British Journal of Pharmacology (2004) 141, 1303–1312
www.nature.com/bjp

REVIEW

Neurokinin NK_1 and NK_3 receptors as targets for drugs to treat gastrointestinal motility disorders and pain

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NK_1 and NK_3 receptors do not appear to play significant roles in normal GI functions, but both may be involved in defensive or pathological processes. NK_1 receptor antagonists are antiemetic, operating *via* vagal sensory and motor systems, so there is a need to study their effects on other gastro-vagal functions thought to play roles in functional bowel disorder's. Interactions between NK_1 receptors and enteric nonadrenergic, noncholinergic motorneurones suggest a need to explore the role of this receptor in disrupted colonic motility. NK_1 receptor antagonism does not exert consistent analgesic activity in humans, but similar studies have not been carried out against pain of GI origin, where NK_1 receptors may have additional influences on mucosal inflammatory or 'irritant' processes. NK_3 receptors mediate certain disruptions of intestinal motility. The activity may be driven by tachykinins released from intrinsic primary afferent neurones (IPANs), which induce slow EPSP activity in connecting IPANs and hence, a degree of hypersensitivity within the enteric nervous system. The same process is also proposed to increase C-fibre sensitivity, either indirectly or directly. Thus, NK_3 receptor antagonists inhibit intestinal nociception *via* a 'peripheral' mechanism that may be intestine-specific. Studies with talnetant and other selective NK_3 receptor antagonists are, therefore, revealing an exciting and novel pathway by which pathological changes in intestinal motility and nociception can be induced, suggesting a role for NK_3 receptor antagonism in irritable bowel syndrome.
British Journal of Pharmacology (2004) **141,** 1303–1312. doi:10.1038/sj.bjp.0705742

Keywords: Tachykinins; NK_1; NK_3; emesis; intestinal motility; visceral pain; osanetant; talnetant

Abbreviations: ACh, acetylcholine; AP, area postrema; cmH_2O, cm of water; DMVN, dorsal motor vagal nucleus; DRG, dorsal root ganglia; EC cel, enterochromaffin cell; ENS, enteric nervous system; FBD, functional bowel disorder; GI, gastrointestinal; 5-HT, 5-hydroxytryptamine; IBS, irritable bowel syndrome; ICC, interstitial cells of cajal; IPAN, intrinsic primary afferent neurone; LES, lower esophageal sphincter; NANC, non-adrenergic, non-cholinergic; NKA, neurokinin A; NKB, neurokinin B; NK_1, neurokinin$_1$; NK_2, neurokinin$_2$; NK_3, neurokinin$_3$; NK_1-Ir, NK1 receptor immunmoreactivity; NO, nitric oxide; NTS, nucleus *Tractus solitarius*; Slow EPSP, slow excitatory postsynaptic potential; VIP, vasoactive intestinal polypeptide

Introduction

The presence of tachykinins in the gut and their actions when exogenously-applied have been extensively studied (Holzer & Holzer-Petsche, 2001). Mammalian tachykinins (substance P, neurokinins A and B) activate tachykinin NK_1, NK_2 and NK_3 receptors, with a characteristic rank-order of affinity. Neurokinin B (NKB), for example, has the highest affinity for NK_3 receptors, followed by NKA > substance P, which in turn, have greater affinity for NK_2 and NK_1 receptors, respectively (Maggi, 2000). However, the differences in affinities are not large, so in terms of an ability to interact with each of the NK receptors, a degree of 'agonist-promiscuity' becomes a real possibility. This concept is discussed later, with respect to the role of the NK_3 receptor in the intestine.

The availability of selective NK receptor antagonists makes its possible to investigate the actions of endogenous tachykinins on normal, defensive and pathological gastrointestinal (GI) functions. This review focuses on the NK_1 and NK_3 receptors and their possible involvement in functional bowel disorders (FBDs).

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Advance online publication: 15 March 2004

NK_1 Receptors

Nausea and vomiting

NK_1 receptor antagonists such as aprepitant, vofopitant and ezlopitant can inhibit acute and delayed emesis induced by cancer chemotherapeutic agents when given alone and more importantly, when given in combination with 5-HT_3 receptor antagonists and/or dexamethasone (Andrews & Rudd, 2004). For optimal activity, NK_1 receptor antagonists must cross the blood–brain barrier to reach a number of different brain regions. The relative importance of these regions in determining the antiemetic efficacy is unclear. A prevailing view is that NK_1 receptors within the nucleus *tractus solitarius* (NTS) of the brainstem play a major role, probably by acting 'upstream' from the vagal nerve terminal (Watson *et al*., 1995; Andrews & Rudd, 2004). In addition, NK_1 receptors elsewhere play important roles, noteably those within the Botzinger complex

(part of the ventral respiratory group of neurones) and the dorsal motor vagal nucleus (DMVN, from where a majority of vagal efferents project back to the gut). In the latter, NK_1 receptors are present on motor neurones projecting to the greater curvature of the stomach (Ladic & Buchan, 1996; Lewis & Travagli, 2001), implicating these receptors in physiological (e.g., fundic accomodation) and pathological behaviours of the stomach (e.g., gastric relaxation, leading to nausea or stasis), probably *via* release of nitric oxide (NO) and VIP within the enteric nervous system (ENS) (Krowicki & Hornby, 2000). NK_1 receptors are not expressed on all vagal terminals. Blondeau *et al* (2002) reported that NK_1-receptor immunoreactivity (NK_1-Ir) was present in 19% of vagal neurones innervating the rat stomach, rising to 46% for the duodenum, but being absent on vagal neurones innervating the ileum and caecum.

The NTS is regarded as a major integrative region, receiving the majority of the abdominal vagal afferent neurones as well as information from other brain areas, and sending projections to regions involved in different motor components of the emetic reflex. The widespread distribution of NK_1 receptors to the NTS (Watson *et al*., 1995) and to the DMVN (e.g., Maubach & Jones, 1997; Krowicki & Hornby, 2000) is consistent with the observation that in animals, anti-emetic activity is observed regardless of the emetic stimulus. Thus, NK_1 receptors can be considered to 'gate' an emetic stimulus arising from 'peripheral' (e.g., *via* the blood to the Area Postrema (AP) or via vagal afferent fibres to the NTS and the AP) or 'central' sources (i.e., projecting to the NTS for integration and ultimately involving the DMVN).

Gastro-vagal activity in functional bowel disorders

The antiemetic activity of NK_1 receptor antagonists is currently the only function that is observed in both animals and humans. This activity plus the widespread distribution of the receptor to brainstem nuclei receiving and driving vagal activity suggests that NK_1 receptor antagonists might also affect certain symptoms of FBD's (e.g., functional nausea and/or functional dyspepsia), which may be mediated *via* the vagus (see Andrews & Sanger, 2002, for a discussion on this possibility). Three areas are considered.

Subcomponents of the emetic reflex The antiemetic activity of NK_1 receptor antagonists indicates a role for the receptor in mediating a defensive behaviour of the gut, but the absence of significant GI 'adverse events' during trials with these compounds (see Andrews & Rudd, 2004) suggests little or no role in normal GI physiology. However, it is not known if NK_1 receptors can mediate symptoms of FBDs that are less severe than emesis, but that superficially appear to resemble 'components' of the emetic response. For example, are NK_1 receptors involved in the generation of transient lower oesophageal sphincter relaxations, a vago-vagal reflex that vents gas from the stomach, but that is also involved in the aetiology of gastro-oesophageal reflux (Holloway & Dent, 2000) ? Does the receptor have a role in symptoms of nausea or early satiety in patients with functional nausea and functional dyspepsia ? The answers to these questions are important not just to develop the full therapeutic potential of NK_1 receptor antagonists but also to investigate the degree to which tachykinin functions are activity-dependent, with efficacy increasing in proportion to the severity of symptoms.

Affective pain An involvement of the vagus in affective-emotional components of visceral pain is recognised clinically (Ness *et al*., 2001) and in animals (e.g. , Traub *et al*., 1996, using rat gastric distension as a model). Further, Jocic *et al* (2001) and Michl *et al*. (2001) exposed the rat stomach to noxious levels of acid and measured *cfos* activity in the brainstem. NK_1 receptor antagonism attenuated the acid-induced transcription of *cfos* mRNA in the NTS and augmented it in the subfornical organ. The authors suggested that these changes play some role in dyspepsia, a consideration given weight by the ability of the vagus to signal and evoke many of the different components of dyspepsia (Andrews & Sanger, 2002).

Gastro-vagal-inflammation or irritation Anterograde tachykinin release from vagal neurones may play at least some role in exacerbating or mediating relaxation of the lower oesophageal sphincter (LES) and/or fundus. Thus, in the ferret isolated LES, relaxation evoked by capsaicin was reduced by NK_1 receptor antagonism (Smid *et al*., 1998). In anaesthetised ferrets, repeated oesophageal acidification evoked LES relaxation, thought to be due to substance P released from extrinsic afferent nerve endings, activating local inhibitory pathways *via* NK_1 receptors (Blackshaw & Dent, 1997). Similar data were obtained in guinea-pig gastric fundus, where NKA induced relaxation (Jin *et al*., 1998). In human isolated LES, [Sar^9, $Met(O_2)^{11}$]-SP had no effects on muscle tension (Huber *et al*., 1993), but further experiments are required to examine the influence of this ligand on nerve-mediated activity.

Substance P is stored in large amounts within enterochromaffin (EC) cells of the mucosa, where it can be released to act at NK_1 receptors on vagal afferent nerve terminals situated in close proximity (Minami *et al*., 2001). The role played by this source of substance P is unclear. Interestingly, raised levels of substance P and NK_1-Ir have been reported in the crypt epithelia of rats with experimentally induced colitis or ileal pouch inflammation; in the ileal pouch, the inflammation was reduced by NK_1 receptor antagonism (see Stucchi *et al*., 2003). Investigations into the pathophysiological functions of substance P-containing EC cells are, therefore, warrented.

Intestinal motility and secretion

NK_1-Ir has been found in the mouse ileum ENS and on the interstitial Cells of Cajal (ICC's; Vannucchi & Faussone-Pellegrini, 2000). In human colon, NK_1-Ir has been observed in circular but not usually the longitudinal muscle, and possibly also in the ICCs. Intense staining around the blood vessels and in the muscle precluded any ability to say that the receptors were located on enteric nerves (Rettenbacher & Reubi, 2001). In guinea-pig ileum, NK_1 receptors were found mostly on NO synthase-containing myenteric neurones, with some on neurones expressing choline acetyltransferase and other markers projecting to the circular muscle, mucosa and submucosa. In the submucosal plexus, most NK_1-Ir had neuropeptide Y immunoreactivity (Lomax *et al*., 1998).

In general, NK_1 receptors do not play a major role in normal intestinal motility. However, although NK_1 receptor antagonism has little or no effects on normal peristalsis in

guinea-pig or pig isolated intestine (Holzer *et al*., 1998; Tonini *et al*., 2001; Schmidt & Hoist, 2002), villous agitation may evoke substance P-mediated, tetrodotoxin-sensitive internalisation of NK_1 receptors in myenteric neurones of guinea-pig ileum (Southwell *et al*., 1998), and NK_1 receptor antagonism can abolish the noncholinergic component of peristalsis measured during muscarinic receptor antagonism (Holzer *et al*., 1998; Tonini *et al*., 2001; Schmidt & Hoist, 2002). These and other data suggest a role for NK_1 receptors in noncholinergic, nonadrenergic (NANC) neurotransmission. NANC excitatory effects may be induced by NK_1 receptor activation in human isolated ileum (Zagorodnyuk *et al*., 1997). In rat isolated colon, NK_1 receptor antagonism reduced electrically evoked, ascending, excitatory nerve-mediated contractions (Hahn *et al*., 2002). Similarly, observations with NK_1 receptor knockout mice (Saban *et al*., 1999) suggest that both the knockout and NK_1 receptor antagonism increase NANC inhibitory motor nerve activity. By contrast, in guinea-pig ileum, NK_1 receptor antagonism had no effect on ascending enteric reflex contractions evoked by distension (Holzer *et al*., 1993) but reduced the descending inhibitory reflex (Johnson *et al*., 1998), these data being consistent with the effects of NK_1 receptor agonists on guinea-pig inhibitory neurotransmission (Lecci *et al*., 1999; Bian *et al*., 2000).

The ability of NK_1 receptors to modulate NANC activity currently has no clear function, although a role in colonic motility is suggested. In human colon circular muscle, NK_1 receptor activation facilitated electrically-induced, neuronally-mediated after-contractions (Mezies *et al*., 2001), and this effect was greater in preparations resected from patients with idiopathic chronic constipation, in which smaller responses to electrical stimulation were observed (Mitolo-Chieppa *et al*., 2001). Further, close intra-arterial injections of NK_1 receptor agonists stimulate giant migrating contractions of dog colon (Tsukamoto *et al*., 1997) and antagonism at the receptor blocked increased defaecation induced by restraint stress in rats (Ikeda *et al*., 1995) and blocked substance P- and stress-induced defaecation by Monglolian gerbils, without affecting increases in defaecation evoked by 5-HT or carbachol (Okano *et al*., 2001). In conscious dogs, De Ponti *et al*. (2001) found that NK_1 (or NK_2 or NK_3) receptor antagonism had no effects on propagated colonic myoelectrical events induced by 5-HT_4 receptor activation, but reduced the associated increase in electrical spike or mechanical activity, an effect not observed with these antagonists in the small intestine. Together, these studies are consistent with an action of NK_1 receptors within the large bowel, although for the studies *in vivo*, a spinal site of action cannot always be ruled out. However, two other studies seem to be at variance with this view. Bradesi *et al* (2002) reported that NK_1 receptor antagonism inhibited substance P-induced histamine release from the colon of rats exposed to restraint stress, but this effect was absent in pre-ovariectomised rats, suggesting an involvement of ovarian steroids in the response. Secondly, prokinetic activity was observed in response to NK_1 receptor antagonism in rabbit isolated colon (Onori *et al*., 2003).

Gastrointestinal nociception or hypersensitivity

In spite of excellent antinociceptive properties of NK_1 receptor antagonists in animals, this promise has not been reproduced in humans (Hill, 2000). Suggested reasons for this failure include a requirement to predose with the antagonist, receptor/neurotransmitter redundancy in pain-conducting systems or a mismatch between the complex animal appreciation of 'nociception' and the human sensation of pain (see Laird *et al*., 2000a, for an expansion of this argument).

For the gut, antinociceptive actions of NK_1 receptor antagonists have been observed using rat models of discomfort induced by gastric or colorectal distension (e.g., Julia *et al*., 1994; Anton *et al*., 2001; but see Kamp *et al*., 2001) and also in guinea-pigs, where antinociceptive activity was apparent only in animals with a sensitised response to colo-rectal distension (Greenwood-van Meerveld *et al*., 2003). Using NK_1 receptor knockout mice, different intestinal nociceptive stimuli were suggested to operate *via* NK_1-dependent (neurogenic inflammation) and –independent (non-neurogenic inflammation or mechanical) pathways (Laird *et al*., 2000a, b). These data are consistent with an involvement of NK_1 receptors in intestinal inflammation induced by infection (Sonea *et al*., 2002), intracolonic infusion of the proteinase-activated receptor-2 ligand SLIGRL (Cenac *et al*., 2003) or following ischaemia and reperfusion (Souza *et al*., 2002). In patients with irritable bowel syndrome (IBS), raised numbers of mucosal mast cells have been reported and these changes were not clearly correlated with symptoms in one study (O'Sullivan *et al*., 2000), but increased numbers of activated colonic mast cells in close proximity to nerve fibres were correlated to symptoms of abdominal pain or discomfort in another (Barbara *et al*., 2004). Ion secretion evoked by mast cell stimulation, capsaicin or electrical field stimulation was each blocked by NK_1 receptor antagonism in human isolated mucosa (Moriarty *et al*., 2001). Further, gastric hyperalgesia involving mast cell degranulation may be inhibited by NK_1 receptor blockade (Anton *et al*., 2001), raising the possibility that NK_1 receptor antagonism might reduce human GI pain at least partly by modulating mechanisms by which extrinsic sensory neurones become sensitised.

Summary and potential for treatment of FBDs

NK_1 receptor antagonists do not greatly affect normal GI functions. However, their antiemetic activity suggests an activity-dependent, defensive role for the NK_1 receptor, operating *via* vagal sensory and motor pathways. It is not known if the same pathways play similar roles in conditions such as functional dyspepsia, but there is a need to study the effects of NK_1 receptor antagonism on such disorders. Interactions between the NK_1 receptor and NANC transmission also suggest a need to explore the role of this receptor in disrupted colonic motility. Finally, NK_1 receptor antagonism may exert analgesic activity in humans, but similar studies have not been carried out for the gut, where an opportunity exists for NK_1 receptor involvement in mucosal inflammatory or 'irritant' processes.

NK_3 Receptors

Gastro-vagal functions

Any involvement of the NK_3 receptor in gastro-vagal neuropathophysiology is not clear. This lack of clarity exists in spite of the presence of 'moderate' levels of NK_3-Ir within

the rat NTS and DVMN regions of the brainstem (Mileusnic *et al*., 1999). Electrophysiological studies indicate that the neurones of the NTS and DMVN do not respond to NKB or senktide (Maubach & Jones, 1997). However, intracerebroventricular, but not systemic administration of the NK_3 receptor antagonist osanetant (SR-142,801) may block the ability of rectal distension to evoke colonic water secretion *via* capsaicin-sensitive, vagus-dependent mechanisms (Eutamene *et al*., 1997).

Gastrointestinal motility

Receptor and ligand distribution: Tachykinins have been localised to intestinal intrinsic primary afferent neurones (IPANs) by immunohistochemistry and the cell bodies of myenteric IPANs bear NK_3 receptors (Costa *et al*., 1996; Mann *et al*., 1997; Jenkinson *et al*., 1999; Lomax & Furness, 2000). These neurones are sensitive to chemical and/or mechanical stimuli and are characterised by morphology (short axon; multiple dendrites projecting to multiple enteric neurones), location (projecting circumferentially from the mucosa, with cell bodies in the myenteric and submucosal plexus) and electrophysiology (ability to generate slow excitatory postsynaptic potentials (EPSPs) or slow postsynaptic excitation) (Clerc *et al*., 1999). An individual IPAN will synapse with additional IPANs, generating a slow-EPSP and thereby forming a self-reinforcing network. IPANs also transmit to longitudinally projecting excitatory and inhibitory inter-/ motor-neurones *via* fast- and slow-EPSPs. NK_3 receptors are distributed to myenteric ascending excitatory and descending inhibitory motorneurones, and to secretomotor neurones. Finally, NK_3 receptors are found in the submucosal plexus on secretomotor/vasodilator neurones, but not on IPANs (Jenkinson *et al*., 1999). Of the species examined, the receptors are found on myenteric neurones in guinea-pig gastric antrum and on myenteric and submucosal neurones in the ileum (Schemann & Kayser, 1991; Jenkinson *et al*., 1999). A similar distribution of the receptor has been reported in rat intestine myenteric and (to a lesser extent) submucosal neurones. However, NK_3-Ir was absent in the stomach and oesophagus, where IPAN-like neurones are also thought to be absent (Grady *et al*., 1996; Mann *et al*., 1997). In the human sigmoid colon, intense NK_3-Ir was detected in the myenteric and submucosal plexus, with no apparent difference in the relative intensities (Dass *et al*., 2002; Figure 1); NK_3-Ir was not detected on longitudinal and circular muscle, or on the muscularis mucosa. A similar localisation of NK_3-Ir was found in the human gastric fundus, but with apparently lower levels in the myenteric plexus.

Each of the mammalian tachykinins is present within the gut, although compared with NKA and substance P, the amount of NKB may be low (Tsuchida *et al*., 1990). Nevertheless, since large amounts of NKA and substance P occur within the ENS, it is argued that NK_3 receptors are activated by each of these tachykinins (Grady *et al*., 1996) released in amounts sufficent to activate the receptor, and in an 'activity-dependent' manner (see Introduction). This is consistent with observations that enteric tachykinin-containing neurones are not associated with clusters of NK receptors, and that tachykinins diffuse for significant distances within the gut, as indicated by the detection of tachykinins in venous effluent (*in vivo*) or bathing solutions (*in vitro*) surrounding an intact intestine (see Jenkinson *et al*., 1999).

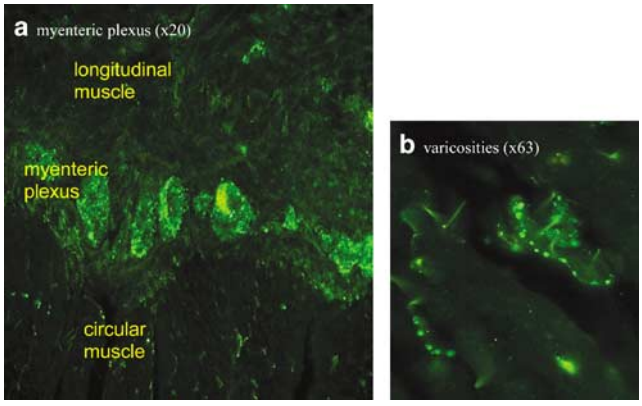

Figure 1 NK_3 receptor immunoreactivity in the myenteric plexus of human sigmoid colon (NB Dass; reported in Dass *et al*., 2002). A rabbit anti-NK_3 (438-452) receptor polyclonal antibody was used with an Alexa 488 conjugated secondary antibody. Intense NK_3-Ir was detected in (a) the myenteric and submucosal plexus and (b) was clearly evident on varicose nerve fibres. NK_3-Ir was not detected in smooth muscle cells, or in muscularis mucosa.

Gastric motility NK_3 receptor antagonists do not affect gastric tone or compliance in conscious dogs (Crema *et al*., 2002), or the gastric emptying of a liquid phenol red meal in conscious rats (Table 1). These data suggest that NK_3 receptors play no role in the control of normal gastric emptying. Further experiments are required to determine if NK_3 receptors can mediate disturbances in gastric motility.

Intestinal motility A major function of intestinal NK_3 receptors appears to be linked to the role of the IPANs. Transmission between IPANs, *via* slow EPSPs, is unaffected by NK_1 receptor ligands (Neunlist *et al*., 1999) but greatly inhibited by NK_3 receptor antagonists in guinea-pig intestine (Neunlist *et al*., 1999; Alex *et al*., 2001; Sanger *et al*., 2002). NK_3 receptors may also be involved in polarised reflexes of the intestine, possibly *via* transmission between IPANs or between IPANs and ascending or descending interneurons. Thus, experiments with senktide, which activates and desensitises NK_3 receptors, suggest that IPAN-evoked activation of the ascending excitatory pathway in guinea-pig intestine may be mediated by ACh and/or by tachykinins, acting at NK_3 receptors (Johnson *et al*., 1996). NK_3 receptors may also play a role in the descending inhibitory pathways (Johnson *et al*., 1998), involving NO-dependent and -independent neurotransmission (Lecci *et al*., 1996).

Distension-evoked activation of IPANs may not normally release tachykinins. This suggestion is possible because NK_3 receptor antagonism does not affect distension-evoked peristalsis in guinea-pig ileum (Holzer *et al*., 1998; B. Tuladhar, unpublished), in pig ileum (Schmidt & Hoist, 2002) or the hexamethonium-resistant propulsive movements of rat isolated colon (Lecci *et al*., 1996). Similarly, the NK_3 receptor antagonist talnetant (0.01, 0.1, 1.0 μM) did not affect neuronally-mediated, electrically-evoked contractions of human isolated taenia coli (unpublished) or rat gastro-caecal transit times (Table 1). Finally, NK_3 receptor antagonism did not affect baseline short-circuit current in guinea-pig colonic

Table 1 Effects of the NK_3 receptor antagonist talnetant (SB-223412), on gastrointestinal motility in conscious rats. (a) The effects on gastric emptying were measured 45 min after administration of a liquid phenol-red test meal in conscious rats (Tache *et al*., 1987). (b) Effects on GI motility were measured 30 min after oral dosing of a charcoal meal to conscious rats (Takemori *et al*., 1969)

Group[a]	*Oral treatment*	*Dose (mg/kg)*	*% gastric emptying*
1	Phenol red only	—	0.0
2	Vehicle	—	60.6
3	Talnetant	5	55.2
4	Talnetant	15	50.7
5	Talnetant	50	62.4
6	Morphine sulphate	20	30.8**

Group[b]	*Oral treatment*	*Dose (mg/kg)*	*Group mean distance travelled by charcoal as % of total gut length ± s.d.*	*% change from vehicle-treated animals*
1	Vehicle	—	53.0 ± 7.6	–
2	Talnetant	5	46.9 ± 5.3	−11.5
3	Talnetant	15	52.1 ± 7.1	−1.7
4	Talnetant	50	50.4 ± 8.6	−4.9
5	Morphine sulfate	10	37.4 ± 12.4	−29.4

[a]Significance of difference from the vehicle-treated group: $^{**}P<0.01$ (ANOVA followed by William's test; talnetant groups, or Student's *t*-test; morphine).
[b]Statistical significance of difference from the vehicle-treated group: $^{**}P<0.01$ (as above).
The doses of talnetant were selected to antagonise at the rat NK_3 receptor (Sarau *et al*., 1997) and were similar to those inhibit rat intestinal anti-nociceptive activity (Fioromonti *et al*., 2003).
s.d. = standard deviation.

mucosa (e.g., Goldhill & Angel, 1998; Frieling *et al*., 1999). Exceptions to these findings of inactivity are the ability of NK_3 receptor antagonism to facilitate the amplitude of giant contractions in rat isolated colon (Gonzalez & Sarna, 2001), or 'submaximal' propulsion in rabbit isolated colon (Onori *et al*., 2001), a preparation in which prokinetic activity may also be evoked by NK_1 receptor antagonism (Onori *et al*., 2003).

Evidence is now emerging to suggest that when IPANs are activated by a stimulus more intense than that required to evoke peristalsis, these neurones release tachykinins to activate NK_3 receptors on connecting IPANs and possibly elsewhere, to change intestinal motility and sensations. Prior to the availability of selective NK_3 receptor antagonists, clues to this function could be found using exogenously applied tachykinins. For example, the NK_3 receptor agonist senktide may activate NO-dependent and –independent, neuronally mediated circular muscle relaxation in guinea-pig colon (Giuliani & Maggi, 1995), facilitate ACh release from the myenteric plexus (Yau *et al*., 1992), stimulate and inhibit propulsive activity in rabbit distal colon (Onori *et al*., 2001), increase colonic spike bursts in conscious rats (Julia *et al*., 1999), facilitate the sensitivity to induction of peristalsis by intraluminal distension (Holzer *et al*., 1995), increase intestinal transit in conscious rats (Chang *et al*., 1999) and induce Cl^- secretion by guinea-pig colonic mucosa (but not in pig jejunum; Thorboll *et al*., 1998) (e.g., Goldhill & Angel, 1998; Frieling *et al*., 1999). To investigate the role of endogenous tachykinins in 'disrupted' intestinal movements, we studied the effects of talnetant on intestinal reflexes evoked by 'supramaximal' mechanical stimuli. We looked for an ability to influence polarised reflexes in intact guinea-pig isolated colon (Sanger *et al*., 2002), by the application of 6, 12 and 20 g weights to evoke contractions oral to the distension and small relaxations on the anal side; the amplitude of the contractions appeared to be independent of the load applied and all changes were prevented by nicardipine. Talnetant reduced the amplitudes of ascending excitatory reflexes and antagonised or even reversed the descending inhibitory reflexes. Significantly, these effects appeared to be greater when the heavier weights were used to generate the reflexes. Thus, these data could be explained if tachykinins were released from the IPANs by the increasingly greater degrees of stretch, to induce slow EPSPs *via* activation of NK_3 receptors and as a consequence, changes in the excitability of the polarised reflexes. Consistent with this hypothesis are electrophysiological experiments which show that slow EPSPs are graded with the frequency of activity in presynaptic fibres, with maximum amplitudes of slow EPSPs occurring in response to stimulation of inputs at 10 Hz or more (Morita & North, 1985).

Furness *et al*. (2002) showed that the NK_3 receptor antagonist SR 142901 did not affect excitatory enteric reflex activity in rat isolated colon induced by a submaximal degree of stretch (1.5 g). However, an effect of NK_3 receptor antagonism became apparent after sensitisation by repeat-distensions and during the combined presence of an NK_1 receptor antagonist. Consequently, these data remain consistent with the hypothesis that NK_3 receptors play a role in disrupted patterns of intestinal motility induced by relatively intense IPAN stimulation, while sparing reflexes evoked by milder stimulation. To further explore this idea, the effects of talnetant 250 nM were studied on peristalsis evoked in guinea-pig isolated ileum by 'optimal' and 'excessive' intraluminal distension (B Tuladhar, unpublished). Peristaltic waves elicited by optimal distension pressures (1.2–3 cmH_2O) were unaffected by talnetant, but the number of peristaltic events and thus, the efficiency of the peristaltic reflex was increased by talnetant when higher pressures (3.5 or 4 cmH_2O) were used. Together with the stretch experiments described above, it is suggested that NK_3 receptor antagonists have a role in mediating intestinal reflexes evoked by 'supraoptimal' stimuli.

Pain

NK_3 receptors are present within the intrinsic neurones of the spinal cord, where they have functional activity (see Fioramonti *et al*., 2003 for references). Further, intrathecal administration of NK_3 receptor antagonists reduces rat behavioural responses to noxious colo-rectal distension (Kamp *et al*., 2001; Gaudreau & Plourde, 2003). NK_3-Ir appears to be absent in normal adult rat dorsal root ganglia (DRG; Ding *et al*., 1996; Seybold *et al*., 1997), but NKB-evoked responses were detected by whole patch clamp in cultured capsaicin-sensitive neurones from rat DRG (Inoue *et al*., 1995) and a

functional NK_3 receptor may be present on capsaicin-sensitive nerve terminals within rat spinal cord, possibly activated by NKB released from intrinsic spinal neurones (Schmid *et al*., 1998). The latter suggests that NK_3 receptors will also be distributed to C-fibre nerve terminals within the intestine and this is supported by the ability of NK_3 receptor antagonism to reduce capsaicin-evoked, tetrodotoxin-sensitive contractions of guinea-pig isolated ileum or oesophagus (Bartho *et al*., 1999).

Selective NK_3 receptor antagonists, administered systemically, reduce nociceptive behaviour caused by colo-rectal distension in conscious rats (Julia *et al*., 1999; Fioramonti *et al*., 2003). Such activity was not seen when measuring pseudoaffective vascular responses to jejunal distension in anaesthetised rats (McLean *et al*., 1998), but mismatches between these methods of assessing intestinal nociception have previously been reported (Banner & Sanger, 1995). Julia *et al*. (1999) found that the antinociceptive effect was not mimicked by intracereboventricular administration of the same antagonist. Further, NK_3 receptor antagonism reduced distension-evoked spinal afferent nerve discharge from the intestine but did not do the same when distension was applied to the urinary bladder, an organ that does not possess IPAN's. The explanation for this organ-dependent difference is not clearly understood (see editorial by Mayer & Marvizon, 1999, for a discussion of these data), but the following was suggested. Firstly, central NK_3 receptors play only a minimal role in modulating intestinal nociception. Secondly, although a supraspinal site of action could not be excluded, it becomes a possibility that IPAN activity plays a role in the mechanism of action on the gut. Thus, the tachykinins released from intestinal IPANs may increase the sensitivity of spinal afferent nerve terminals within the intestine *via* a localised change in muscle tension and/or by direct activation of NK_3 receptors on these nerve terminals.

The hypothesis of a 'peripheral' site of action to explain the antinociceptive activity of NK_3 receptor antagonists receives support from Fioramonti *et al*. (2003). These authors used the rat colo-rectal distension model to compare the anti-nociceptive activity of orally administered talnetant with that of SB-235375, a selective NK_3 receptor antagonist with no measureable ability to enter the brain or spinal cord (see also Shafton *et al*., 2004, for pharmacokinetic data on SB-235375). SB-235375 exerted antinociceptive activity of potency and magnitude similar to that of talnetant (Figure 2). Shafton *et al*. (2004) also used SB-235375 to show that this compound inhibited nociceptive responses to brief colo-rectal distension, at doses that had no effects on similar nociceptive responses evoked by skin pinch; both nociceptive behaviours were measured as a visceromotor response *via* electromyograph recordings from the external oblique muscle of the abdomen. These results are consistent with an intestinal specificity of antinociceptive activity, first reported by Julia *et al*. (1999).

Summary and potential for treatment of FBDs

Antagonism at the receptor has little or no ability to modulate normal GI motility. However, NK_3 receptors may play a role in disrupted intestinal motility (Figure 3). It is hypothesised that during these conditions, the current data also point to a 'peripheral', possibly IPAN-mediated mechanism whereby NK_3 receptor antagonists inhibit intestinal nociception. Thus, the above hypothesis further proposes that neurokinins released from the IPANs can affect C-fibre sensitivity, either indirectly by affecting smooth muscle tension or directly by activation of NK_3 receptors located on C-fibre terminals within the intestine. To achieve this activity, IPANs and intestinal C-fibre terminals do not necessarily have to make synaptic contact, as tachykinins are known to diffuse for significant distances.

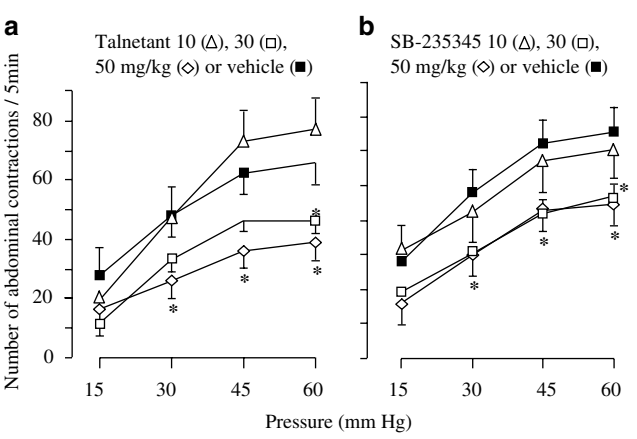

Figure 2 Inhibition of responses to colo-rectal distension in conscious rats by the selective NK_3 receptor antagonists talnetant and SB-235375 (Fioramonti *et al*., 2003). The effects of isobaric colo-rectal distension were assessed by measuring the number of abdominal contractions induced by increasing pressures of distension. Talnetant (a) or SB-235345 (b) was dosed orally and the effects were compared with vehicle; $n = 10$ each, $^*P < 0.05$ vs vehicle. Unlike talnetant, SB-235345 had no measurable ability to cross the rat blood–brain barrier into the brain or spinal cord (see also, Shafton *et al*., 2004).

NK_3 receptor antagonism may also reduce certain effects of immune (IgE and IgG serum litres reduced after sensitisation to cow's milk, but the increase in intestinal mast cell numbers was reduced only by NK_1 receptor antagonism; Gay *et al*., 1999) and inflammatory (TNBS-induced neurogenic colitis reduced by NK_3 or NK_2 receptor antagonism in guinea-pigs; Mazelin *et al*., 1998) stimuli on gut function. The mechanisms of these actions are unclear, but as for the above studies on intestinal motility and sensation, the data are consistent with an activity-dependent role for the receptor.

Conclusions

NK_1 and NK_3 receptors do not seem to play major roles in normal GI physiology, but evidence suggests that the receptors can be activated in an activity-dependent manner, indicating roles in 'defensive' and/ or pathological GI functions. In terms of FBDs, the role of the NK_1 receptor is unclear, but clinical studies involving gastro-vagal reflexes, intestinal motility and GI pain have yet to be performed. Recent studies with talnetant and other selective NK_3 receptor antagonists are beginning to open up an exciting and novel pathway by which pathological changes in intestinal motility and nociception can be induced, suggesting a role for NK_3 receptor antagonism in IBS.

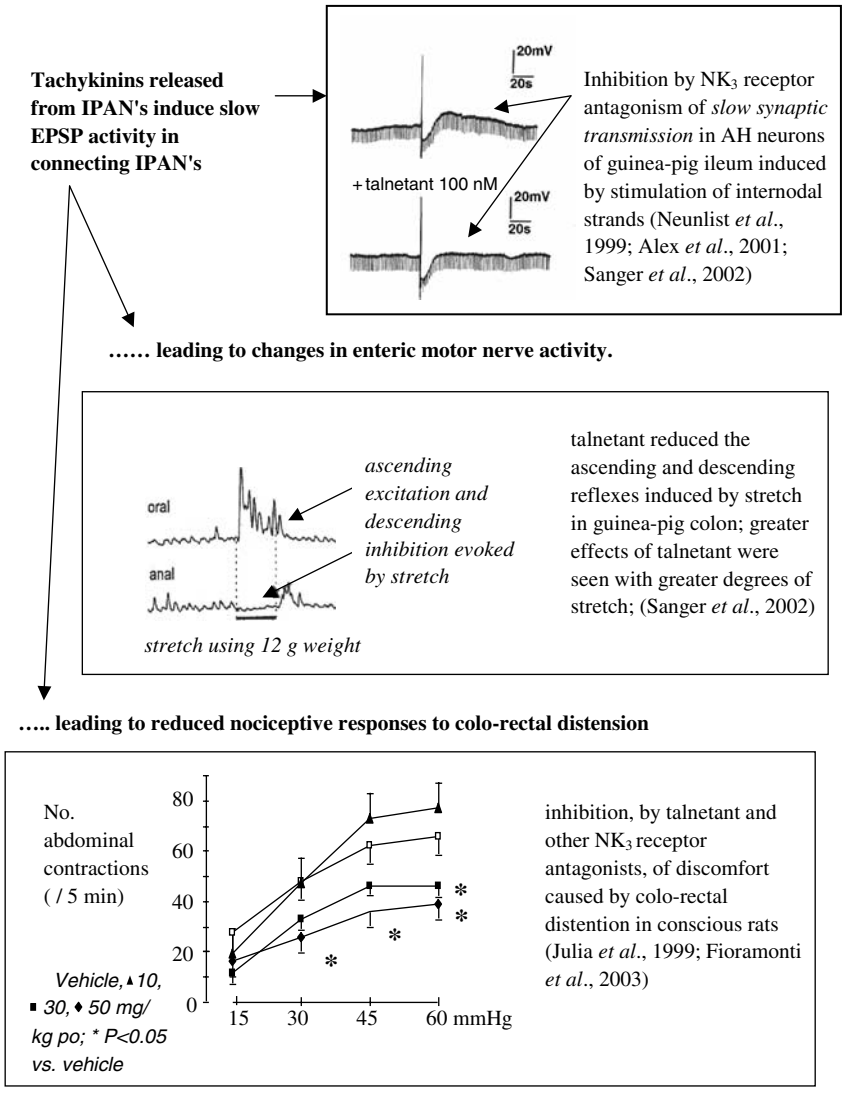

Figure 3 Mechanisms by which NK_3 receptors may play a role in disrupted intestinal motility and sensations.

Collegues and collaborators are acknowledged for stimulating discussions over the years: Drs Narinda Dass, Douglas Hay, Tachi Yamada, Lionel Bueno and Bishwa Tuledhar, and Professors John Furness and Paul Andrews.

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(Received January 20, 2004
Revised February 12, 2004
Accepted February 13, 2004)