et al.*, 1991; Karakida *et al.*, 1991).

10.3. *Tachykinins in Gastrointestinal Tumors*

Endocrine tumors of the midgut, notably carcinoids, have long been known to synthesize SP and to secrete this peptide into the blood plasma (Pernow, 1983; Otsuka and Yoshioka, 1993) because many patients with endocrine ileal tumors have elevated plasma levels of the tachykinin, whereas pancreatic endocrine tumors rarely express SP (Emson *et al.*, 1984; Ahlman *et al.*, 1985; Conlon *et al.*, 1985; Martensson *et al.*, 1985; Souquet *et al.*, 1987; Öberg *et al.*, 1989; Vinik *et al.*, 1990). The range of tachykinin-like peptides found in the tumor tissue and plasma of carcinoid patients has been expanded meanwhile to include NKA, NPK and shorter fragments of these peptides, notably NPK-(1-24) (Norheim *et al.*, 1984, 1986; Theodorsson-Norheim *et al.*, 1985, 1987; Conlon *et al.*, 1987, 1988; Ahlman *et al.*, 1988; Balks *et al.*, 1989; Wangberg *et al.*, 1991). This spectrum of peptides indicates that it is primarily β-PPT-A from which the tachykinins in midgut carcinoid tumors are derived, whereas PPT-B seems to be absent from the tumor cells (Roth *et al.*, 1985; Bishop *et al.*, 1989; Conlon *et al.*, 1988; Kage and Conlon, 1989). Long-term cultures of metastases derived from midgut carcinoid tumors have revealed that there is an endocrine cell-like and a neuron-like phenotype of tumor cells, tachykinins being expressed primarily in the neuron-like cell type (Ahlman *et al.*, 1989).

The release of tachykinins from carcinoid tumors can be stimulated by ingestion of a meal, alcohol or administration of pentagastrin (Norheim *et al.*, 1986). Many attempts have been made to correlate basal and stimulated plasma levels of tachykinins with symptoms of the carcinoid syndrome, notably flushing and diarrhea. The plasma levels of NKA and NPK rise in a majority of patients subjected to a provocation test, whereas SP immunoreactivity increases in a more erratic manner (Norheim *et al.*, 1986; Conlon *et al.*, 1987, 1988; Balks *et al.*, 1989; Vinik *et al.*, 1990). Somatostatin and the long-acting somatostatin analog octreotide inhibit both basal and stimulated release of tachykinins from carcinoid tumor cells and reduce the flushing attacks (Conlon *et al.*, 1987; Souquet *et al.*, 1987; Balks *et al.*, 1989; Öberg *et al.*, 1989; Vinik *et al.*, 1990; Wangberg *et al.*, 1991). However, the correlation between incidence and intensity of flushing and plasma concentration of SP and NKA is low, which indicates that tachykinins cannot be

solely responsible for the carcinoid flush (Conlon *et al.*, 1987; Balks *et al.*, 1989; Vinik *et al.*, 1990).

Comparatively little attention has been devoted to the possible role of tachykinins in diarrhea and intestinal hypermotility associated with the carcinoid syndrome. Carcinoid patients who develop diarrhea display major alterations of gut motor function (von der Ohe *et al.*, 1993), which in some way may be related to the hypersecretion of tachykinins into the circulation. The finding that the 5-HT_2 antagonist ketanserin prevents the gastrointestinal manifestations of the carcinoid syndrome (Ahlman *et al.*, 1985) does not rule out any involvement of tachykinins, given that 5-HT can activate, via 5-HT_2 and 5-HT_3 receptors, enteric neural pathways that involve tachykininergic neurons (Kirchgessner *et al.*, 1992b; Li and Owyang, 1996) and in this way may cause hypersecretion and hyperperistalsis.

Although interesting from a functional point of view, the presence of tachykinin receptors in gastrointestinal tumors is largely unknown. Of a number of human colon cancer cell lines, about 10% have been found to contain SP binding sites (Frucht *et al.*, 1992), whose expression is similarly low in adenocarcinomas of the colon and pancreas (Hennig *et al.*, 1995).

10.4. *Pathophysiological Implications of Tachykinins in Gastrointestinal Secretion*

10.4.1. Salivary secretion. Very little is known about pathological changes of the tachykinin system in the salivary glands and their potential significance for salivary gland function. Irradiation as is used in radiotherapy has been reported to increase the expression of SP in periacinar nerve fibers and intraglandular ganglion cells within the rat submandibular gland (Forsgren *et al.*, 1992), but it is unclear whether this change relates to a decrease in salivary function, as is frequently seen in patients undergoing radiotherapy in the head or neck. Changes in the expression of tachykinins might not only affect the secretory activity, but also the structural organization of salivary glands, since SP has been found to exert a trophic effect on the rat parotid gland subjected to parasympathetic denervation (Mansson *et al.*, 1990). The presence of SP in human saliva, the decrease of salivary SP levels in chronic pain patients and the increase of salivary SP levels in headache patients (Nicolodi and Del Bianco, 1990; Parris *et al.*, 1990, 1993; Takeyama *et al.*, 1990; Pikula *et al.*, 1992; Nicolodi, 1994) are observations that have not been evaluated yet in their possible significance for, or reflection of, salivary gland function.

10.4.2. Intestinal secretion. Increasing evidence suggests that endogenous tachykinins are of relevance for pathological disturbances of intestinal secretory processes. This applies, in particular, to the hypersecretory states that are associated with infection and inflammation. A tachykinin-mediated reduction of fluid absorption or even secretion may be responsible in part for the increase in defecation, which in the rat, is caused by *Salmonella* endotoxin or idazoxan and which is reduced by the NK_2 antagonist SR-48,968 (Croci *et al.*, 1994). The secretory and tissue-destructing reactions, which in the rat ileum are caused by C. *difficile* toxin A, are efficiently inhibited by the NK_1 receptor antagonist CP-96,345 (Pothoulakis *et al.*, 1994). The site of action by which endogenous tachykinins mediate the secretory action of C. *difficile* toxin A has not been delineated yet, but there is evidence that both capsaicin-sensitive extrinsic afferents and mast cells are involved (Castagliuolo *et al.*, 1994; Pothoulakis *et al.*, 1994). In contrast, the fluid secretion and diarrhea induced by *Vibrio cholerae* toxin does not depend on tachykinins and is left unaltered by tachykinin antagonists (Sjöqvist *et al.*, 1993; Pothoulakis *et al.*, 1994).

The effects of infection and inflammation on secretory disturbances in the intestine may involve distinct interactions between immune cells and tachykinin neurons, which are portrayed by the ability of interleukin-1β to cause hypersecretion in the rat colon, a response that depends on nerves, activation of NK_1 and NK_2 receptors in cascade, as well as the formation of NO (Eutamene *et al.*, 1995). Similarly, antigen challenge of the guinea-pig colon sensitized to *Trichinella spiralis* enhances the I_{sc} responses to SP and electrical field stimulation (Wang *et al.*, 1991), which in part may be related to the down-regulation of the SP-degrading enzyme, neutral endopeptidase (Hwang *et al.*, 1993). Pretreatment with a neurotoxic dose of capsaicin reduces the antigen-evoked rise of I_{sc} in the jejunal mucosa of ovalbumin-sensitized rats and blunts the intestinal fluid secretion induced by *Escherichia coli* enterotoxin Sta (Crowe *et al.*, 1990; Nzegwu and Levin, 1996), but it is not known whether this observation reflects a possible contribution of tachykinins.

Although an ever increasing number of data points to a participation of tachykinins in inflammatory hypersecretion, it is still difficult to draw a comprehensive scheme that would incorporate all pertinent observations. This uncertainty is due in part to the ambiguity of some experimental findings that, like the reduction of the maximal I_{sc} response to SP in the rat mitomycin C colitis model (Kachur *et al.*, 1995), can be explained in more than one way. A very plausible pathophysiological mechanism, though, has been delineated from analysis of the secretory response of the feline jejunum to acid-induced peritonitis. The hypersecretion seen in this model is very likely due to stimulation of intramural sensory neurons that use SP and NKA as their messengers and activate VIP-releasing secretomotor neurons (Brunsson *et al.*, 1990, 1995).

10.4.3. Therapeutic prospects. If tachykinins are factors in certain states of pathological hypersecretion in the intestinal mucosa, it would be logical to target therapeutic interventions at the blockade of tachykinin receptors and at interruption of the cholinergic and noncholinergic relays in the tachykinin-stimulated secretory reflex pathways (Fig. 2). The ability of tachykinin receptor antagonists to prevent the secretory effects of C. *difficile* toxin A and interleukin-1β (Pothoulakis *et al.*, 1994; Eutamene *et al.*, 1995)

and the likely implication of tachykinins in the hypersecretory responses to intestinal anaphylaxis and peritonitis mark potentially important directions to novel therapeutic strategies. Additional possibilities to control SP-induced hypersecretion include the use of somatostatin-14 and its stable analog, octreotide, both of which inhibit the SP-induced increase in I_{sc} in the mucosa of the rat colon (Fassler *et al.*, 1990; Ferrar *et al.*, 1990). The findings that methionine-enkephalin and sympathetic nerve stimulation are able to suppress SP-evoked VIP release and net fluid secretion in the feline small intestine (Brunsson *et al.*, 1995) present another therapeutic perspective.

10.5. *Pathophysiological Roles of Tachykinins in Gastrointestinal Inflammation and Tissue Injury*

10.5.1. Implications of tachykinins in the vascular components of inflammation. The pronounced alterations of the tachykinin system in inflammatory bowel disease (Table 2) and the spectrum of vascular and immunological actions of SP and NKA in the gut are compatible with the conjecture that these peptides have a bearing on gastrointestinal inflammation (Sharkey, 1992). In a number of mammalian species, SP and NKA are able to increase blood flow and vascular permeability in the gut, which suggests that prolonged tachykinin release could contribute to the vascular components of the inflammatory reaction. It is important to recall in this context that NK_1 binding sites on small blood vessels may be up-regulated by a factor of more than 1000 in the inflamed intestine of patients with Crohn's disease or ulcerative colitis (Mantyh, C. R. *et al.*, 1988, 1994, 1995) and in patients with pseudomembranous colitis caused by C. *difficile* infection (Mantyh *et al.*, 1996). In order to understand the functional significance of these observations, it will be necessary to identify the source of tachykinins that are targeted at these receptors and to analyze the functional significance of receptor up-regulation for the vascular system. Some of the features of inflammatory bowel disease are reproduced by delayed-type hypersensitivity reactions, and it is, therefore, of particular relevance to note that the NK_1 receptor antagonist RP-67,580 reduces the increase in vascular permeability caused by a delayed-type hypersensitivity reaction in the mouse small intestine (Kraneveld *et al.*, 1995). As capsaicin pretreatment prevents the plasma protein leakage caused by oral dinitrobenzene sulphonic acid challenge in mice subjected to dinitrofluorobenzene contact sensitization, it would appear that primary afferent nerve fibers are the tachykinin system responsible for the delayed-type hypersensitivity-induced rise of vascular permeability (Kraneveld *et al.*, 1995).

Since SP, capsaicin and splanchnic nerve stimulation cause plasma protein extravasation in the guinea-pig gallbladder and bile duct (Lundberg *et al.*, 1984), it may be hypothesized that tachykinins released from afferent nerve fibers under conditions of bile stone obstruction, biliary infection or cholecystitis may contribute to inflammation of the biliary system.

10.5.2. Implications of tachykinins in the immunological components of inflammation. The possibility that tachykinins represent messengers in the interface between the nervous and immune system may be of major importance for inflammatory disease. From the available data, it would seem that mast cells, lymphocytes and granulocytes may be those immune cells that preferentially are under the influence of tachykinins. SP-positive nerve fibers lie in close proximity to mast cells in the mesentery and gastrointestinal mucosa (Skofitsch *et al.*, 1985; Stead *et al.*, 1987; Crivellato *et al.*, 1991), and SP can act on mast cells in the rat intestinal mucosa to cause release of histamine and other factors (Shanahan *et al.*, 1985; Marshall *et al.*, 1994). Conversely, mast cells isolated from the normal human colon are not responsive to SP (Lowman *et al.*, 1988; Rees *et al.*, 1988), but it is not known whether this is also true for mast cells in the inflamed intestine. The interactions between tachykininergic neurons and mast cells in intestinal inflammation have not been studied in detail yet, but it need be recalled in this context that the mucosal inflammation provoked by C. *difficile* toxin A in the rat ileum (Pothoulakis *et al.*, 1994) and the plasma protein leakage caused by a delayed-type hypersensitivity reaction in the mouse small intestine (Kraneveld *et al.*, 1995) depend on both tachykinins and mast cell-derived factors. Furthermore, it is quite probable that mast cells participate in the tachykinin-mediated motor and secretory disturbances evoked by anaphylaxis, infection and inflammation (Perdue and McKay, 1994).

Immune cells isolated from many tissues including the gut are responsive to SP. The proliferation of lymphocytes isolated from Peyer's patches of the mouse and their synthesis of immunoglobulins (Igs) (notably IgA and, to a lesser extent, IgM) is stimulated by SP both *in vitro* and *in vivo* via interaction with specific SP receptors (Stanisz *et al.*, 1986, 1987; Scicchitano *et al.*, 1988). Similarly, SP increases the release of Igs, interferon-γ and interleukin-2 from cultures of the human duodenal mucosa, whereas the release of interleukin-1β is reduced (Hart *et al.*, 1988, 1990). The ability of SP immunoneutralization to reduce SP up-regulation, inflammation and lymphocyte proliferation in the inflamed small intestine of *Trichinella spiralis*-infected mice (Agro and Stanisz, 1993) indicates that the SP/lymphocyte axis indeed may be of pathophysiological relevance. This view is supported by the observation that T lymphocytes from hepatic granulomas of *Schistosoma*-infected mice express mRNA for NK_1, but not NK_2 or NK_3, receptors, whereas lymphocytes isolated from normal liver devoid of granulomas exhibit none of the three tachykinin receptor subclasses (Cook *et al.*, 1994). The perspective that emerges from these findings attributes tachykinins an immune-modulatory role in inflammatory bowel disease, given that in Crohn's disease, ulcerative colitis and C. *difficile*-induced pseudomembranous colitis, there is a remarkable up-regulation and ectopic expression of NK_1 binding sites on both lymphoid aggregates and small blood vessels (Mantyh, C. R. *et al.*, 1988, 1994, 1995, 1996). T lymphocytes play a crucial role in delayed-type hypersensitivity reactions, and

experimental data are compatible with the surmise that afferent nerve-derived SP and NKA stimulate NK_1 receptors on lymphocytes and in this way, contribute to delayed-type hypersensitivity inflammation in the murine gut (Kraneveld *et al.*, 1995).

The ability of SP to promote the adherence, emigration and activity of granulocytes (Holzer, 1992) is another important factor in the interactive contribution of the tachykinin and immune system to chronic inflammation. SP enhances adhesion of leukocytes to the endothelium of rat mesenteric venules (Zimmerman *et al.*, 1991) and plays a role in the subsequent accumulation of granulocytes in the inflamed tissue since NK_1 antagonists attenuate the granulocyte infiltration, which in the rat colon, is induced by TNBSA and in the rat ileum is provoked by *C. difficile* toxin A (McCafferty *et al.*, 1994; Pothoulakis *et al.*, 1994). Macrophages are another population of immune cells that can be activated by tachykinins, and it is pathologically relevant to note that the expression of NK_1 receptor mRNA in rat peritoneal macrophages is up-regulated by exposure to endotoxin (Bost *et al.*, 1992).

The interrelationship between the tachykinin and immune system is very likely of a bidirectional nature, as stimulation of immune cells releases a broad spectrum of mediators that act on a variety of target cells including tachykininergic afferent neurons (Holzer, 1992). Although these interactions have been little studied in the gut, it need be noted that the ability of interleukin-1β to cause hypersecretion in the rat colon depends on endogenous tachykinins acting via NK_1 and NK_2 receptors in cascade (Eutamene *et al.*, 1995).

Immune cells are not only targets at which tachykinins act to modify immune responses, but under pathological conditions, can be induced to synthesize and release tachykinins themselves. Rat peritoneal macrophages contain SP-encoding mRNA that can be up-regulated by endotoxin stimulation (Bost *et al.*, 1992). Infection of mice with *Salmonella dublin* induces the expression of both β-PPT-A and γ-PPT-A mRNA in mononuclear leucocytes that contribute to the *Salmonellosis*-induced up-regulation of PPT expression in mesenteric lymph nodes and spleens (Bost, 1995). Eosinophils from intestinal granulomas of *Schistosoma*-infected mice synthesize SP and release it in response to histamine or a calcium ionophore (Weinstock *et al.*, 1988; Weinstock and Blum, 1990). Similarly, eosinophils isolated from the mucosa of the inflamed human colon express mRNA for SP and contain the authentic peptide (Metwali *et al.*, 1994). Although the functional implications of tachykinins released from immune cells have not been deciphered yet, it might well turn out that the ectopic up-regulation of tachykinin receptors in inflammatory bowel disease is related to an ectopic synthesis and release of tachykinins.

10.5.3. Tissue injury and protection. Capsaicin-sensitive extrinsic afferent neurons have a bearing on gastric mucosal defense against injury because capsaicin-induced stimulation of sensory neurons enhances, and ablation of these neurons weakens, the resistance of the rat gastric mucosa against experimentally imposed injury (Holzer, 1992). CGRP released locally within the stomach and subsequent formation of NO are thought to be primarily responsible for the protective action of sensory nerve stimulation (Lambrecht *et al.*, 1993), whereas the role of tachykinins is less well defined. SP is devoid of a protective action (Evangelista *et al.*, 1987, 1989) and in fact, has been found to exaggerate gastric mucosal damage caused by acidified taurocholate or ethanol (Soper and Tepperman, 1986; Karmeli *et al.*, 1991). The deleterious effect of SP is mimicked by septide and senktide, which points to an implication of both NK_1 and NK_3 receptors (Karmeli *et al.*, 1991). Analysis of the mode of action suggests that SP aggravates ethanol injury by mast cell degranulation and subsequent release of histamine, platelet-activating factor, leukotriene B_4 and leukotriene C_4 (Karmeli *et al.*, 1991, 1993). The discharge of peptide-degrading proteases from mast cells is thought to explain why in the rat gastric mucosa exogenous SP inhibits the CGRP-mediated hyperemic response to capsaicin (Grønbech and Lacy, 1994).

The adverse action of SP on gastric mucosal integrity in the rat is shared by endogenous tachykinins, since tachykinin antagonists have been found to reduce ethanol-induced injury and to counteract the effect of VIP to exacerbate mucosal damage (Karmeli *et al.*, 1991, 1993; Cho *et al.*, 1994; Hayashi *et al.*, 1996). The findings that ethanol damage leads, within 10 min, to an increase in the SP concentration of the gastric mucosa and that the protective effect of somatostatin is associated with a diminished rise of the gastric mucosal SP concentration (Karmeli *et al.*, 1993, 1994) have been used to further advance the concept of a deleterious role of endogenous SP. However, the rapid rise of the gastric SP level after exposure to ethanol is difficult to understand in terms of mechanism and significance and, furthermore, is at variance with the ethanol-induced release of SP into the gastric lumen (Hayashi *et al.*, 1996).

The ability of the NK_1 receptor antagonist CP-96,345 to inhibit the increase in epithelial permeability and the necrosis, which in the rat ileum, is caused by *C. difficile* toxin A (Pothoulakis *et al.*, 1994), indicates that SP is a major mediator of the cytotoxic effects of this bacterial toxin on the intestinal mucosa. The adverse action of SP in this model also seems to be related to mast cells, since CP-96,345 effectively prevents the toxin-evoked release of rat mast cell protease II (Pothoulakis *et al.*, 1994). The implication of capsaicin-sensitive afferent neurons in the deleterious effects of *C. difficile* toxin A on the rat ileum (Castagliuolo *et al.*, 1994) is an exception inasmuch as in all other models of gastrointestinal injury that have been tested the role of these neurons is to strengthen defense against injury (Holzer, 1992). Any function of endogenous tachykinins in these models remains to be deciphered, although a beneficial effect of exogenous SP has been noted in a model of acute colitis (Murthy *et al.*, 1991). The mechanism by which SP reduces the plasma protein leakage, which in the

rat colon is caused by immune complex-formalin treatment or exposure to ethanol, could be related to vascular decongestion (Murthy *et al.*, 1991).

Although most data indicate that NK_1 receptor activation impairs mucosal homeostasis, there is evidence that NK_2 receptor stimulation is beneficial for the gastric mucosa, at least under certain experimental conditions. Thus, NKA and related analogs that act preferentially on NK_2 receptors inhibit ethanol-induced injury to the rat gastric mucosa, in spite of a decrease in gastric mucosal blood flow (Evangelista *et al.*, 1989, 1990a; Heinemann *et al.*, 1996; Stroff *et al.*, 1996). The protective effect of NK_2 agonists depends on stimulation of afferent nerve fibers, release of CGRP and formation of NO (Stroff *et al.*, 1996). Since MEN-10,627 blocks the gastroprotective action of both NK_2 receptor agonism and sensory nerve stimulation, it follows that endogenous tachykinins acting via NK_2 receptors participate in the sensory nerve-mediated maintenance of gastric mucosal integrity (Stroff *et al.*, 1996). The effect of NKA on gastric mucosal integrity, though, depends on the experimental model under study. While ethanol injury is reduced, damage caused by acid backdiffusion through a disrupted gastric mucosal barrier is exacerbated by NKA because tachykinins suppress the gastric hyperemia, which is evoked by acid backdiffusion and which is important to counteract the deleterious consequences of acid influx into the mucosa (Heinemann *et al.*, 1996). This antivasodilator action of NKA is mediated by NK_2 receptors and thus, is independent of the tachykinins vasoconstrictor action, which is due to NK_1 receptor activation (Heinemann *et al.*, 1996).

10.5.4. Therapeutic prospects. From a synopsis of the pertinent data, it would seem that NK_1 receptor antagonists may be useful anti-inflammatory drugs that interfere with both the vascular and immunological manifestations of the inflammatory process. NK_1 binding sites on small blood vessels and lymphoid aggregates are up-regulated in inflammatory bowel disease and pseudomembranous colitis (Mantyh, C. R. *et al.*, 1988, 1994, 1995, 1996), and it is NK_1 receptors that mediate the inflammatory and cytotoxic reactions to delayed type-hypersensitivity challenge, TNBSA and *C. difficile* toxin A (McCafferty *et al.*, 1994; Pothoulakis *et al.*, 1994; Kraneveld *et al.*, 1995). These pathological alterations may be exploited therapeutically if the tachykinin receptors, which in chronic inflammation are expressed by mast cells, lymphocytes, granulocytes, macrophages and blood vessels, can be identified and selective ligands for these receptors can be developed. Another therapeutic approach may be targeted at interference with the processes that lead to pathological tachykinin expression in immune cells and to up-regulation of tachykinin receptors in the inflamed tissue. Whether NK_1 receptor antagonists or NK_2 receptor agonists are useful in ulcerative disorders of the upper gastrointestinal tract requires further study.

The therapeutic usefulness of tachykinin antagonists as anti-inflammatory drugs is difficult to predict as long as it is not known whether tachykinins are factors that are essential in the initiation of the disease, factors that are important to sustain the disease, or secondary factors that contribute to the symptoms of the disease. It is obvious, though, that any rational approach to causative therapy needs to take account of the complex phenotypic and functional changes that in chronic inflammatory disease cause an imbalance of the factors that normally maintain tissue homeostasis. It need also be considered that chronic inflammation is likely to cause perturbations that reach beyond the gut and generate secondary symptoms and complications. Inflammatory pain is a premier example of this. The change in the environment of nociceptive afferent nerve terminals in the chronically inflamed tissue produces sensitization of nociceptors, recruitment of additional nociceptors, windup of the central processing of afferent input, hyperalgesia, allodynia and overt pain (Cervero, 1994). There is circumstantial evidence that a disturbance of the peripheral tachykinin system is a contributory factor to inflammatory pain. The increase in the SP concentration of the colonic wall and blood plasma of rats affected with dextran sulfate-induced colitis is associated with a writhing behavior that is indicative of pain and that in normal rats, can be reproduced by intraperitoneal injection of SP (Kishimoto *et al.*, 1994). This experimental finding and the analogous up-regulation of SP in the gastric mucosa of patients suffering from painful nonulcer dyspepsia (Kaneko *et al.*, 1993) may reflect a role of tachykinins in the peripheral sensitization of nociceptive afferents. Whether tachykinins also contribute to the central sensitization of nociceptive pathways from the gut remains to be substantiated.

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