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Review

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Neurokinin receptors in the gastrointestinal muscle wall: cell distribution and possible roles

Abstract: The neurokinin receptors are G-protein-linked receptors; three distinct molecules, called neurokinin-1, neurokinin-2, and neurokinin-3 receptors, have been identified. Their physiological ligands are the tachykinins, which, in the mammalian gut, correspond to substance P, neurokinin A, and neurokinin B. In this apparatus, the main source of tachykinins is represented by intrinsic neurons located either in the myenteric plexus and projecting mainly to the muscle coat, or in the submucous plexus and projecting to the mucosa and submucosal blood vessels. The availability of specific antibodies has allowed identifying the sites of distribution of the neurokinin receptors in the gut, and important differences have been found among cell types and animal species. The complexity of the receptor distribution, either intraspecies or interspecies, is in agreement with the variegated picture coming out from physiological and pharmacological experiments. Interestingly, most of the knowledge on the tachykinin systems has been obtained from pathological conditions. Here, we tried to collect the main information available on the cellular distribution of the neurokinin receptors in the gut wall in the attempt to correlate their cell location with the several roles the tachykinins seem to play in the gastrointestinal apparatus.

Keywords: gastrointestinal apparatus; neurokinin; tachykinin.

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Introduction

The neurokinin receptor family consists of three distinct proteins called NK1r, NK2r, and NK3r, all sharing the capacity to be internalized in response to agonist binding (1–3). The neurokinin receptors are G-protein-linked receptors. Their physiological ligands are small peptides called tachykinins (TKs), among which the most represented in the mammalian gut are substance P (SP), neurokinin A (NKA), and, to a much lesser extent, neurokinin B (NKB) (4). Each of the TKs bind to the neurokinin receptors with different affinity, NK1r being the preferred receptor of SP, NK2r the preferred receptor of NKA, and NK3r the preferred receptor of NKB (5).

Unlike other systems, the most abundant source of TKs in the gastrointestinal tract is represented by intrinsic (enteric) neurons, localized in the myenteric and submucosal plexuses and projecting to all tissue layers of the gut. Most of the TK nerve fibers originating from the myenteric neurons project to the circular muscle layer (CML), although 50% of the nerve fibers present in the longitudinal muscle layer (LML) are SP positive. The innervations of the circular and longitudinal smooth muscle layers mediate most of the excitatory motor activity.

Conversely, the majority of the SP-containing neurons of the submucosal plexus project to the mucosa (6, 7) and submucosal blood vessels (8), and almost all are sensory neurons likely involved in the secretomotor reflexes and vasodilatation (9). TKs stored in enteric neurons can be released by mechanical (i.e., intestinal wall distension) or chemical (synaptic input) stimuli having a physiological relevance (4, 10). Spinal and vagal primary afferent neurons and immune cells contribute the rest of the TK content in gastrointestinal organs.

Studies using agonists and antagonists have shown that the actions of TKs depend on the different distribution of the neurokinin receptors and on their distinct affinities for them (11–13). The availability of specific antibodies has allowed identifying the sites of distribution of the neurokinin receptors in the gut wall of several laboratory mammals (13–21), showing an important difference in neurokinin receptor distribution among cell types and animal species. The present review attempts to update the knowledge on the cell distribution and roles of the neurokinin receptors and related TKs in mammals. Special

attention will be placed on their presence and role in the gut muscle coat.

Neurokinin receptor distribution in the gastrointestinal tract

Neurokinin-1 receptor (NK1r)

NK1r-immunoreactivity (IR) was first detected in the enteric neurons and in the interstitial cells of Cajal located in the deep muscular plexus (ICC-DMP) (16, 19, 22), using an antibody made by Vigna and co-workers (23). Further, Vannucchi and co-workers (20) showed that, in the rat and guinea pig ileum, NK1r was also located on the ICC-MP but their staining was less intense. Moreover, only the NK1r-IR ICC-DMP were closely associated with the SP-IR nerve endings, suggesting that this ICC population is the main target of TK neurotransmission (18).

In the mouse ileum, NK1r-IR was also found in special cells located in the stroma of the villi, which, under the transmission electron microscope, were identified as the myoid cells (13). The lack of detection of NK1r in the villi of rat and guinea pig was attributed to receptor scarcity or a different conformation rather than to its real absence. In this regard, several pharmacological findings suggested the existence of a variety of NK1r, the so-called septide-sensitive receptor (24). Further, by pharmacological manipulation (25, 26) or using different antibodies (3, 27), it was possible to demonstrate that the smooth muscle cells (SMCs) also express NK1r, a possibility that was already envisaged from pharmacological studies (28). In humans, NK1r has also been detected in the muscularis mucosae and in the media of the submucosal blood vessels (28).

In neurons, NK1r-IR is mainly distributed along the plasma membrane (13, 16); double-labeling experiments have clarified that this receptor is primarily located at the postsynaptic sites, with a net prevalence, up to 85% in the guinea pig small intestine, on nitrergic inhibitory neurons (Figure 1) (16, 29, 30) and in minor part on excitatory (cholinergic) neurons [see ref. (28) for a review]. With regard to a possible control of TK release (presynaptic location), NK1r has been found on very few myenteric cholinergic and some submucosal acetylcholine (Ach)/SP-positive neurons of the mouse ileum (29). In the SMCs, the receptor is still located along the plasma membrane of the majority of the cells. In both types of cells, neurons and SMCs, the interaction with agonists induces receptor endocytosis (internalization), causing a loss of affinity by the same cells to other molecules of the agonist (downregulation in the presence of the agonist) (1).

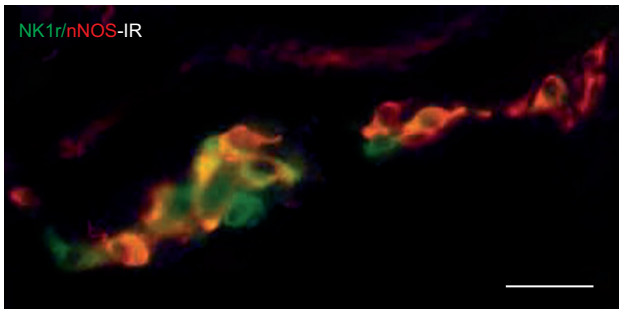

Figure 1 Mouse stomach.
Double labeling with NK1r and nNOS antibodies. In a myenteric ganglion, some neurons are NK1r-IR (in green), some are nNOS-IR (in red), and some others are NK1r/nNOS-IR (in orange). Calibration bar, 40 μm [From Ref. (30) with permission].

Neurokinin-2 receptor (NK2r)

NK2r-IR has been described in the SMCs of the entire gastrointestinal muscle coat of the most common laboratory mammals and humans (3, 13, 14, 17, 30, 31). Interestingly, in the CML of the mouse ileum, it has been reported that the inner portion [inner CML (ICML)] has a higher density of NK2r-IR compared with the outer portion (13). The receptor is located along the cell contour of the majority of the SMCs, and its binding to the agonist causes internalization (Figure 2) (2, 3).

Interestingly, NK2r was also found in nerve varicosities located in the muscle layers, in the myenteric and submucosal plexuses of rats and guinea pigs, and in the DMP nerve endings of the mouse ileum (13, 14, 17). This distribution indicates a presynaptic role for this receptor. Double-labeling studies demonstrated a co-distribution for many of the NK2r-IR varicosities with SP (Figure 3) (21) or with neuronal nitric oxide synthase (nNOS) (17), suggesting a complex control by NK2r of either excitatory (SP and likely Ach) or inhibitory (nNOS) neurotransmission. Furthermore, the richness in NK2r-positive varicosities at the DMP associated with the consistent presence of NK2r in the ICML seems to indicate a prevalence of this receptor as compared with NK1r in this region (13). Finally, NK2r-IR was found in the enterocytes of the guinea pig ileum and colon (13, 17).

Neurokinin-3 receptor (NK3r)

NK3r-IR has been detected in the myenteric and submucosal neurons of rats, mice, and guinea pigs (13–15). The labeling was mainly distributed along the plasma membrane of the perikaryon and of the more proximal

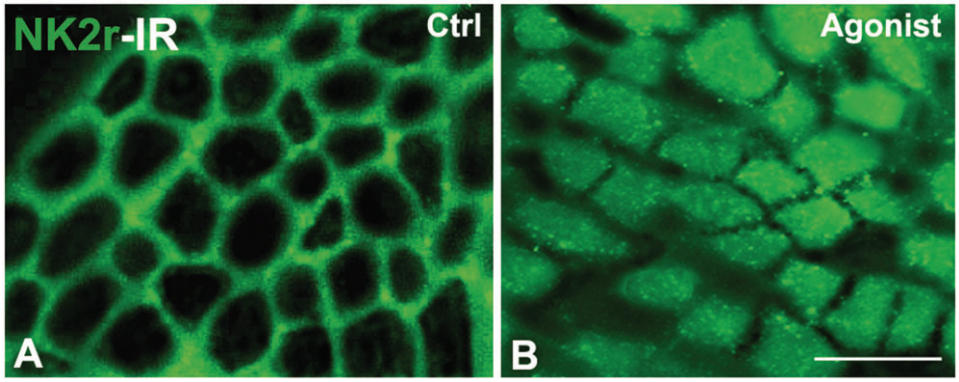

Figure 2 Human colon.
NK2r labeling under a confocal microscope. IR appears as small brilliant green granules. (A) Cells with a non-internalized NK2r. IR is distributed along the cell contour. (B) Cells with internalized NK2r particles. The specimen was treated with 1 μM of the NK2r agonist [βAla^8]NKA (4–10). The IR granules are deeply located within the cytoplasm. In some cells, the nucleus is completely masked. Calibration bar, 10 μm (Vannucchi and Evangelista, Unpublished data).

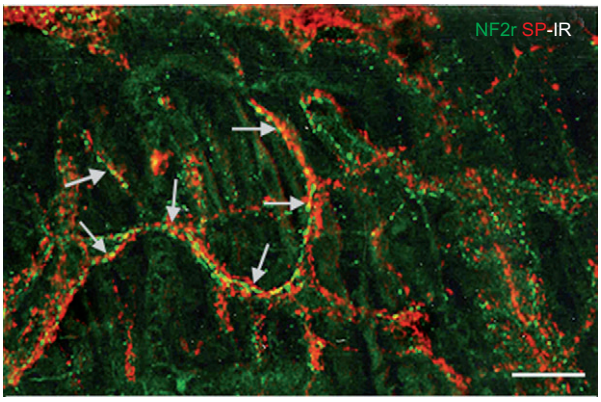

Figure 3 Rat ileum.
Double labeling with NK2r and SP antibodies (whole mount). Numerous NK2r-IR (in green) and SP-IR (in red) varicose fibers at the myenteric plexus area. Many but not all of the NK2r-IR varicosities overlap the SP-IR ones (arrows). In the background, the spindle-shaped and green-labeled cells are the smooth muscle cells of the LML that are NK2r-IR. Confocal microscope. Calibration bar, 20 μm [From, Ref. (21), with permission].

portion of neuronal processes but also partly in the cytoplasm (13, 32). Although double labeling with TK markers are lacking, physiological investigations suggest a main presynaptic role for NK3r that should be located on SP- and SP/ACh-carrying neurons (autoreceptors) (33–35).

NK3r-IR has been found in the LML of the guinea pig (36), rat (37), and mouse (13) small intestine. Furthermore, the latter authors reported the presence of NK3r in the SMCs forming the ICML. NK3r labeling in the SMCs is distributed along the cell surface, although in the ICML, because of the extreme thinness of the cells, the entire cytoplasm seems labeled (Figure 4) (13).

Biological actions mediated by the neurokinin receptors having physiological/trophic relevance

Several pharmacological, functional, and morphological studies (38–42) have identified TKs as major non-cholinergic excitatory neurotransmitters at the gastrointestinal level and shown that TKs play numerous roles in the gut, some of which even opposite (4, 5, 28) (Table 1).

Neurokinin-1 receptor (NK1r)

The role of NK1r in muscle contractility is complex and not yet well defined. Its presence, with important differences in intensity, on almost all the cell types responsible for this function might explain the often unexpected responses obtained after functional and/or pharmacological stimuli. By using specific NK1r agonists or antagonists, it was possible to measure an increase or a decrease, respectively, of muscle contractility (4, 10, 43, 44) and these responses were attributed to the presence of NK1r in the SMCs. However, the role in mediating direct muscle contraction is likely in support of that due to NK2r (see below) and, with respect to NK2r, is much less significant in the economy of the TK excitatory effects on gut motility.

A main role of NK1r is instead in mediating the inhibitory component attributed to the TKs. In several species and gut regions, it has been reported that administration of NK1r agonists led to an inhibitory response that was prevented by specific antagonists (45). Interestingly, this effect is not always immediately appreciable and sometimes needs to

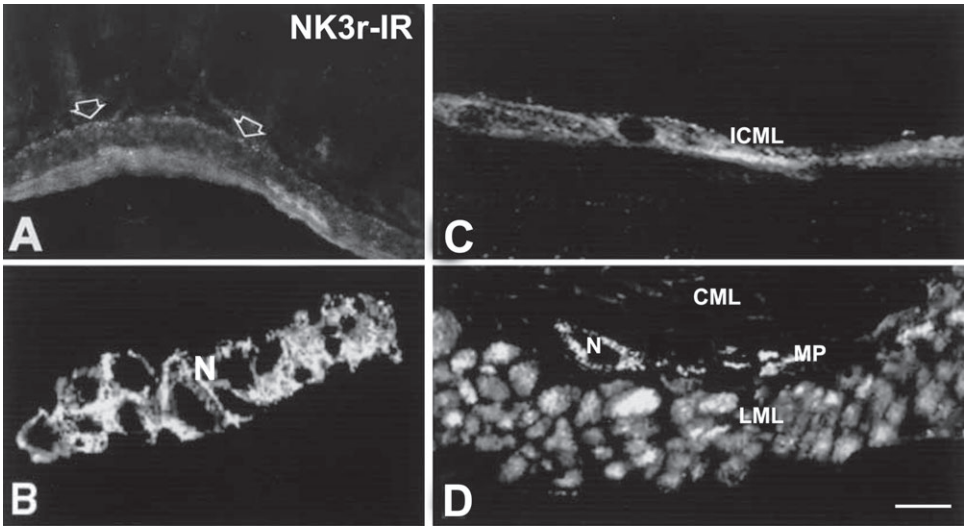

Figure 4 Mouse and guinea pig ileum NK3r-IR.
(A) Mouse. NK3r-IR is present at the myenteric plexus, the LML, and the innermost portion of the CML (arrows). (B) Mouse. Detail of NK3r-IR neurons at the myenteric plexus. (C, D) Guinea pig. NK3r-IR is present at the innermost portion of the CML (ICML), (C) and at the myenteric plexus (MP) and LML. (D) Transverse sections: (A) conventional microscope; (B–D) confocal microscope. Calibration bars: (A) 55 μm, (B) 6 μm, (C) 8 μm, and (D) 10 μm (From: Ref (13), with permission).

Table 1 Main pathophysiological effects mediated by the neurokinin receptors.

Effect	Receptors
Gastrointestinal smooth muscle contraction of both circular and longitudinal layers; contribution to peristalsis (under physiological conditions) or to exaggerated intestinal motility (associated with inflammatory or infectious diseases, e.g., diarrhea)	NK1r/NK2r/NK3r
Gastrointestinal smooth muscle relaxation (afforded by inhibitory transmitters released from enteric neurons); contribution to postsurgical intestinal atony	NK1r/NK3r
Neuro-neuronal communications in enteric plexuses, leading to release of excitatory (Ach, TKs) or inhibitory (NO, VIP) transmitters from enteric neurons	NK3r/NK1r/NK2r
Water/ion secretion from the intestinal epithelium	NK1r/NK2r/NK3r
Genesis and/or maintenance of symptoms associated with acute or chronic inflammatory diseases (Chron's disease, ulcerative colitis, etc.) and pancreatitis	NK1r
Pain arising from gastrointestinal system (visceral nociception)	NK2r (NK3r)

be unmasked by blocking the excitatory activity mediated by NK2r. Furthermore, the inhibitory component of TKs on gut contractility was reduced in the presence of drugs able to block NOS activity, indicating that the inhibitory role attributed to SP was likely mediated by NK1r located on nNOS-positive neurons (Figure 5) (30, 45–47). Finally, the observation that NK1r agonists inhibit, and NK1r antagonists cause some facilitation of, peristaltic motor activity (45) suggests an inhibitory role of NK1r also in peristalsis.

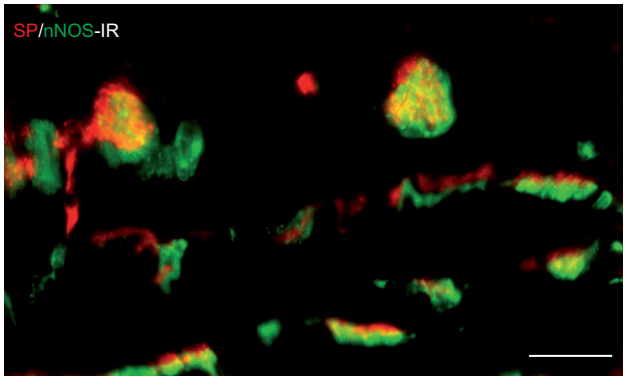

Figure 5 Rat colon.
Double labeling with SP and nNOSβ antibodies. Numerous SP-IR nerve endings (in red) are close to the nNOS-IR neurons (in green) and the intramuscular SP-IR nerve fibers run parallel to the nNOS-IR ones. Calibration bar, 40 μm (Vannucchi, Unpublished data).

Neurokinin-2 receptor (NK2r)

NK2r plays a main role in muscle contractility (48) and mediates most of the spasmogenic effects produced by TKs

on human gastrointestinal organs such as the ileum (49), colon (50), and esophagus (51). Less clear is the role of the neurokinin receptors in (gastro)intestinal peristalsis. *In vivo* studies in which intestinal contractions were evoked by mechanical stimuli (e.g., intestinal wall distension) have shown that endogenous TKs were actually involved [see ref. (35) for review] but their contribution to intestinal peristalsis was hardly appreciable under normal conditions, unless muscarinic receptors were blocked (52–55). This concept was reinforced by the studies performed in unanesthetized dogs (56) and in healthy human volunteers (57), in which the NK2r selective antagonist nepadutant (58) did not affect gastrointestinal normal motor activity *per se*. Nevertheless, other authors (59) have shown that simultaneous administration of NK1r, NK2r, or NK3r antagonists produced a 50% inhibition of velocity of propulsion of a balloon inserted in isolated segments of the guinea pig distal colon. This study indicates that for a conclusive statement on the contribution of TKs to normal peristalsis, further studies in which all neurokinin receptors are simultaneously blocked are required.

Neurokinin-3 receptor (NK3r)

Similarly to NK2r, NK3r mainly mediates excitatory responses on the muscle coat (28); however, for a better understanding of its role, it is important to remember that NK3r binds with the highest affinity to NKB, but this peptide is very scarce in the gut (4). Therefore, it might be reasonable to hypothesize that the recruitment of NK3r occurs when the TK system is overactivated and/or the other two neurokinin receptors are downregulated. In the small intestine of rats and guinea pigs, NK3r agonists cause LML contraction (60, 61). Atropine inhibits the contraction owing to NK3r activation, but is less effective than tetrodotoxin (60, 61), as the contractile response is mediated by the release of both ACh and TKs stimulating NK3r on the muscle (61, 62).

Neurokinin receptors and the intestinal stretch receptor

As mentioned above, TKs stored in enteric neurons can be released by mechanical stimuli such as wall distension. Local distension stimuli mediate food absorption, which, indeed, is the main function of the gastrointestinal apparatus. The importance of the local response to the presence of food has brought about the hypothesis of the existence of a stretch receptor (63), whose anatomical components will be restricted to those forming the inner portion of the CML, such as the DMP, the ICC, and the thin layer of SMCs forming the ICML (13). Intriguingly, as each of these components express one or even two neurokinin receptors (nerve varicosities are NK2r-IR; ICC are NK1r-IR; SMCs of the ICML are either NK2r-IR or NK3r-IR), it appears that the TKs have a main role in mediating the stretch function and all the three peptides seem to be actively involved.

NK1r as trophic factor during intestinal development

An area that deserves mention is the study of the time course of TK expression during gut development. Experiments done in fetuses, neonates, and adult rats have shown an early appearance (7 days after birth) of SP and NK1r expression in the myenteric neurons and in the ICC located at the DMP. In particular, at 7 days of postnatal life, the NK1r in the ICC was expressed at level comparable to those detected in the adult. This finding has brought about the hypothesis that NK1r might exert a trophic role either on the ICC, facilitating their differentiation, or on the nerve terminals carrying TKs, stimulating the production of the neurotransmitter (19, 64). Finally, the high expression of NK1r in the ICC-DMP during the period of changing from a liquid to a solid diet is in favor of a very early role of the TK system in mediating local stimuli such as distension.

Neurokinin receptors as regulators of gut homeostasis

The richness of neurokinin receptors in the mucosa and submucosa of the gastrointestinal tract has been related to a possible trophic role of these receptors in regulating (enhancement) ion and fluid secretions in the small and large intestines. At this level, TKs would act as intermediate modulators by stimulating secretomotor neurons, which in turn release Ach and other non-cholinergic transmitters, which would be the final mediators (4, 10, 43). All three neurokinin receptors likely mediate the effects of TKs (65). To this regard, it should be mentioned that, in the rat colon mucosa, the NK2r mediating increase in short-circuit current (Isc) responses has been found to be functionally distinguishable from the NK2r mediating contractions in the muscularis mucosae (66). Thus, TK-containing primary afferent neurons could play a homeostatic role in the intestine as well as in the stomach. At this level, the neuropeptide CGRP (co-released from the

sensory nerve terminals with TKs) has been reported to play a trophic role by increasing blood flow in the gastric mucosa to facilitate the removal of the noxae (43).

Biological effects mediated by neurokinin receptors having pathological relevance (Table 1)

Neurokinin receptors and gastrointestinal inflammatory diseases

The importance of the neurokinin receptors in mediating motor and sensitive signs and symptoms associated with various inflammatory and infectious intestinal diseases (67, 68) has been well established by several experimental evidences. It has been shown that NK2r (and partially NK1r) antagonists may prevent increased fecal excretion and colonic giant contraction in castor oil-induced diarrhea in rats, without producing constipation (69). Likewise, the NK2r antagonist MEN 10.627 reduced stress-induced defecation and mucin release in rats (Figure 6) (68). Carini et al. (70) have shown that the exaggerated intestinal motility caused by chemical irritation of the rat colonic mucosa depends on an enhancement of cholinergic transmission mediated by NK2r. The latter could be located on nerve varicosities as presynaptic heteroreceptors (14). In a rat model of trinitrobenzensulfonic (TNBS)-induced colitis, a decrease in TK content and NK1r and NK2r mRNAs has been reported as an early consequence of the inflammatory reaction (71).

In human chronic inflammatory bowel diseases, NK1r has been found markedly upregulated compared with the concentration in normal subjects, and the application of the NK1r antagonist SR 140333 was able to reduce the associated hypersecretory activity (72). Renzi et al. (73) have shown that, in addition to NK1r, NK2r expression was also markedly increased in inflammatory cells (eosinophils) of the lamina propria of patients with Crohn's disease or ulcerative colitis, suggesting their possible involvement in the above pathologies. The role of NK1r in mediating inflammatory reactions of the gut has recently been supported by the use of NK1r-knockout mice, which showed reduced intestinal symptoms and tissue damage produced by *Clostridium difficile* toxin A compared with control animals (74, 75).

Changes in the expression of NK1r in neurons and the ICC and NK2r in the SMCs of the CML, were reported in rats infected with *Nippostrongylus brasiliensis*, which could explain the loss of contractile activity associated with the disruption of migrating motor complex present in these animals (76).

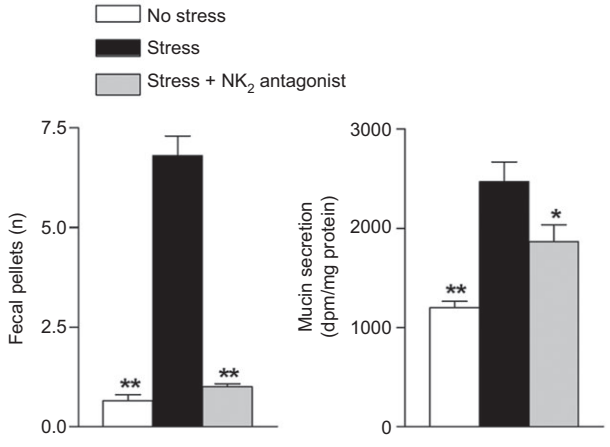

Figure 6 Effect of the TK NK2r antagonist MEN 10627 on stress-induced defecation and mucin release in rats.
Stress was induced by a 30-min restraining period; MEN 10627 (100 μg/kg, i.v.) or vehicle was given 5 min before the immobilization (data from Castagliuolo and Evangelista). * and **, $p<0.05$ and $p<0.01$, respectively, as compared with the stress group (filled bars, treated with vehicle). Mean±SEM of four to six rats for each group.

Neurokinin receptors and visceral nociception

Recent studies on the use of neurokinin receptor antagonists in various animal models of visceral nociception have shown that these receptors mediate pain arising from the gastrointestinal system. NK2r antagonists have been found effective in reducing reflex responses (e.g., abdominal contractions) caused by painful stimuli, such as rectal wall distension (77) or intraperitoneal administration of acetic acid (78). Moreover, NK2r antagonists have been proven effective in animal models of visceral hyperalgesia induced by inflammation (79) or stress (80, 81). In particular, the results obtained by Toulouse et al. (80), showing the ability of nepadutant to inhibit rectal hypersensitive responses in rats pretreated with TNBS or previously subjected to restraining, suggest that NK2r has a main role in mediating visceral allodynia/hyperalgesia. This main role is further supported by the studies of Birder et al. (82), who showed that the increased expression of either *c-fos* and *c-jun* protooncogene markers in the spinal cord and dorsal root ganglia neurons of rats pretreated with TNBS is prevented by nepadutant, and by Laird et al. (83), who found nepadutant to be capable of preventing the hypersensitivity of single spinal cord neurons responding to colorectal distension or pelvic nerve stimulation in rats pretreated with intracolonic acetic acid.

Laird et al. (84) have shown that intracolonic instillation of acetic acid or capsaicin fails to produce either acute (cardiovascular) responses or primary hyperalgesia in NK1r-knockout mice, thus suggesting a role for even this latter receptor as a mediator of visceral hyperalgesia.

Also, NK3r (located at the peripheral or/and central level) could play a role in visceral hyperalgesia owing to the reported effectiveness of either intraperitoneal (85) or intrathecal (86) administration of SR 142801 (a NK3r selective antagonist) in reducing reflex abdominal contractions elicited by various nociceptive stimuli.

Neurokinin receptors and functional gut diseases

Neurokinin receptor antagonists have been proposed for the treatment of functional gastrointestinal disorders characterized by visceral pain associated with motor disorders, such as the irritable bowel syndrome (IBS) (87). With regard to this, it should be mentioned that an NK2r antagonist (nepadutant) has been proven effective in preventing the increase of gastrointestinal motility provoked by systemic administration of NKA in healthy volunteers (57). Interestingly, the action of NKA on gastrointestinal motility was accompanied by a number of adverse effects, part of which resemble symptoms characterizing IBS. Furthermore, otilonium bromide, a drug successfully used to treat IBS (88), acts as an NK2r antagonist preventing the receptor internalization in the human colon (3).

However, it has also been reported that TKs produce inhibitory motor effects following stimulation of NK1r, NK2r, or NK3r (35) present in enteric inhibitory neurons, which in turn release nitric oxide (45, 89, 90). With regard to this, Toulouse et al. (91) have shown that the NK2r selective antagonist nepadutant significantly shortens the surgery-induced inhibition of intestinal motility (ileus) in rats, thus proving the involvement of NK2r in this model and suggesting a possible use of NK2r antagonists in postoperative ileus.

Neurokinin receptors and genetic diseases

In *mdx* mice, an animal model for Duchenne muscular dystrophy, decreased responsiveness and expression of NK2r in the gastric SMCs have been associated with the increased gastric tone present in these mice (92). Of note, the change in NK2r was accompanied by a significant decrease in myogenic nNOS, with both conditions likely due to the absence of the structural protein dystrophin. Mice with a mutation in the W locus (c-kit mutant mice) showed important changes in NK1r expression in the enteric neurons (internalization) and in the ICC located at the DMP (loss), associated with a significant increase in SP content interpreted as an attempt to compensate the NK1r loss at the ICC-DMP (93). In the ileum of caveolin 1 knockout (Cav-$1^{-/-}$) mice, a reduction in the pacing frequencies and an impairment of contractile activity and both attributed to the loss of several signaling molecules commonly bound to the Cav1 protein (Daniel et al., 2006). Investigation on the neurokinin receptors showed significant changes in the cell distribution of NK1r and NK2r (internalization) in the SMCs associated with a significant decrease in SP content (27).

Conclusion

Neurokinin receptors mediate the different actions of the TKs released from enteric neurons. The complexity of the distribution of this receptor, either intraspecies or interspecies, is in agreement with the variegated picture coming out from physiological and pharmacological experiments. Interestingly, most of the knowledge on the TK systems has been obtained from pathological conditions. Indeed, a large body of preclinical evidence indicates that neurokinin receptors mediate the genesis and/or the maintenance of signs and symptoms accompanying various human diseases such as inflammatory bowel diseases and IBS. Up to now, among the numerous clinical trials testing different neurokinin receptors in human gastrointestinal diseases, none has produced a commercially available drug yet (94). Nevertheless, giving maximal attention to the potential side effects, the ability of these drugs to prevent or reduce neurokinin receptor-mediated effects in experimental animal models of gut diseases justifies the expectation that neurokinin receptor-based drugs will afford therapeutically relevant effects in humans.

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