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Tachykinin receptors and gastrointestinal motility: focus on humans

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Abstract. – Peptides of the tachykinin (TK) family were first discovered in the gastrointestinal tissue about 75 years ago and supposed to be involved in gastrointestinal (GI) motility. This hypothesis has been repeatedly proven, although the role of TKs on motility is modulatory rather than pivotal. Furthermore, beyond the well known excitatory role, it has been acknowledged that TKs can also inhibit GI motility. TKs act at 3 receptors termed as TK NK_1 (NK_1r), NK_2 (NK_2r), and NK_3 (NK_3r) receptors. The view gained through intense preclinical research suggested that motor effects induced by the stimulation of NK_2r were prominently mediated by a direct action on smooth muscle, those produced by the stimulation of NK_1r were due to both muscular and neuronal effects, whereas the motor effects induced by NK_3r were exclusively mediated by neuronal effects. Recent functional and anatomical findings in humans are challenging this concept since NK_2r have been found in several kinds of myenteric neurons and selective NK_2r antagonists can, in particular conditions, produce GI motor effects likely related to a neuronal site of action. Furthermore, the evidence for a myotropic role of NK_1r is scarce, and very few studies, if any, have documented a functional role for NK_3r. The findings that an acute or a long lasting blockade of NK_2r does not alter normal GI functions and that these receptors can modulate visceral sensitivity are good starting points for testing this class of drugs in GI diseases characterised by altered GI motility.

Key Words:

Clinical studies, Aprepitant, Nepadutant, Talnetant.

Introduction

Substance P (SP), the most famous component of the tachykinin (TK) peptide family, was first extracted from the horse brain and intestine by von Euler and Gaddum in 1931. The same authors observed that this extract had myotropic activity on isolated intestinal segments and postulated that SP (P means powder, the physical form of the extract) was released in the intestine and was the mediator responsible for its movements. This hypothesis was verified 50 years later when it became evident that TKs were, together with acetylcholine, the main excitatory transmitters to the gastrointestinal (GI) smooth muscle[1]. During the course of these 50 years the TK family enlarged. Erspamer discovered, characterized and sequenced from non-mammalian species peptides with SP-like biological activity which were named tachykinins[2], i.e, fast relaxants of vascular smooth muscle, which were later shown to have sequence homology to SP[3]. In the early 1980s, three independent groups discovered novel mammalian TKs which were named neurokinin A (NKA) and B (NKB), and soon after elongated forms of NKA were described (neuropeptide-kappa and -gamma). At the eve of the new millennium, novel mammalian TKs were identified: hemokinin-1 (HK-1) and its elongated forms endokinin A and B[4,5] (Table I).

TKs must share the common amidated C-terminal motif Phe-X-Gly-Leu-Met-NH_2 (were X has to be a non-polar amino acid) to exert biological functions through TK receptors[5-7] which have been termed NK_1 (NK_1r), NK_2 (NK_2r), and NK_3 receptors (NK_3r) (Table I). N-terminal SP metabolites, such as SP[1-5], also exert biological effects, although these effects are not mediated by TK receptors but could involve a site regulating the expression of delta opioid receptors[8].

TKs are encoded by 3 genes termed TAC1 (SP, NKA, neuropeptide-gamma and -kappa), TAC3 (NKB), and TAC4 (HK-1, endokinin A, B, C, and D) and each of these genes produces multiple mRNA isoforms. Thus, both the beta- and gamma-TAC1 mRNAs produce both SP and NKA, whereas both alpha- and delta-TAC1 only encode SP.

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Table I. Amino acid sequence of tachykinins and tachykinin-related peptides and their receptor preference. In bold, the common C-terminal sequence. Receptor preference has been assessed in functional experiments on Ca^{2+} mobilization or luciferase assay (both responses are dependent on phospholipase C activation) in cells expressing human NK_1 NK_2 NK_3 receptors[5-7].

Peptides	Amino acid sequence	Receptor preference
Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	$NK_1 > NK_3 > NK_2$
Neurokinin A	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2	$NK_2 > NK_1 > NK_3$
Neuropeptide-gamma	Asp-Ala-Gly-His-Gly-Gln-Ile-Ser-His-Lys-Arg-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2	$NK_2 > NK_1 > NK_3$
Neuropeptide-kappa	Asp-Ala-Asp-Ser-Ser-Ile-Glu-Lys-Gln-Val-Ala-Leu-Leu-Lys-Ala-Leu-Tyr-Gly-His-Gly-Gln-Ile-Ser-His-Lys-Arg-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2	$NK_2 > NK_1 > NK_3$
Neurokinin B	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2	$NK_3 > NK_2 > NK_1$
Hemokinin-1	Thr-Gly-Lys-Ala-Ser-Gln-Phe-Phe-Gly-Leu-Met-NH_2	$NK_1 > NK_3 > NK_2$
Endokinin A	Asp-Gly-Gly-Glu-Glu-Gln-Thr-Leu-Ser-Thr-Glu-Ala-Glu-Thr-Trp-Val-Ile-Val-Ala-Leu-Glu-Glu-Gly-Ala-Gly-Pro-Ser-Ile-Gln-Leu-Gln-Leu-Gln-Glu-Val-Lys-Thr-Gly-Lys-Ala-Ser-Gln-Phe-Phe-Gly-Leu-Met-NH_2	$NK_1 > NK_3 > NK_2$
Endokinin B	Asp-Gly-Gly-Glu-Glu-Gln-Thr-Leu-Ser-Thr-Glu-Ala-Glu-Thr-Trp-Glu-Gly-Ala-Gly-Pro-Ser-Ile-Gln-Leu-Gln-Leu-Gln-Glu-Val-Lys-Thr-Gly-Lys-Ala-Ser-Gln-Phe-Phe-Gly-Leu-Met-NH_2	$NK_1 > NK_3 > NK_2$
Endokinin C	Lys-Lys-Ala-Tyr-Gln-Leu-Glu-His-Thr-Phe-Gln-Gly-Leu-Leu-NH_2	Antagonist at NK_1
Endokinin D	Val-Gly-Ala-Tyr-Gln-Leu-Glu-His-Thr-Phe-Gln-Gly-Leu-Leu-NH_2	Antagonist at NK_1
Hemokinin-1*	Arg-Ser-Arg-Thr-Arg-Gln-Phe-Tyr-Gly-Leu-Met-NH_2	$NK_1 > NK_2 > NK_3$

*Rat and mouse sequence.

TK receptors belong to class 1 (rhodopsin-like) seven transmembrane G-protein-coupled receptors. All tachykinin receptors can activate classical transduction mechanisms linked to phospholipase C activation, and all natural TKs having the common C-terminal sequence act as full agonists on these pathways, whereas their intrinsic activity can vary when measured on other G-protein mediated pathways (e.g., increase of cyclic adenosine monophosphate). TKs can also exert biological effects through non G-protein mediated mechanisms (e.g., sustained activation of mitogen-activated kinases) but the agonist order of potency or intrinsic activity is unknown for this transduction pathway[9]. Interestingly, endokinin C and D, which have been termed as TK-related peptides because both of them have a Leu residue replacing the common C-terminal Met of TKs[10] exert an antagonist effect through NK_1 receptors[11].

In the GI tract TKs and their receptors are localized on many cell types, including neurons and nerve fibres and exhibit a remarkable degree of plasticity in response to environmental changes or diseases. As a matter of fact, there is evidence that TKs play a role in several aspects of GI function such as immune-inflammatory processes, tissue integrity, intestinal barrier function, carcinogenesis, blood supply, secretion, afferent signaling and motor regulation. Although all these aspects can influence each other, this article is focussed on the role of TKs and their receptors on GI motor regulation exerted at the peripheral level with particular emphasis on evidence collected in humans. In this respect, it is worth noting that TKs can also modulate GI motility by acting at sites located in the central nervous system, as it occurs for the anti-emetic action produced by blood-brain barrier-penetrating NK_1r antagonists.

Expression of TKs and Their Receptors in the GI Tract

In the GI tract the most abundant TK-encoding mRNAs are beta- and gamma-TAC1. TAC4 transcripts have also been detected, although these are likely to be related to immune, rather than motor function. A few papers have described the expression of NKB and none that of TAC3, in spite of the robust evidence (especially

in animals) for the expression of NK_3r in the gut[12]. This pattern of TK gene expression implies that SP and NKA are the most abundant TKs in the GI tract and are always colocalized. Thus, SP and NKA are expressed on nerve fibres from both extrinsic neurons and intrinsic neurons as well as in somata and varicosities of intrinsic neurons. A scheme of TK expression in the GI tract has been outlined on the basis of morphological evidence obtained in laboratory animals[13]. According to this scheme, TKs are expressed in: (i) intrinsic primary afferent neurons (IPANs) which contain both choline acetyltransferase and calbindin and project to both circular muscle (CM) and mucosa; (ii) secretomotor neurons having the same chemical coding as above; (iii) ascending myenteric interneurons which contain both choline acetyltransferase and calretinin; (iv) ascending (excitatory) motor neurons to the CM with long projections colocalizing choline acetyltransferase, neurofilament protein and enkephalin; (v) ascending (excitatory) motor neurons to the CM with short projections colocalizing choline acetyltransferase, gamma-aminobutyric acid and enkephalin; (vi) ascending (excitatory) motor neurons to the longitudinal muscle (LM) which contain both choline acetyltransferase and calretinin; (vii) nerve fibres of capsaicin-sensitive extrinsic primary afferent neurons which have their cell bodies in dorsal root ganglia and send collaterals to the sympathetic prevertebral ganglia. Capsaicin-sensitive neurons have the peculiarity to release TKs at both peripheral and central varicosities, so that they can influence motility through mechanisms involving spinal reflexes or through an axon reflex involving a local release of TKs upon stimulation.

In the human GI tract the expression of TKs (SP in particular) has been detected in the following neurons classified according to morphological characteristics proposed by Brehmer[14]. SP was found in: (i) a minority of type I stubby neurons (small cell body, one axon, short, non-branched lamellar dendrites) some of which have been hypothesized to be ascending interneurons, whereas those expressing enkephalin and projecting to the LM and CM could be cholinergic motor neurons; (ii) type II neurons (multi-axonal, non dendritic) which project to the mucosa, express calretinin and/or somatostatin and have been proposed to be intrinsic primary afferent neurons; (iii) type III neurons (non-nitrergic, one axon and several long, branched dendrites radially emerging from the cell body) where SP could colocalize with either somatostatin or calretinin; iv) a small minority (0.5%) of type V neurons (neurofilament-positive, small smooth cell body with a single stem process from which very long dendrites and one axon emerge).

Likewise, Holzer and Holzer-Petsche[13] proposed a scheme for the distribution of NK_1r, NK_2r and NK_3r based on animal studies. According to this scheme, smooth muscle cells (both in the CM and LM) and enterocytes express both NK_1r and NK_2r, whereas blood vessels and interstitial cells of Cajal (ICC) seem to express NK_1r only. Indeed, evidence for the expression of NK_3r in smooth muscle cells (functional evidence in cultured cells, morphological evidence in colonic human specimens) and ICC (mRNA in cultured or freshly isolated cells) has been described but their functional meaning remains to be clarified[12,15]. NK_1r are particularly dense in ICC located in the deep muscular plexus and some immunoreactivity has also been observed in ICC located close to the myenteric plexus, whereas in the human antrum NK_1r have been detected in intramuscular ICC only[16,17].

NK_1r and NK_3r have been invariably localized to cell bodies and nerve fibres of the GI nerve plexuses. NK_3r were found on vasomotor neurons in the submucous plexus (SMP) where they play a physiological role in mucosal stroking- and distension-induced vasodilatation[18]. Still in the SMP, NK_1r have been localised to IPANs (those expressing TKs, calbindin and choline acetyltransferase), whereas secretomotor neurons containing either vasoactive intestinal polypeptide (VIP) or neuropeptide Y (NPY) express both NK_1r and NK_3r[16,19-21]. Likewise, NK_1r and NK_3r have been found on SMP neurons of the human gut, but the functional role and the neurochemical characterization of these neurons remain unknown[23]. In the myenteric plexus (MP) of rodents, both NK_1r and NK_3r are expressed on IPANS (calbindin-positive), ascending interneurons and motor neurons (both calretinin-positive), descending interneurons and motor neurons (both positive for nitric oxide synthase, the latter ones also containing VIP). Colocalization of NK_1r or NK_3r with NPY has also been described in the small intestine[16,24].

In rodents, NK_2r were only described in nerve terminals[22] which in guinea-pigs were shown to belong to descending interneurons expressing nitric oxide synthase (NOS) or gastrin-releasing peptide[25]. NK_2r were also detected in nerve fibres

within the muscle layers and some of these fibres expressed TKs[17]. At variance with laboratory animals, in humans specific immunolabeling of NK_2r is also present on somata, and in the human colon NK_2r are widely expressed on both neuronal cell bodies and fibres of the MP[23]. The chemical coding of neurons expressing NK_1r, NK_2r and NK_3r in the human gut is at present not known, except for the finding of some colocalization of NK_2r and TKs in nerve fibres within muscle layers, as previously observed in guinea-pigs[26].

TK Receptors and GI Motility

Given the extensive distribution of TKs and their receptors in GI neurons and effector cells respectively, it is no wonder that TKs can affect GI motility by acting through multiple mechanisms and sites of action (even with opposite consequences) within the GI wall. In this respect preclinical models have highlighted that NK_1r or NK_3r agonists can elicit either (or both) excitatory and inhibitory effects on GI motility, whereas the NK_2r agonists almost invariably produce excitatory motor effects. However, the indiscriminate access to receptors of exogenously administered agonists renders this approach physiologically unreliable, whereas studies with antagonists allow to appreciate the role of discrete tachykininergic pathways in well-defined physiological and pathological conditions. Overall preclinical evidence indicates that TKs produce excitatory effects by acting on smooth muscle (NK_1r and NK_2r), ICC (NK_1r) and neurons (NK_1r, NK_2r, and NK_3r), whereas TK-induced inhibitory effects are attributable to neuronal effects (NK_1r, NK_2r, and NK_3r) although other mechanisms cannot be excluded.

The involvement of TK receptors in three aspects of motility will be discussed: (i) active contractions elicited by the activation of excitatory motor neurons that are part of the ascending excitatory reflex involved in peristalsis; (ii) regulation of basal smooth muscle tone which is thought to tune the threshold for both the activation of motor reflexes and visceral sensitivity; and (iii) direct activation of neuronal afferent pathways leading to an inhibition of reflex motility.

Active Contractions

As pointed out in the previous paragraph, TKs are colocalized with acetylcholine in motor neurons providing excitatory inputs to both CM and LM, and NK_1r and NK_2r are located on both CM and LM to mediate contractions due to the reflex activation of these neurons induced by mucosal stimulation or by stretch. Indeed, SP is released from the guinea-pig small intestine when peristalsis is evoked by increasing intraluminal pressure through a capsaicin-resistant but partially hexamethonium-sensitive mechanism[27]. Depolarisation-induced co-release of similar amounts of both SP and NKA from guinea-pig colon motor neurons has also been demonstrated[28]. Likewise, similar amounts of SP and NKA were contained[29] and released during the ascending excitatory reflex in the human small intestine[30]. Whether TKs and acetylcholine are simultaneously co-released is yet an unsolved issue. Functional studies based on the effect of TK antagonists suggest that moderate intestinal distension would induce the release of acetylcholine only, whereas more intense stimuli would be required to induce the release of TKs in both humans and animals[30-32]. However, the difference between mild and intense stimulation in terms of release of mediators could be quantitative rather than qualitative.

In this context it should be pointed out that the efficiency of cholinergic mechanical coupling on GI smooth muscle is much greater than that observed with TKs[33], and this implies that TK-mediated components of contractions are not evident unless muscarinic receptors are blocked. Indeed, this actually occurs in most of animal and human GI preparations when motility is elicited through electrical stimuli or GI wall distension since NK_1r or NK_2r antagonists have a very small inhibitory effect (if any) on atropine-sensitive contractions or peristalsis[13]. A notable exception is represented by the colonic CM, where NK_2r antagonists decrease the amplitude of contractions induced by electrical field stimulation (EFS) even when muscarinic receptors are viable[34-36]. Indeed, in all human segments examined so far, i.e., esophagus[37], ileum[38], and colon[39], NK_1r and NK_2r are expressed on both CM and LM. Despite the fact that selective NK_1r and NK_2r agonists evoke contractions, the inhibitory effect of NK_1r antagonists on non-adrenergic, non-cholinergic (NANC) components of EFS-induced contractions has been only observed in the human ileum and it was limited to short-lasting stimuli[38,40]. An inhibition of the cholinergic component of EFS-induced twitches by an NK_1r antagonist has also been described in the human

colon LM, but this effect has been observed with antagonist concentrations which far exceed those specific for NK_1r[41]. The relative roles of TK receptors in eliciting direct smooth muscle contractions by exogenous application of selective agonists or by the release of endogenous TKs from ascending motor neurons in human GI segments are summarized in Table II.

In the human colon, the largest component of NK_2r-mediated contractions is due to a direct effect on smooth muscle. A small atropine-sensitive component has been described when NKA^{4-10} was used as the contractile agent[42]. However, this finding was not replicated when the human colon CM was contracted by NK_2r selective agonists[43]. In this latter case, a small indomethacin-sensitive component has been detected, indicating that the stimulation of NK_2r produces prostanoid release[43].

The issue whether stimulation of NK_2r can induce the release of acetylcholine is not yet solved. Although NK_2r have been localised to nerve varicosities colocalizing TKs and acetylcholine[23], no direct evidence for NK_2r-induced acetylcholine release has been provided yet. Despite this, several results (mostly preclinical) suggest that such an interaction occurs. In both small intestine (guinea-pig) and colon (guinea-pig and rabbit) the inhibitory effect of sub-effective concentrations of muscarinic antagonists on peristalsis is enhanced by the addition of NK_2r antagonists[44-46]. Furthermore, NK_2r antagonists reduce a component of colonic hypermotility (giant contractions) elicited by intraluminal irritation, whereas the inhibitory effect of atropine is complete, indicating that endogenous TKs act through NK_2r to enhance cholinergic motility[47]. A similar conclusion can be drawn on the basis of the analysis of sequential addition of muscarinic and NK_2r antagonists or vice-versa on EFS-induced contractions in the human colon CM[35]. As is shown in Figure 1, the sum of the inhibitory effects produced by the muscarinic receptor and NK_2r antagonists on their own is larger than 100% at all frequencies, indicating that part of the cholinergic component is reduced by NK_2r antagonists and that part of the TK-mediated component is inhibited by muscarinic receptor antagonists.

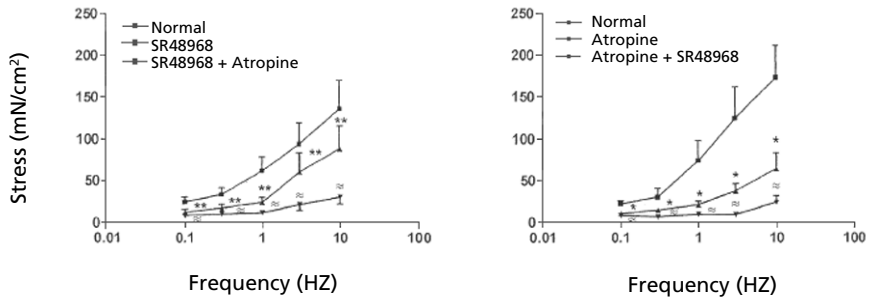
In conclusion, in human GI segments, contractions due to the activation of motor neurons is mediated by both acetylcholine and TKs acting through muscarinic and NK_2r, respectively. In most of intestinal segments the NK_2r-mediated component is not evident unless transmission via muscarinic receptors is blocked. In the human colon, a part of the cholinergic component is modulated by NK_2r, and selective antagonists are able to reduce part of the atropine-sensitive contractions.

Spontaneous Motility

In addition to propulsive contractions outlined above, intestinal movements include two other aspects: small amplitude rhythmic phasic contractions and smooth muscle tone[48]. Rhythmic smooth muscle contractions and tone are thought to underlie the mixing of the luminal contents and to regulate the threshold for the activation of motor and secretory reflexes but also for visceral sensitivity, respectively. Rhythmic smooth muscle contractions developing spontaneously in vitro and in vivo are believed to be largely myogenic due to the propagation of slow waves generated by ICC pacemakers to the smooth muscle. However, these rhythmic contractions are subject to neural regulation through both excitatory and inhibitory inputs[49]. In the rat colon especially, the neural inhibitory modulation prevails since in the presence of tetrodotoxin or a NOS inhibitor the amplitude of CM contractions is increased and the effect of the combination of these drugs is not additive[50,51]. Nevertheless in NANC conditions,

Table II. Contractile effect induced by selective NK_1r, NK_2r, and NK_3r agonists and inhibitory effect by selective NK_1r, NK_2r, NK_3r antagonists (ant) on EFS- or NANC EFS-induced contractions. Legend: + = contraction; 0 = no effect; - = inhibition; n.a. = not assessed.

GI Segment	NK_1r agonist	NK_2r agonist	NK_3r agonist	EFS+	NANC-EFS+
Oesophagus CM	0	+	0	n.a.	NK_2r ant -
Oesophagus LM	0	+	0	n.a.	0
Ileum CM	+	+	0	0	NK_1r ant - NK_2r ant -
Ileum LM	+	+	0	n.a.	n.a.
Colon CM	+	+	0	NK_2r ant -	NK_2r ant -
Colon LM	0	+	0	n.a.	n.a.



Human colon CM: inhibition produced by atropine or SR48968 expressed as % of inhibition produced by both antagonists

Hz	*Atropine*	*SR48968*
0.1	100%	100%
0.5	72%	83%
1.0	82%	85%
5.0	78%	47%
10.0	72%	50%

Figure 1. Effect of sequential addition of NK_2r and muscarinic antagonists or muscarinic plus NK_2r antagonists on frequency-response curves on human colon CM (from 35 with permission of the publisher).

there is evidence that TKs play a modulatory role on spontaneous, rhythmic, hexamethonium-resistant contractions since NK_2r antagonists reduce spontaneous mechanical activity by about 50% 50). A similar effect has been observed in the isolated human colon, where NK_2r antagonists produce a concentration-dependent inhibition (max 80%) of spontaneous phasic contractions[52]. Interestingly, in this latter preparation neither NK_1r antagonists nor atropine have any effect whereas tetrodotoxin substantially inhibits these contractions, suggesting that TKs could be released from tetrodotoxin-sensitive nerves other than excitatory motor neurons[52].

A further effect of TKs which contributes to spontaneous motility of GI smooth muscle concerns the modulation of basal tension and intraluminal pressure. In the mouse stomach neither NK_1r nor NK_2r antagonists affect spontaneous mechanical activity under normal conditions. However, NK_2r but not NK_1r antagonists produce a concentration-dependent gastric relaxation in mdx dystrophic mice or in normal mice pretreated with a NOS inhibitor[53]. Likewise, in isolated rat small intestinal segments the application of NK_2r but not NK_1r antagonists decreases resting tone (up to 70% of the maximal effect of isoprenaline) in a concentration-dependent manner. The source from which TKs are released to mediate this effect remains obscure since the relaxant effect of NK_2r antagonists was resistant to apamin, NOS inhibitors, capsaicin pretreatment, indomethacin, tetrodotoxin, omega-conotoxin, hexamethonium, nifedipine and to the removal of the mucosa[54]. No evidence for a relaxant effect by NK_2r antagonists has been provided in isolated human preparations. The selective NK_2r antagonist nepadutant, however, produced a significant increase of rectal compliance during ascending limit balloon distension in volunteers who had previously received a glycerol enema[12]. Interestingly, glycerol enema has been shown to induce a mild mucosal inflammation and a reduction of rectal air nitric oxide[55], suggesting that, as it occurs in animals[56], NK_2r antagonists could be able to reduce colonic CM tone following NOS inhibition in humans, too.

Neuronal Modulation of Motility

Beyond the relatively well characterized excitatory effects at the neuromuscular junction, TKs

exert a neurotransmitter role in the communication amongst neurons in both MP and SMP. In this context, both NK_1r and NK_3r contribute to slow excitatory postsynaptic potentials of MP IPANs during the activation of ascending excitatory pathways while NK_3r also participate in descending inhibitory circuitries[19,57]. Indeed, GI motor activity can be altered by TK agonists and antagonists also through an interaction with receptors located on neuronal excitatory or inhibitory motor pathways. In the preclinical setting, either the selective stimulation of NK_3r or the concomitant stimulation of both NK_1r and NK_3r activates acetylcholine- and TK-expressing excitatory motor neurons[58,59], whereas a possible neuronal excitatory role of NK_2r has been identified in hexamethonium-resistant guinea-pig small intestine peristalsis[60]. On the other hand, the selective stimulation of NK_1r by SP produces a nitric oxide-dependent inhibitory effect on ongoing guinea-pig small intestine peristalsis[61]. NK_3r-mediated nitric oxide-dependent and -independent inhibitory motor effects have been reported in guinea-pig small and large intestine preparations[62,63]. As a matter of fact, the selective antagonism of NK_1r, NK_2r, or NK_3r has been shown to accelerate rabbit isolated colon peristalsis[64] or intestinal transit in a rat model of surgical ileus[65]. Unfortunately, no evidence has been provided to indicate if such neuronal NK_1r, NK_2r, or NK_3r-mediated excitatory or inhibitory mechanisms operate in isolated human specimens, too.

Other neuronal targets through which TKs can potentially affect GI motility are fibres of extrinsic primary afferent (DRG) neurons. The subset of capsaicin-sensitive neurons is known to be a source of TKs but rarely a target for these peptides. Nevertheless, NK_3r have been detected in mouse nodose ganglion neurons projecting to the viscera[66], and functional evidence for the expression of NK_2r in capsaicin-sensitive pelvic DRG neurons has been also provided[67,68]. Obviously an interference with reflex GI motility exerted through the activation of extrinsic sensory fibres is expected to occur in vivo rather than in vitro, although it should not be forgotten that capsaicin-sensitive neurons are capable to release mediators locally upon stimulation, which allows for a modulatory effect to occur even in vitro.

A peripheral modulation of visceral sensitivity in preclinical models of colonic distension has been clearly demonstrated by either NK_2r or NK_3r antagonists. In fact, NK_2r antagonists revert inflammation- or stress-induced hypersensitivity to colonic distension without altering visceral nociception under normal conditions, whereas NK_3r antagonists produce a clear visceral hyposensitivity in normal animals[19,56]. Interestingly, the effect of NK_2r antagonists was associated with a specific reversal of colonic distension-induced hyperexcitability and c-fos expression in spinal cord dorsal horn neurons following colonic inflammation, whereas the responses elicited by electrical stimulation of the pelvic nerve or exposure to somatic noxious stimuli remained unaffected[56]. Alterations of the sensory transmission elicited by colonic distension have been described in NK_1r knock-out mice[69], and reversal of visceral hypersensitivity by NK_1r antagonists has been also reported[70] but it is unclear if these effects are exerted at the peripheral or central nervous system level.

Clinical Studies

The availability of potent and selective antagonists at the human TK receptors could have theoretically allowed a progress in the understanding about the role of these receptors in human GI diseases. Unfortunately, the progress made was quite modest. Ezlopitant, a selective NK_1r antagonist, showed a promising effect in a pilot trial in IBS, suggesting a role of these receptors in the modulation of visceral sensitivity[71]. However, blockade of NK_1r had no effect on acid-induced hypersensitivity in the human esophagus[72]. At the colonic level, a NK_1r antagonist reduced both the compliance and the volume threshold for discomfort under isobaric conditions but not when distension was performed by the method of ascending limits in healthy volunteers[73]. Interestingly, the stimulation of NK_1r triggers a relaxation on precontracted human colon CM and LM, but this response was impaired in specimens taken from patients with inflammatory bowel diseases[74]. These patients suffer from diarrhea, and diarrhea has been reported as a common adverse event in trials in which NK_1r antagonists were tested as analgesic-antimigraine drugs[75]. Overall, these results suggest that descending motor neurons regulating colonic tone are tonically activated by NK_1r and that blockade of this mechanism could stimulate colonic motility.

The blockade of NK_3r by talnetant did not affect colonic compliance nor sensory threshold recorded by the method of ascending limits or

random phasic distensions in healthy volunteers[76]. The same antagonist had no effect on IBS symptoms in 2 phase II studies where a wide range of doses (20-800 mg/day) was administered to a consistent number of patients[77].

The effect of NK_2r antagonists in patients suffering from GI diseases has not yet been reported. The available evidence indicates that the intravenous infusion of NKA increases small intestinal motility recorded by manometry in healthy volunteers. The fasting pattern of the migrating motor complex was disrupted after NKA administration, since the total duration of phase II motility (>2 contractions/min, without burst organization) as well as the amplitude and frequency of contractions was increased, whereas the duration of phase I motility (periods of relative quiescence with <2 contractions/min) was decreased. In contrast, phase III motility (bursts of propagating contractions with a frequency of 11-12 contractions/min) was not altered by NKA. Nepadutant, a selective NK_2r antagonist, prevented the motor effects induced by NKA administration in healthy volunteers without altering the normal pattern of the migrating motor complex in volunteers treated with saline. Interestingly, volunteers treated with NKA and placebo (n=10) experienced a series of gastrointestinal adverse events (n=10) including abdominal pain, nausea, vomiting and borborygmi, whereas no such adverse events were recorded in the group receiving both NKA and nepadutant[78]. Overall these results indicate that the blockade of NK_2r does not affect physiological motility of the small intestine or GI sensitivity, whereas the stimulation of these receptors increases motility and alters GI sensitivity.

The effect of nepadutant, at the same dose used as in the above mentioned study, was also investigated on sensory thresholds and compliance tested by rectal distension with a barostat by the method of ascending limits. This study was carried out in healthy volunteers challenged with a glycerol enema. Nepadutant did not significantly alter sensory thresholds, which were on the other hand only marginally decreased by glycerol. However, a small but significant increase in rectal compliance (larger balloon volume at constant pressure) was recorded after nepadutant as compared to placebo[12], suggesting that in the presence of mild inflammation, NK_2r blockade facilitates the accomodation of smooth muscle in response to radial stretch. A question arises whether this modulatory effect of a NK_2r antagonist on accommodation occurs during inflammation only or under normal conditions, too. Animal studies indicate that NK_2r antagonists do not affect GI compliance under normal conditions but do so following pharmacologically induced or genetically determined NOS inhibition[53,56]. However, NK_2r antagonists tend to decrease compliance during guinea-pig small intestinal peristalsis[44] or in the rat colon during physiological isovolumetric recordings[47].

The dose of nepadutant used in the above mentioned clinical studies (8 mg intravenously) allowed to block the GI effects of exogenously administered or endogenously released TKs acting at NK_2r, but the duration of this blockade was limited to 2-3 hours[78]. Since the time frame of GI functions outlasts this period, a further study was aimed at evaluating the effects of a longer lasting blockade of NK_2r. Therefore, a larger dose of nepadutant (16 mg intravenously every 12 h) was administered for 8 days in healthy male and female subjects who had to fill in a questionnaire reporting the bowel habits (number of bowel movements and stool consistency according to the Bristol Scale of Stool Form) of the day before. Nepadutant did not alter bowel habits as compared to the baseline (the day before starting the treatment). Interestingly, in the group treated with placebo, bowel movements significantly decreased during the first day of treatment and thereafter returned to the baseline value; no such decrease was observed in the group treated with nepadutant[12]. We interpreted the drop of bowel movements observed in the placebo group as stress-related due to the expectation of receiving 2 injections a day and to be confined to the clinical unit for several days. Whatever the cause of this drop, the fact that nepadutant can prevent an inhibition of motility (whatever the cause of this inhibition), provides a sort of proof of concept for the possibility to normalise bowel habits by blocking NK_2r in intestinal (or other peripheral) neurons. The presence of NK_2r on varicosities of guinea-pig myenteric neurons expressing either NOS or gastrin-releasing peptide[25] provides an anatomical substrate to explain a NK_2r-mediated inhibitory modulation of GI motility.

Conclusions

TKs are important modulators of GI physiology, being able to affect motor, secretory and sen-

sory functions. Animal studies have provided evidence that the stimulation of both NK_1r and NK_2r located on effector cells (either ICC and smooth muscle or smooth muscle only, respectively) mediate the excitatory NANC component due to activation of motor neurons. An increase of motility through stimulation of neuronal NK_1r or NK_3r has also been shown. On the other hand, in adequate preclinical models it is possible to appreciate an inhibitory modulation of motility exerted by neuronal NK_1r, NK_2r or NK_3r.

In humans, NK_2r play a pivotal role in motility by acting on either smooth muscle or neurons. No NK_3r-mediated motor effect has been yet described. The NANC excitatory input to both CM and LM is almost exclusively provided by NK_2r. Neuronal NK_1r or NK_2r exert an inhibitory brake on motility, although these receptors seem to operate in different contexts. Overall the results obtained in humans indicate that NK_1r antagonists could be useful in motility disorders characterized by constipation whereas NK_2r antagonists could be tested in disorders characterized by either diarrhea or constipation.

A recent paper[79] has shown that administration of anti-emetic doses of aprepritant (a NK_1r antagonist) does not alter GI or colonic propulsion in healthy volunteers.

References

1) Bartho L, Holzer P. Search for a physiological role of substance P in gastrointestinal motility. Neuroscience 1985; 16: 1-32.

2) Severini C, Improta G, Falconieri-Erspamer G, Salvatori S, Erspamer V. The tachykinin peptide family. Pharmacol Rev 2002; 54: 285-322.

3) Chang MM, Leeman SE. Isolation of a sialogogic peptide from bovine hypothalamic tissue and its chracterisation as substance P. J Biol Chem 1970; 245: 4784-4790.

4) Pennefather JN, Lecci A, Luz Candenas M, Patak E, Pinto FM, Maggi CA. Tachykinin and tachykinin receptors: a growing family. Life Sci 2004; 74: 1445-1463.

5) Page NM. Characterisation of the gene structures, precursor processing and pharmacology of the endokinin peptides. Vasc Pharmacol 2006; 45: 200-208.

6) Stratowa C, Machat H, Burger E, Himmler A, Schafer R, Spevak W, Weyer U, Wiche-Castanon M, Czerniliofsky AP. Functional characterisation of the human neurokinin receptors NK_1, NK_2, and NK_3 based on a cellular assay system. J Rec Signal Transd Res 1995; 15: 617-630.

7) Kurtz MM, Wang R, Clements MK, Cascieri MA, Austin CP, Cunningham BR, Chicchi GG, Liu Q. Identification, localisation, and receptor characterisation of novel mammalian substance P-like peptides. Gene 2002; 296: 205-212.

8) Guan JS, Xu Z-Z, gao H, He S-Q, Ma GQ, Sun T, Wang L-H, Zhang Z-N, Lena I, Kitchen I, Elde R, Zimmer A, He C, Pei G, Bao L, Zhang X. Interaction with vesicle luminal protachykinin regulates surface expression of delta-opioid receptors and opiod analgesia. Cell 2005; 122: 619-631.

9) Lecci A, Maggi CA. Tachykinins and their receptors. Encyclopedic reference of molecular pharmacology. S. Offermanns and W. Rosenthal, eds. Springer, Heidelberg 2008.

10) Patacchini R, Lecci A, Holzer P, Maggi CA. Newly discovered tachykinins raise new questions about their periphearl roles and the tachykinin nomenclature. Trends Pharmacol Sci 2004; 25: 1-3.

11) Naono R, Nakayama T, Ikeda T, Matsushima O, Nishimori T. Leucine at the carboxyl-terminal of endokinins C and D contributes to elicitation of the antagonistic effect on substance P in rat pain processing. Brain Res 2007; 1165: 71-80.

12) Lecci A, Capriati A, Altamura M, Maggi CA. Tachykinins and tachykinin receptors in the gut, with special reference to NK_2 receptors in humans. Auton Neurosci Basic Clin 2006; 126-127: 232-249.

13) Holzer P, Holzer-Petsche U. Tachykinin receptors in the gut: physiological and pathological implications. Curr Opin Pharmacol 2001; 1: 583-590.

14) Brehmer A. Structure of enteric neurons. Adv Anat Embriol Cell Biol 2006; 186: 1-94.

15) Lecci A, Santicioli P, Maggi CA. Pharmacology of transmission to gastrointestinal muscle. Curr Opin Pharmacol 2002; 2: 630-641.

16) Harrington AM, Hutson M, Southwell BR. Immunohistochemical localisation of substance P NK_1 receptor in guinea pig distal colon. Neurogastroenterol Motil 2005; 17: 727-737.

17) Faussone-Pellegrini M-S. Relationship between neurokinin receptor-expressing interstitial cells of Cajal and tachykininergic nerves in the gut. J Cell Mol Med 2006; 10: 20-32.

18) Patton D, O'Reilly M, Vanner S. Sensory peptide neurotransmitters mediating mucosal and distension evoked neural vasodilator reflexes in guinea-pig ileum. Am J Physiol Gastrointest Liver Physiol 2005; 289: G785-G790.

19) Sanger GJ, Tuladhar BR, Bueno L, Furness JB. Defensive and pathological functions of the gastrointestinal NK_3 receptors. Vasc Pharmacol 2006; 45: 215-220.

20) Poole DP, Matsuyama H, Nguyen TV, Eriksson EMY, Fowler CJ, Furness JB. Effects of inflammation and inflammatory agents on PKCepsilon translocation and excitability of guinea-pig submucosal neurons. Gastroenterology 2007; 133: 1229-1239.

21) Larsson MH, Sapnara M, Thomas EA, Bornstein JC, Lindstrom E, Svensson DJ, Sjovall H. Pharmacological analysis of components of the change in transmural potential difference evoked by distension of the rat small intestine in vivo. Am J Physiol Gastrointest Liver Physiol 2008; in press.

22) Grady EF, Baluk P, Bohm S, Gamp PD, Wong H, Payan DG, Ansel J, Portbury AL, Furness JB, McDonald DM, Bunnett NW. Characterisation of antisera specific to NK_1, NK_2, and NK_3 neurokinin receptors and their utilisation to localize receptors in the rat gastrointestinal tract. J Neurosci 1996; 16: 6975-6986.

23) Jaafari N, Liberge M, Christen M-O, Jule Y. Comparative distribution of NK_1, NK_2 and NK_3 receptors in the ganglionated plexus and external muscle layers of the human colon. Gastroenterology 2004; 126 (Suppl 2): A-217.

24) Jenkinson KM, Morgan JM, Furness JB, Southwell BR. Neurons bearing NK_3 tachykinin receptors in the guinea-pig ileum revealed by specific binding of fluorescently labelled agonists. Histochem Cell Biol 1999; 112: 233-246.

25) Portbury AL, Furness JB, Southwell BR, Wong H, Walsh JH, Bunnett NW. Distribution of neurokinin-2 receptors in the guinea-pig gastrointestinal tract. Cell Tissue Res 1996; 286: 281-292.

26) Jaafari N, Khomitch-Baud A, Christen M-O, Julé Y. Distribution pattern of tachykinin NK_2 receptors in human colon: involvement in the regulation of intestinal motility. J Comp Neurol 2007; 503: 381-391.

27) Donnerer J, Bartho L, Holzer P, Lembeck F. Intestinal peristalsis associated with release of immunoreactive substance P. Neuroscience 1984; 11: 913-918.

28) Lippi A, Santicioli P, Criscuoli M, Maggi CA. Depolarisation evoked co-release of tachykinins from enteric nerves in the guinea-pig proximal colon. Naunyn-Schmiedeberg's Arch Pharmacol 1998; 357: 245-251.

29) Maggi CA, Giuliani S, Patacchini R, Santicioli P, Theodorsson E, Barbanti G, Turini D, Giachetti A. Tachykinin antagonists inhibit nerve-mediated contractions in the circular muscle of the human ileum. Gastroenterology 1992; 102: 88-96.

30) Grider JR. Identification of neurotransmitters regulating intestinal peristaltic reflex in humans. Gastroenterology 1989; 97: 1414-1419.

31) Giuliani S, Lecci A, Giachetti A, Maggi CA. Tachykinin and reflexly evoked atropine-resistant motility in the guinea-pig colon in vivo. J Pharmacol Exp Ther 1993; 265: 1224-1231.

32) Giuliani S, Tramontana M, Lecci A, Maggi CA. Tachykinin receptors mediate atropine-resistant duodenal reflex contractions in vivo. Naunyn-Schmiedeberg's Arch Pharmacol 1996; 354: 327-335.

33) Maggi CA, Giuliani S, Zagorodnyuk V. Sequential activation of the triple excitatory transmission to the circular muscle of the guinea-pig colon. Neuroscience 1997; 79: 263-274.

34) Cao W, Pricolo VE, Zhang L, Behar J, Biancani R, Kirber MT. Gq-linked NK_2 receptors mediate neurally induced contraction of human sigmoid circular smooth muscle. Gastroenterology 2000; 119: 51-61.

35) Maselli MA, Piepoli AL, Guerra V, Caruso ML, Pezzolla F, Lorusso D, Demma I, De Ponti F. Colonic smooth muscle responses in patients with diverticular disease of the colon: effect of the NK_2 receptor antagonist SR48968. Dig Liver Dis 2004; 36: 348-354.

36) Auli M, Farré R, Gallego D, Martinez E, Marti Rague J, Sunol X, Jimenez M, Clave P. Cholinergic and tachykinergic co-neurotransmission in "on" and "off" contractions in human sigmoid colon. Neurogastroenterol Motil 2006; 18: 788.

37) Kovac JR, Chrones T, Preiksaitis HG, Sims SM. Tachykinin receptor expression and function in human esophageal smooth muscle. J Pharmacol Exp Ther 2006; 318: 513-520.

38) Zagorodnyuk V, Santicioli P, Turini D, Maggi CA. Tachykinin NK_1 and NK_2 receptors mediate non-adrenergic non-cholinergic excitatory neuromuscular transmission in the human ileum. Neuropeptides 1997; 31: 265-271.

39) Mitolo-Chieppa D, Mansi G, Nacci C, De Salvia MA, Montagnani M, Potenza MA, Rinaldi R, Lerro G, Siro-Brignani G, Mitolo CI, Rinaldi M, Altomare DF, Memeo V. Idiopathic chronic constipation: tachykinins as cotransmitters in colonic contraction. Eur J Clin Invest 2001; 31: 349-355.

40) Patacchini R, Giuliani S, Turini A, Navarra G, Maggi CA. Effect of nepadutant at tachykinin NK_2 receptors in human intestine and urinary bladder. Eur J Pharmacol 2000; 398: 389-397.

41) Guagnini F, Valenti M, Mukenge S, Matias I, Bianchetti A, Di Palo S, Ferla G, Di Marzo V, Croci T. Neural contractions in colonic strips from patients with diverticular disease: role of endocannabinoids and substance P. Gut 2006; 55: 946-953.

42) Kolbel CB, Mayer EA, Holtmann G, Layer P, Petschulat W, Erhard J, Goebell H. Effects of neurokinins on human colonic motility. Neurogastrenterol Motil 1994; 6: 119-127.

43) Burcher E, Shang E, Warner FJ, Du Q, Lubowski DZ, King DW, Liu L. Tachykinin NK_2 receptors and functional mechanisms in human colon: changes with indomethacin and in diverticular disease and ulcerative colitis. J Pharmacol Exp Ther 2007; DOI:10.1124/jpet.107.130385.

44) Holzer P, Maggi CA. Synergistic role of muscarinic acetylcholine and tachykinin NK_2 receptors in intestinal peristalsis. Naunyn-Schmiedeberg's Arch Pharmacol 1994; 349: 194-201.

45) Tonini M, Spelta V, De Ponti F, De Giorgio R, D'Agostino G, Stanghellini V, Corinaldesi R, Sternini C, Crema F. Tachykinin-dependent and -independent components of peristalsis in the guinea-pig isolated distal colon. Gastroenterology 2001; 120: 938-945.

46) Onori L, Aggio A, Taddei G, Tonini M. Contribution of NK_2 tachykinin receptors to propulsion in the rabbit distal colon. Am J Physiol Gastrointest Liver Physiol 2000; 278: G137-G147.

47) Carini F, Lecci A, Tramontana M, Giuliani S, Maggi CA. Tachykinin NK_2 receptors and enhancement of cholinergic transmission in the inflamed rat colon: an in vivo motility study. Br J Pharmacol 2001; 133: 1107-1113.

48) Sarna SK. Tachykinins and in vivo gut motility. Dig Dis Sci 1999; 44: 114S-118S.

49) Forrest AS, Ordog T, Sanders KM. Neural regulation of slow-wave frequency in the murine gastric antrum. Am J Physiol Liver Physiol 2006; 290: G486-G495.

50) Mule F, D'Angelo S, Tabacchi G, Serio R. Involvement of tachykinin NK_2 receptors in the modulation of spontaneous motility in rat proximal colon. Neurogastroenterol Motil 2000; 12: 459-466.

51) Gonzales A, Sarna SK. Neural regulation of in vitro giant contractions in the rat colon. Am J Physiol Gastrointest Liver Physiol 2001; 281: G275-G282.

52) Cao W, Harnett KM, Pricolo VE. NK_2 receptor-mediated spontaneous phasic contractions in normal and ulcerative colitis human sigmoid colon. J Pharmacol Exp Ther 2006; 317: 1349-1355.

53) Mule F, Amato A, Vannucchi MG, Faussone-Pellegrini MS, Serio R. Altered tachykinergic influence on gastric mechanical activity in mdx mice. Neurogastroenterol Motil 2006; 18: 844-852.

54) Maggi CA, Giuliani S. Evidence that tachykinin NK_2 receptors modulate resting tone in the rat isolated small intestine. Br J Pharmacol 1996; 118: 1262-1268.

55) Bouin M, Delvaux M, Blanc C, Lagier E, Delisle MB, Fioramonti J, Bueno L, Frexinos J. Intrarectal injection of glycerol induces hypersensitivity to rectal distension in healthy subjects without modifying rectal compliance. Eur J Gastroenterol Hepatol 2001; 13: 573-580.

56) Lecci A, Capriati A, Maggi CA. Tachykinin NK_2 receptor antagonists for the treatment of irritable bowel syndrome. Br J Pharmacol 2004; 141: 1249-1263.

57) Johnson PJ Bornstein JC. Neurokinin-1 and -3 receptor blockade inhibits slow excitatory synaptic transmission in myenteric neurons and reveals a slow inhibitory input. Neuroscience 2004; 126: 137-147.

58) Patacchini R, Maggi CA, Holzer P. Tachykinin autoreceptors in the gut. Trends Pharmacol Sci 2000; 21: 166.

59) Furness JB, Kumano K, Larsson H, Murr E, Kunze WAA, Vogalis F. Sensitisation of enteric reflexes in the rat colon in vitro. Autonomic Neurosci 2002; 97: 19-25.

60) Holzer P, Lippe ITh, Heinemann A, Bartho L. Tachykinin NK_1 and NK_2 receptor-mediated control of peristaltic propulsion in the guinea-pig small intestine in vivo. Neuropharmacology 1998; 37: 131-138.

61) Holzer P. Involvement of nitric oxide in the substance P-induced inhibition of intestinal peristalsis. Neuroreport 1997; 8: 2857-2860.

62) Maggi CA, Patacchini R, Meini S, Giuliani S. Nitric oxide is the mediator of tachykinin NK_3 receptor-induced relaxation in the circular muscle of the guinea-pig ileum. Eur J Pharmacol 1993; 240: 45-50.

63) Giuliani S, Maggi CA. Effect of SR142801 on nitric oxide-dependent and independent responses to tachykinin NK_3 receptor agonists in isolated guinea-pig colon. Naunyn-Schmiedeberg's Arch Pharmacol 1995; 352: 512-519.

64) Onori L, Aggio A, Taddei G. Regulatory role of tachykinins on intestinal propulsive activity. Ital J Gastroenterol Hepatol 1999; 31: 346-351.

65) Ciechanowicz R, Sein-Anand J, Chodorowski Z, Bitel M, Petrusewicz J, Korolkiewicz RP. Salutary effects of tachykinin receptor antagonists in a rat model of postoperative ileus. J Surg Res 2006; 133: 197-202.

66) Peeters PJ, Aerssens J, de Hoogt R, Stanisz A, Gohlmann HW, Hillsley K, Meulemans A, Grundy D, Stead RH, Coulie B. Molecular profiling of murine sensory neurons in the nodose and dorsal root ganglia labeled from the peritoneal cavity. Physiol Gen 2006; 24: 252-263.

67) Sculptoreanu A, de Groat WC. Protein kinase C is involved in neurokinin receptor modulation of N- and L-type Ca2+ channels in DRG neurons of the adult rat. J Neurophysiol 2003; 90: 21-31.

68) Sculptoreanu A, de Groat WC. Neurokinins enhance excitability in capsaicin-responsive DRG neurons. Exp Neurol 2007; 205: 92-100.

69) Laird JMA, Olivar T, Roza C, De Felipe C, Hunt SP, Cervero F. Deficits in visceral pain and hyperalgesia of mice with a disruption of the tachykinin NK_1 receptor gene. Neuroscience 2000; 98: 345-352.

70) Okano S, Ikeura Y, Inatomi N. Effects of tachykinin antagonists on the viscerosensory response caused by colorectal distension in rabbits. J Pharmacol Exp Ther 2002; 300: 925-931.

71) Lee OY, Munakata J, Naliboff BD, Chang L, Mayer EA. A double-blind parallel group pilot study of the effect of CJ-11,974 and placebo on perceptual and emotional responses to rectosigmoid distension in IBS patients. Gastroenterology 2000; 118: A-846.

72) Willert RP, Hobson AR, Delaney C, Hicks KJ, Dewit OE, Aziz Q. Neurokinin-1 receptor antagonism in a human model of visceral hypersensitivity. Aliment Pharmacol Ther 2007; 25: 309-316.

73) Akyuz F, Mimidis K, Nikolai H, Vos R, Tack J. Influence of NK-1 receptor antagonist aprepritant on rectal sensitivity and compliance in healthy volunteers. Gastroenterology 2007; 32(Suppl 2): A-684.

74) Smith AS, Smid S. Impaired capsaicin and neurokinin-evoked colonic motility in inflammatory bowel disease. J Gastroenterol Hepatol 2005; 20: 697-704.

75) Goldstein DJ, Wang O, Gitter BD, Iyengar S. Dose-response study of the analgesic effect of lanepitant in patients with painful diabethic neuropathy. Clin Neuropharmacol 2001; 24: 16-22.

76) Houghton LA, Cremonini F, Camilleri M, Busciglio I, Fell C, Cox V, Alpers DH, Dewit OE, Dukes GE, Gray E, Lea R, Zinsmeister AR, Whorwell PJ. Effect of the NK_3r antagonist, talnetant, on rectal sensory function and compliance in healthy humans. Neurogastroenterol Motil 2007; 19: 724-731.

77) Duks GE, Dewit OE, Sanger GJ, Ameen VZ, Northcutt AR, Perschy TL, Schmith GD, Hamedani AG, Alpers DH. Lack of effect of the NK_3 receptor antagonis, talnetant SB223242 on symptoms of IBS results of 2 randomised, double blind, placebo-controlled dose ranging trials. Gastroenterology 2007; 32: A-60.

78) Lordal M, Navalesi G, Theodorsson E, Maggi CA, Hellstrom PM. A novel tachykinin NK_2 receptor antagonist prevents motility-stimulating effects of neurokinin A in small intestine. Br J Pharmacol 2001; 134: 215-223.

79) Madsen JL, Fuglsang S. A randomized, placebo-controlled, crossover, double-blind trial of the NK_1 receptor antagonist aprepitant on gastrointestinal motor function in healthy humans. Aliment Pharmacol Ther 2008; 27: 609-615.