

Review

Tachykinins in regulation of gastric motility and secretion

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Received 24 August 1999; received after revision 1 December 1999; accepted 3 December 1999

Abstract. The tachykinins constitute a family of neuropeptides with a common C-terminal amino acid sequence. The best known tachykinin is substance P. Tachykinins are found in the nerve plexuses and nerve fibers in the stomach of all species examined. The circular muscle layer is densely innervated, whereas the longitudinal layer and the mucosa are less intensively innervated. Tachykinins are also found in a significant number of afferent neurons with cell bodies in the dorsal root ganglia. Release of tachykinin can be demonstrated in response to both electrical stimulation of the vagus nerves and application of capsaicin. In the stomach all three known tachykinin receptors seem to be present. Although species variations exist, NK-2

receptors are generally present on the musculature, NK-1 receptors on both neurons and muscles, and NK-3 receptors on neurons only. Tachykinins stimulate motility in all parts of the stomach, but tachykinins also appear to inhibit motility in certain situations. Also, motility initiated centrally, mediated through the vagus nerves, is influenced by tachykinins. The precise role of tachykinin in the various motor programs in the stomach is not clear. Gastric acid secretion is influenced by tachykinins in several species. Tachykinins do not seem to act as neurotransmitters directly on parietal cells, but may have a modulatory function. The importance of tachykinins for the regulation of pepsinogen and hormone secretion from the stomach remains unclear.

Key words. Capsaicin; substance P; neurokinin A; neuropeptides; NK-receptors; receptor antagonists; gastrin.

General biology of the tachykinins

Chemistry

The tachykinins constitute a family of neuropeptides with a common C-terminal amino acid sequence, Phe-X-Gly-Leu-Met-NH₂, where X is an aromatic or hydrophobic residue. Mammalian tachykinins include substance P (SP), neurokinin A (NKA), neurokinin B (NKB) and two N-terminally extended forms of neurokinin A, neuropeptide K and neuropeptide μ . The tachykinins are encoded by two different genes, desig-

nated the preprotachykinin A (PPT-A) and PPT-B genes, [1]. PPT-A encodes SP and NKA, whereas PPT-B encodes NKB. In the rat gastrointestinal tract, the PPT-A, but not the PPT-B gene, is expressed [2]. This corresponds to the finding that only SP and NKA peptides can be detected in gastrointestinal tissue, whereas NKB is undetectable (see below).

The tachykinins can activate three different receptors (NK-1, NK-2 and NK-3). The mammalian tachykinins can activate all three receptors, but SP is the preferred ligand for the NK-1 receptor, NKA for the NK-2 receptor and NKB for the NK-3 receptor. Selective agonists and antagonists have been constructed for all three receptors.

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Capsaicin in tachykinin studies

Capsaicin is found in chili pepper and has turned out to be a very useful tool in physiological and pharmacological research. Capsaicin administered acutely stimulates unmyelinated (C) or thin myelinated (A) primary sensory fibers. When administered in high doses or for longer periods, capsaicin can ablate the primary sensory neurons [3]. Capsaicin can thus be used in two different ways. First, it can be used to acutely activate primary sensory nerves. Second, the primary sensory fibers can be ablated, and the functions under study can be compared between animals with or without ablation. Also, the presence and localization of primary sensory fibers can be investigated by immunohistochemistry, comparing capsaicin-treated and -untreated animals [4–6]. The most important neurotransmitters in the primary sensory nerve fibers are tachykinins and calcitonin gene-related peptide (CGRP). The functions of tachykinins and CGRP in primary sensory fibers can thus be investigated using capsaicin.

Localization in the stomach

SP immunoreactivity has been found in stomach tissue extracts from various species, including mouse, rat, cat, dog, pig and humans [7–12]. NKA and SP immunoreactivity were found in equal amounts in the pig fundus, antrum and pylorus [9]. During rat pre- and postnatal development the amounts of SP and NKA in the stomach varies, but from the second postnatal week the amounts are stable and equal [11]. NKB is not present in pig stomach [13], and has not been found in gut tissue of any species, whereas small amounts of the precursor of NKA, neuropeptide K, are present in the porcine antrum [13].

In humans extractable SP immunoreactivity was found throughout the stomach, with increasing concentrations towards the distal part [12]. The aboral rise in SP concentration was seen in both the mucosa, the submucosa and the muscle layer. The distribution of TK immunoreactivity has been examined in various species using immunohistochemistry. A large number of SP-immunoreactive nerve fibers or neurons have been found in the myenteric plexus and circular muscle layer of all species examined, including rat, mouse, guinea pig, cat, dog, pig and humans [4, 10, 14–18]. In contrast to the dense innervation in the circular muscle layer in all species, the longitudinal muscle layer is generally sparsely innervated [4, 14–18]. The innervation of the mucosa and submucosa shows large variations among species. Sundler [19] compared the density of SP-immunoreactive nerve fibers in the corpus and antrum of different species. In the corpus, only a few fibers were found in mouse, rat, hamster, guinea pig, ferret and

humans, whereas the mucosa of mole and pig contained numerous fibers. In the antral mucosa of mole, pig and humans, moderate to numerous SP-immunoreactive nerve fibers were found, but in the other species none to a few fibers were found. In the canine stomach mucosa no SP-immunoreactive nerve fibers were found [20]. Large variations among different species with respect to the innervation pattern, therefore, seem to exist, as well as variations between the innervation patterns observed in the oxyntic and the antral mucosa.

The origin of SP-immunoreactive nerve fibers in the stomach has been examined using various procedures, including ligation of the vagus or the splanchnic nerves, neurotomy of the vagal or splanchnic nerves, dorsal ganglionectomy, coeliac ganglionectomy, retrograde or anterograde labeling, capsaicin treatment and myectomy.

Denervation of sensory afferent fibers using capsaicin have resulted in conflicting results. Sharkey, Green and Dockray [4, 5] found that mucosal and submucosal SP-immunoreactive nerve fibers disappear or are markedly reduced in response to neonatal capsaicin pretreatment in rats. Holzer [8] found that capsaicin treatment did not affect the amount of SP immunoreactivity in extracts of rat stomach. Suzaki [6] studied adult rats treated with capsaicin and found no effect on the density of SP-immunoreactive nerve fibers in the stomach, whereas CGRP-immunoreactive nerve fibers were almost abolished.

SP immunoreactivity is present in mouse, cat and human vagal nerve fibers [21–23] and in the ganglia related to the vagus nerve (nodose and jugular ganglia) of mouse, rat and guinea pig [5, 24]. Minagawa [16] found no change in SP-immunoreactive nerve fiber density in the stomach wall after vagotomy in rats, whereas in the same species Suzuki [6] found a significant decrease in the number of SP-immunoreactive fibers in the mucosa. In cats, vagus ligation resulted in a decrease in the number of SP-immunoreactive fibers in myenteric plexus of the corpus, but an unchanged number in the antrum [22]. In the canine fundus, vagotomy in combination with sympathetic denervation did not change the number of SP-immunoreactive nerve fibers or neurons [25]. Using retrograde tracing with injection of dye in the stomach wall, the extrinsic afferent nerve fibers can be labeled. In vagal afferent nerve fibers from corpus or antrum/pylorus of rat, mouse and guinea pig the proportion of SP-immunoreactive nerve fibers is low [5, 24]. Taken together, it seems that tachykinin-containing nerves in the stomach represent extrinsic afferent or efferent vagal nerve fibers only to a minor extent. This, of course, does not exclude involvement of tachykinins in central regulation of gastric functions via the vagus nerves. Ladic [26] used retrograde tracing of a marker substance (Fluorogold) injected under the serosal sur-

face along the greater curvature of rat stomach. The localization of the marker substance was compared with SP immunoreactivity and immunoreactivity for the tachykinin NK-1 receptor in the dorsal motor nucleus of the vagus. Both SP immunoreactivity and NK-1 immunoreactivity were associated with the retrogradely labeled neurons. A morphological basis for the involvement of tachykinins in central regulation of stomach functions via vagus therefore seems to exist.

Afferent nerve fibers projecting to the spinal ganglia, however, contain a large number of SP-immunoreactive nerve fibers. In the rat, mouse and guinea pig SP is present in 40–70% of retrogradely labeled nerve fibers projecting from the gastric corpus to the dorsal root ganglia [4, 5]. Minagawa [16] used spinal ganglionectomy and transection of the splanchnic nerves to show that most of the SP-immunoreactive nerve fibers found in rat myenteric plexus originated from the dorsal root ganglia via the splanchnic nerves. The rest of the nerve fibers originate from SP-immunoreactive cells within the myenteric plexus. However, Suzuki [6] found no effect of extirpation of the celiac and superior mesenteric ganglia on the innervation pattern of SP in rat stomach. In the cat transection of the splanchnic nerves reduced SP immunoreactivity markedly in the antrum and slightly in the corpus [22]. When the splanchnic nerves were ligated, SP immunoreactivity was found cranial to the ligation, suggesting that the transmitter is produced centrally to the ligation, possibly in the dorsal root ganglia.

In summary a significant number of stomach afferent tachykininergic neurons with cell bodies in the dorsal root ganglia seem to exist. These fibers probably travel with the splanchnic nerves.

In the intestinal wall, cell bodies in the myenteric plexus are the source of nerve fibers to the circular and longitudinal muscle layers, and cell bodies in the submucosal plexus are the source of nerve fibers in the mucosa and submucosa [25]. This is not the case in the stomach, where a well-developed submucosal plexus is not present [25, 27]. Removal of the longitudinal muscle layer together with the myenteric plexus (myectomy) in the canine corpus resulted in loss of nerve fibers, including SP-immunoreactive nerve fibers, in the circular muscle and mucosa of the area beneath the lesion [25]. This indicates that mucosal nerve fibers derive from myenteric cell bodies. Retrogradely labeling nerve fibers in the mucosa and circular muscle showed that SP-immunoreactive cell bodies in the myenteric plexus mainly project to the circular muscle layer, and to a minor extent to the mucosa [27].

In conclusion, tachykininergic neurons in the stomach densely innervate the circular muscle layer and, to a minor extent, also the mucosa.

Release

The release of tachykinins from stomach preparations has been measured in response to various stimuli. Electrical vagus stimulation enhanced the release of both SP and NKA in isolated vascularly perfused porcine antrum [13], isolated perfused porcine fundus/corpus [28] and vascularly perfused cod stomach [29]. A luminal release of SP has been shown in feline stomach in response to electrical vagus stimulation [30]. Administration of capsaicin increased the release of both SP and NKA in the isolated perfused porcine antrum [13]. In the guinea-pig stomach, *in vivo*, luminal application of capsaicin caused a release of SP, and application of capsaicin to fundic mucosal tissue samples *in vitro* increased the release of SP [31]. In vascularly perfused rat stomach preparations capsaicin evoked release of SP [32], whereas capsaicin did not affect SP release *in vitro* in preparations of the mucosa or the muscle layers of rat stomach, but increased NKA release [33]. Being derived from a common precursor molecule, it is likely that SP and NKA are in fact released in equal amounts, but the lack of a significant measurable release of SP is probably due to faster degradation of SP than NKA [34]. Finally, acetylcholine was shown to increase SP and NKA release in the vascularly perfused cod stomach [29]. Taken together, tachykinins can be released from stomach tissue in response to electrical stimulation of the vagus nerves and application of capsaicin stimulating sensory afferent nerves.

Localization of receptors

Localization of tachykinin receptors in the stomach has been visualized using three methods: autoradiography, immunohistochemistry and assays for expression of messenger RNA (mRNA) encoding tachykinin receptors. Localization of receptors has also been deduced from physiological and pharmacological studies, as will be described below.

Antibodies against fragments of rat NK-1, NK-2 and NK-3 and guinea-pig NK-1 and NK-2 receptors have been developed. In the rat stomach few myenteric neurons were NK-1-immunoreactive [35, 36]. Large amounts of NK-2 receptors were found on the circular muscle layer, whereas there were few binding sites in the longitudinal muscle layer. NK-3 receptors were found on neurons in the myenteric and submucosal plexus in fundus and antrum [36]. In another study, however, no NK-3 receptors were found in the rat stomach [37]. In guinea-pig stomach NK-1 immunoreactivity was found on nerve cell bodies in the myenteric plexus and on nerve cell bodies in the submucosal plexus, except in the corpus [38]. In the guinea-pig stomach NK-2 receptor immunoreactivity was confined to the circular muscle

layer and a few nerve fibers in the muscle layers in the antrum [39].

Antibodies to human NK-1 receptors were used to study localization in the human antrum [40]. Immunoreactivity was found on neurons and nerve fibers in the myenteric and submucosal plexus. No immunoreactivity was found on muscle cells, whereas cells probably representing Cajal's interstitial cells were stained. Finally, epithelial cells and endothelial cells lining blood vessels appeared to be immunoreactive.

In rat fundus, autoradiography using ligands designed for all three receptors revealed binding of NK-2-preferring ligands to the circular muscle and muscularis mucosa. Binding of NK-1-preferring ligand was only seen on the circular muscle, whereas unequivocal specific binding of the NK-3 ligand was not seen [41]. The presence of NK-1 and NK-2 receptors was confirmed in quantitative binding studies, with NK-2 receptors showing the highest binding capacity [41]. In feline stomach SP binding sites were found in mucosa, submucosa, circular muscle and longitudinal muscle. High concentrations of binding sites were found in the circular muscle, especially in the pyloric region [42].

Binding of the three natural ligands, SP, NKA, and NKB, has been studied in canine and human stomach. In the canine stomach SP and NKA binding was found on circular muscle, arterioles, venules, submucosa and in the fundus in the region containing chief and parietal cells. In the pyloric sphincter (inner circular muscle), a high density of binding sites for SP and NKA was found, whereas only NKA binding was found in the outer circular muscle. In the myenteric plexus, binding of SP was seen, but not of NKA. Nowhere in the stomach could binding of NKB be detected [43]. In human stomach, NKA bound to the circular and longitudinal muscle and lamina muscularis mucosa, whereas SP binding was found on circular muscle in the antrum but not in the fundus. Furthermore, SP bound to arterioles, venules and germinal centers of gut-associated lymphatic nodules. No NKB binding was seen [44].

Tsuchida [45] measured the relative amounts of mRNA for NK-1, NK-2 and NK-3 receptors compared with the total amount of RNA in rat tissue. In the stomach large amounts of NK-2 receptor mRNA were found, whereas mRNA for NK-1 and NK-3 receptors was present only in small amounts.

Thus all three tachykinin receptors seem to be present in the stomach. Species variations exist, but in general NK-2 receptors are present only on the musculature, NK-1 receptors on both muscle cells and neurons, and NK-3 receptors only on neurons. Further, more NK-1 receptors may be present on blood vessels and germinal centers of the lymphatics.

Effects of the tachykinins

Motility

The stomach has several motor functions. It is able to store large amounts of foodstuffs and liquids. It can grind and disperse its contents and finally empty the chyme in a highly controlled manner. According to the two-compartment model, the proximal stomach (corpus/fundus) is primarily responsible for storage, whereas the distal stomach (antrum/pylorus) is responsible for dispersing and emptying the contents [46].

Several methods for studying the involvement of tachykinins in stomach motility have been employed, both in vivo and in vitro. These include emptying marker substances from the stomach, pressure and volume recordings using intragastric balloons, motor recordings of muscle strips in tissue baths, and strain-gauge recordings.

Gastric emptying. Conflicting results regarding the effects of SP (and other tachykinins) on gastric emptying have been obtained in rats. SP was found to increase the rate of gastric emptying [47], to decrease the rate [48] or to have no effect [49]. These differences may be explained by the way SP was administered. In one study SP was injected into the aorta (no effect on the emptying rate) and in the two others intraperitoneally (increase and decrease). In the study where SP was found to increase the emptying rate, SP was given 5 min before the test meal, and here SP stimulated emptying only at the highest concentration [47]. In the study where tachykinins were found to decrease the emptying rate, SP and NKA were given together with administration of the meal [48]. The decreased emptying rate was converted to increased emptying when SP and NKA were administered after atropine pretreatment. Thus, the effect of atropine and the tachykinins must be considered in view of the complex nature of stomach emptying. Given systemically, the tachykinins can affect the intrinsic nerves in the stomach, the extrinsic nerves and the musculature, they may stimulate the secretion of hormones affecting stomach emptying and they may possibly have additional effects. The overall effect of drugs on stomach emptying is therefore the result of several, frequently opposing effects. It can be speculated that the inhibitory action of SP and NKA is due to contractions of the pylorus mediated by cholinergic neurons equipped with tachykinin receptors. After atropine pretreatment, the cholinergic pylorus contractions are absent or diminished, whereas SP- and NKA-activated muscular receptors in the rest of the stomach now mediate contractions and, therefore, increased emptying [48].

Tachykinins may be involved in the cerebral regulation of gastric emptying by interference with the activity of extrinsic nerves. Thus, intracerebroventricular injection

of tachykinins inhibits gastric emptying [50]. NKA was the most potent, indicating that the effect is mediated by NK-2 receptors.

As previously mentioned, capsaicin is known to activate sensory afferent fibers. Stomach emptying is decreased by chili ingestion in both rats and humans [51, 52], an effect that could be mediated by tachykinins or CGRP.

Effects on intragastric pressure and volume. As mentioned, the proximal and distal part of the stomach have different motor functions. The proximal part can relax at the same time that the distal part is contracting or vice versa. When measuring changes in volume or pressure with the use of intragastric balloons or intragastric saline, the recorded change is the resultant of effects from both the proximal stomach (corpus fundus) and the distal (antrum/pylorus). The effect of drugs, including tachykinins, can therefore be opposite in the proximal and distal parts, but only the overall effect is registered.

Holzer-Petsche [53] used a fixed intragastric volume of saline and measured pressure changes in rat stomachs in vivo. SP and NKA induced contractions, with SP being more potent than NKA. The effect of SP was reduced by atropine and the nerve conduction blocker tetrodotoxin (TTX), whereas the effect of NKA was unchanged. This indicated that SP acts through both cholinergic neurons and via tachykininergic muscle receptors, whereas NKA acts solely through muscle receptors. Lippe [54] found that tachykinins are also capable of inducing contractions in vitro, using isolated perfused rat stomach. Motility was measured as pressure changes at a fixed intragastric volume. NK-1 and NK-3 agonists had virtually no effect, whereas NK-2 agonist induced strong contractions. Furthermore, NK-2 antagonists reduced the effects of tachykinins. Contractions induced by tachykinins in this model therefore seem to be mediated by NK-2 receptors. Lidberg and Delbro [55, 56] studied feline stomach in vivo using intragastric balloons. SP induced contractions, an effect that was reduced by atropine. In further experiments, electrical stimulation of the distal part of the cut vagus nerves also induced hexamethonium-resistant contractions that were reduced by an SP antagonist [56]. However, in cats with chronic vagotomy there was no effect of SP antagonists [57]. Since the effect of vagus stimulation was resistant to hexamethonium, it was believed to be caused by antidromic stimulation of sensory fibers [56].

In canine stomach SP also induced contractions in vivo [58, 59]. During atropine infusion SP increased basal muscle tone, but not phasic contractions [59].

Muscle strips, in vitro. When studying motility by the use of muscle strips, the tissue is removed from its normal physiological environment and deprived of the normal influence from extrinsic nerves, hormones and

intramural reflexes. On the other hand it is possible to perform a high number of experiments with relatively small tissue samples. The strips can be oriented in the longitudinal or the circular direction. The mucosa and submucosa can be removed, and the intrinsic nerves can be stimulated using field stimulation.

The tachykinins induce contractions in both circular and longitudinal strips from several species. Circular strips from rat corpus contracted in response to NKA and SP, with NKA being most potent [53]. The effect of SP was reduced by atropine and TTX, whereas the effect of NKA was unaltered, similar to the results obtained in rat stomachs in vivo [53].

Longitudinally oriented muscle strips from rat fundus also contracted in response to SP, NKA, NKB and other tachykinin analogs. The activity of each was unaffected by TTX and atropine, indicating that the effect is not mediated by interneurons, but via muscular tachykinin receptors. Using antagonists, the receptors involved were found to be NK-2 and NK-3 receptors, but not NK-1 [60].

Longitudinal strips from rat antrum and pylorus also contracted in response to SP. The activity was not affected by TTX, but partly blocked by atropine. Hexamethonium reduced the effect of SP in antral strips, but not in the strips from the pylorus. SP therefore seems to act partly via cholinergic neurons and partly via muscle receptors also in longitudinal muscle in antrum and pylorus. In the same study it was found that a 5-HT₂ receptor antagonist reduced the effect of SP, indicating that the SP effect also involves serotoninergic neurons [61].

Jin [62] studied strips of circular muscle from guinea-pig fundus. It was found that NK-1 and NK-3 agonists induced relaxation, whereas NK-2 agonists induced contractions. The activity of NK-2 agonists was unaffected by TTX, indicating that muscular NK-2 receptors are activated. On the other hand, the relaxation induced by NK-1 and NK-3 agonists was augmented by atropine, whereas TTX converted the effect of NK-1 agonists to contractions. NK-1 agonists, therefore, appear both to activate inhibitory interneurons and activate muscular NK-1 receptors, inducing contractions. Immunoneutralization showed the inhibitory interneurons to be VIP-ergic [62].

In longitudinal canine antral muscle strips, SP, NKA and NKB all induced contractions. TTX inhibited the activity of SP and NKB, but had only a minor effect on contractions induced by NKA, indicating that NK-2 receptors are located on muscle cells, whereas NK-1 and NK-3 receptors are located on nerve cells and possibly also muscle [63]. Release of acetylcholine was measured in response to stimulation of SP, NKA and NKB. All three tachykinins caused a release of acetylcholine, with NKA being the most potent [63]. In circu-

lar strips from canine antrum and pylorus, NK-1- and NK-2-selective agonists induced contractions, whereas NK-3 agonists had no effect. The activity of NK-1 and NK-2 was unaffected by TTX [64]. Fox [65] studied the effect of SP in canine circular muscle strips from fundus and compared the effect *in vitro* to the effect of SP *in vivo* studied by a strain-gauge technique. SP induced contractions both in muscle strips and *in vivo*. SP-induced contractions were inhibited by TTX and atropine *in vivo* but not *in vitro*. Finally, SP induced contractions in muscle strips from all parts of the porcine stomach, both circular and longitudinal strips. In the proximal parts SP predominantly induced tonic contractions and in the distal part predominantly phasic contractions [66].

Strain-gauge techniques. Strain-gauge transducers can be used to record motility in separate segments of an organ, both *in vivo* and in isolated organs *in vitro*. The transducers are typically sutured onto the serosal side of an organ in either the longitudinal or the circular direction. Several strain-gauge electrodes can be employed at the same time.

The motility of canine stomach has been investigated *in vivo* by several groups using strain-gauge electrodes. Fox [65] found that SP stimulated contractions when motility was recorded in the circular direction in fundus. The effect was inhibited by TTX and atropine. In the canine antrum, *in vivo*, the natural ligands SP, NKA and NKB, as well as selective receptor agonists, induced atropine-sensitive circular contractions [64, 67]. NK-1 agonists were more potent than NK-3 agonists, again being more potent than NK-2 agonists [64]. Shibata [68] used circularly oriented strain gauges in corpus and antrum and vagally denervated fundic pouches. SP had very little effect in the corpus, but induced strong phasic contractions in antrum and the pouches, effects that were inhibited by atropine at both sites. The ganglionic blocker, hexamethonium, on the other hand, enhanced SP-induced contractions in the antrum, but reduced the contractions in the pouches. SP thus appears to stimulate postsynaptic excitatory and presynaptic inhibitory neurons. Furthermore, the vagus nerves appear to influence the activity of SP.

Pyloric flow and pressure. The motor function of tachykinins in the pyloric sphincter has been examined in a few studies. Pyloric contraction and relaxation can be measured as changes in flow from a constant pressure liquid reservoir applied to the pylorus. Pyloric contractions can also be measured with a balloon placed in the pyloric sphincter or by the use of a sleeve sensor.

Edin and Lidberg [55, 69] studied the feline pyloric sphincter, *in vivo*, by applying a constant liquid pressure across the sphincter. Contraction was recorded as reduced flow. Intraaortic administration of SP resulted

in strong contractions that could be inhibited by atropine, but not hexamethonium. Electrical stimulation of the vagus nerves also induced strong pyloric contractions. The vagally induced contractions were inhibited by administration of a peptide SP antagonist. This indicates that tachykinins could mediate extrinsic nervous control of pyloric function, at least in the cat.

Allescher [64] used a sleeve sensor to record pyloric contractions in canine stomach. The responses to SP and selective tachykinin receptor agonists were studied. SP and an NK-1 agonist induced contractions which were reduced by atropine and TTX. An NK-2 agonist induced contraction that was insensitive to atropine and TTX, whereas an NK-3 agonist had only a minor effect. The canine pylorus therefore seems to contain muscular NK-1 and NK-2 receptors and neuronal NK-1 receptors.

Other motility studies. Porcine antral motility has been studied using isolated vascularly perfused antral preparations with intact vagal innervation [70]. Motility was recorded using a suture placed at the serosa and connected to a force transducer, whereas the preparation was placed in an organ bath suspended with rubber bands. The motility recorded primarily represents the circular musculature. SP, NKA, electrical stimulation of the vagus nerves and infusion of capsaicin all induced phasic contractions. The SP- and NKA-induced contractions were inhibited by a nonpeptide NK-1 antagonist, and the vagus-induced contractions were unaffected by tachykinin antagonists, whereas the capsaicin-induced contractions could be blocked by tachykinin antagonists. The tachykinins, therefore, do not seem to be involved in vagal control of porcine antral motility. The physiological significance of the capsaicin-induced contractions is unclear.

Physiological role in motility. Based on published studies it can be concluded that tachykinins stimulate motility in all parts of the stomach, including the pyloric sphincter, but tachykinins also seem to inhibit motility in certain situations. The action of the tachykinins is mediated by muscular NK-1 and NK-2 receptors, and by NK-1 and NK-3 receptors on interneurons (primarily cholinergic neurons). Tachykinins also influence motility initiated in the central nervous system and mediated extrinsically through the vagus. The precise role of tachykinins of the different motor programs in the stomach, i.e. gastric emptying, is not clear. Most studies to date have used relatively unspecific tachykinin peptide agonists or antagonists. With the development of nonpeptide antagonists, the role of tachykinins in stomach physiology can be studied more accurately. Molecular biology with development of animals where tachykinins or tachykinin receptors have been knocked out [71] undoubtedly will provide valuable information.

Exocrine secretion

Acid secretion. Gastric acid secretion is regulated in a complex interplay of nervous, hormonal and paracrine factors. The nervous regulation of acid secretion is mediated by both extrinsic nerves, including the vagus nerves, the splanchnic nerves and reflexes involving antidromically stimulated sensory afferent nerves. The best-established neurotransmitter in regulation of acid secretion is acetylcholine, known to mediate the vagal input. Other transmitters known to affect parietal secretion are gastrin-releasing peptide (GRP), vasoactive intestinal polypeptide (VIP) and histamine from enterochromaffin like (ECL) cells [72].

The involvement of tachykinins in regulation of acid secretion has primarily been studied in rats. Intravenous infusion of SP had no effect on basal acid secretion in rats *in vivo* [73, 74], but inhibited the stimulatory effect of electrical stimulation of the vagus nerves [74]. In an isolated rat gastric fundus preparation SP also did not affect basal acid secretion, but augmented the stimulatory effect of histamine [75]. In isolated rat parietal cells SP failed to influence either basal or acetylcholine-stimulated acid secretion, but inhibited histamine-induced acid secretion [76]. Stimulation of tachykinin receptors in the brain have been shown to inhibit gastric acid secretion. Improtá [50, 77] found that intracerebroventricular administration of tachykinins inhibited acid secretion induced by histamine, but surprisingly not acid secretion induced by pentagastrin or bethanechol. The inhibitory effect was found to be mediated by NK-3 receptors. Okuma [78] found that intrathecal administration of SP inhibited acid secretion induced by electrical stimulation of the vagus nerves. The effect of electrical vagus stimulation could be inhibited by electrical stimulation of the preoptic area. Inhibition resulting from stimulation of the preoptic area could be blocked by intrathecal administration of an SP antagonist. These results indicate that the effect of stimulation of the preoptic area involves tachykininergic neurons. In dogs with chronic gastric fistula and in the Atlantic cods, SP had no effect on acid secretion [79–81], whereas SP inhibited basal acid secretion in dogs with Pavlov pouch [82]. In cats with gastric fistula, on the other hand, SP stimulated acid secretion [75]. In isolated vascularly perfused porcine fundus/antrum, SP and NKA stimulated acid secretion [28].

The involvement of sensory nerve fibers in the regulation of acid secretion has been examined using capsaicin. Limlomwongse [83] found that intraluminal infusion of capsaicin into the rat stomach, *in vivo*, stimulated acid secretion, an effect that was abolished by hexamethonium or atropine. Barrachina [84], however, found that intraluminal capsaicin had no effect on basal acid secretion, but inhibited acid secretion in-

duced by forceful distension of the stomach. Raybould [85] found that pretreatment of the vagus nerves with placement of capsaicin for 30 min around the nerves also inhibited distension-induced acid secretion. Sensory afferent nerve fibers therefore seem to be involved in reflex regulation of acid secretion induced by distension. Also, inhibition of acid secretion by acidification of the small intestine and stimulated acid secretion by protein (peptone) in the stomach seem to be influenced by sensory nerve fibers [86, 87]. These effects may be mediated by vagal sensory nerves stimulated antidromically, because acid secretion induced by electrical stimulation of the vagus nerves could be inhibited by pretreatment of the vagus nerves with capsaicin. The neurotransmitter (or neurotransmitters) mediating sensory nerve activation could be the tachykinins, but further studies are needed to establish this. In conclusion, tachykinins do not seem to act as neurotransmitters directly on parietal cells, but may have a role as modulators of other transmitters influencing acid secretion.

Pepsinogen secretion. Few studies have addressed the involvement of tachykinins in regulation of pepsinogen secretion. In the cod stomach SP potently stimulated pepsinogen secretion [81], and pepsinogen secretion was also stimulated by SP in chief cells isolated from guinea pigs or dogs. The effect involved NK-1 receptors [88, 89]. In isolated vascularly perfused pig corpus/fundus, SP and NKA pig strongly stimulated pepsinogen secretion [28]. Given the fact that tachykininergic nerve fibers are often found in the vicinity of the gastric chief cells and that gastric release of tachykinins can be demonstrated in response to electrical stimulation of the vagal innervation of the pig stomach, the tachykinins should be considered potential regulators of pepsinogen secretion in this species.

Endocrine secretion

Gastrin is secreted from the G-cells in the antral mucosa, whereas somatostatin is secreted from D-cells throughout the stomach mucosa. The effect of tachykinins on gastrin and somatostatin secretion has been examined both *in vivo* and *in vitro*.

In isolated vascularly perfused rat stomach, infusion of tachykinins decreased somatostatin secretion, with NKA and NKB being more potent than SP. The activity was unaffected by atropine. [90–92]. Basal gastrin secretion was not influenced by SP infusion [91]. Capsaicin increased somatostatin secretion, but this effect could be blocked by a CGRP antagonist, indicating that CGRP rather than the tachykinins are involved in the effect of capsaicin in isolated rat stomach preparations [93].

The involvement of tachykinins in regulating gastrin and somatostatin secretion in pig stomach was investi-

gated using isolated perfused porcine antrum or corpus/fundus preparations [28, 94]. Infusions of SP and NKA decreased secretion of gastrin and increased secretion of somatostatin via NK-1 and NK-2 receptors. In this preparation somatostatin acts as a powerful paracrine inhibitor of gastrin release [95]. The tachykinins could therefore potentially decrease gastrin secretion via increased somatostatin secretion. However, infusion of monoclonal somatostatin antibodies did not affect the inhibitory effect of SP on gastrin secretion, indicating that the tachykinins do not act via somatostatin.

Electrical stimulation of the vagus nerves of the isolated perfused porcine antrum leads to increases in gastrin secretion [95], but the vagal trunks also carry inhibitory fibers [96]. Addition of NK-1 and NK-2 antagonists during electrical vagus stimulation augmented gastrin secretion, indicating that tachykinins may mediate the vagal inhibitory regulation of gastrin secretion.

In corpus/fundus preparations SP and NKA decreased somatostatin secretion, in contrast to the stimulatory effect in antrum preparations [13]. A similar differential regulation of antral and fundic somatostatin secretion was observed with respect to the GRP-ergic nerves [97]. In conclusion, the role of tachykinins in regulating gastrin and somatostatin secretion cannot be established from the few studies published, and further studies are needed. A function as modulator of vagal regulation of secretion seems to exist, however.

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