

# Pharmacology and Function of Tachykinin Receptors

## Alessandro Lecci

Clinical Research Department, Menarini Ricerche, via Sette Santi 1, Firenze 50131, Italy.

Following his degree in biology in 1986, Alessandro Lecci has worked closely with the pioneer of tachykinin receptor pharmacology, Dr Carlo Alberto Maggi, at Menarini Ricerche in Florence. The majority of Dr Lecci's work has focused on the pharmacology of tachykinin receptors at the visceral level.

## Historical perspective

Tachykinins are a family of structurally-related peptides of which substance P (SP) is the most famous component. All mammalian tachykinins known up to now share a common C-terminal amino acid sequence (Table 1), i.e. Phe-Xaa-Gly-Leu-MetNH<sub>2</sub>, that is the minimal structural motif for the activation of tachykinin receptors.

The existence of SP was described in the 1930s by Von Euler and Gaddum<sup>1</sup> in mammals, and tachykinin peptides were identified and sequenced by Erspamer in non-vertebrates (eledoisin) and then in non-mammalian species (physalaemin).<sup>2,3</sup> In the 1960s Lembeck continued Von Euler and Gaddum's studies, describing the effects of SP and other tachykinins in the central nervous system (CNS) and peripheral preparations of mammals.<sup>4</sup> The CNS physiology of SP was further developed by Konishi and Otsuka in the 1970s,<sup>5</sup> whereas Hokfelt gave a major contribution<sup>6</sup> in defining the neuroanatomical localisation of this peptide. Meanwhile, Chang and Leeman had defined the amino acid sequence of SP,<sup>7</sup> which was then followed in the 1980s by the discovery of two other mammalian tachykinins, namely neurokinin A (NKA), also known as substance K, and neurokinin B (NKB), also known as neuromedin K, by Matsuo,<sup>8</sup> Munekata<sup>9</sup> and co-workers (Table 1).

In the 1980s, following an intense pharmacological investigation by means of mammalian and non-mammalian tachykinins, selective agonists and prototype antagonists, the existence of 3 tachykinin receptors termed NK<sub>1</sub>, NK<sub>2</sub>, and NK<sub>3</sub> was proposed by Regoli.<sup>10</sup> In the same period, 2 genes for tachykinins, namely preprotachykinin-A, encoding both SP and NKA,<sup>11</sup> and preprotachykinin-B, which encodes NKB,<sup>12</sup> were identified by Nakanishi and co-workers (Table 2). Two elongated forms of NKA originating from alternative splicings of preprotachykinin-A mRNA, defined as neuropeptide-gamma and kappa (Table 1) were then discovered in mammals.<sup>13,14</sup> In between the late 1980s and the begin of the 1990s, the hypothesis of the existence of 3 tachykinin receptors, belonging to the family of G-protein coupled receptors (GPCRs), was demonstrated by the cloning of NK<sub>1</sub>, NK<sub>2</sub>, and NK<sub>3</sub> receptors in various mammal species by Nakanishi's and other independent groups.<sup>15,16,17</sup> In the course of the 1990s, the synthesis of potent and selective peptide and non-peptide tachykinin receptor antagonists has allowed a substantial improvement in the knowledge about the role that tachykinin receptors play in physiological and pathological conditions (Table 3). This information was implemented at the end of 1990s through the construction of mutant mice lacking the preprotachykinin-A gene<sup>18</sup> or tachykinin NK<sub>1</sub> receptor.<sup>19</sup> Two major recent achievements in the field include the discovery of a new tachykinin, hemokinin-1 (HK-1) (Table 1, Figure 1), coded by a new tachykinin gene termed preprotachykinin-C in the mouse<sup>20</sup> or TAC4 in humans (Table 2), and the registration of aprepitant, a selective NK<sub>1</sub> receptor antagonist, for the treatment of emesis and nausea associated with the use of chemotherapy.

Notwithstanding the fact that tachykinins are among the most investigated peptides, basic and applied research in this field is still an exciting challenge because tachykinins regulate a very large number of functions, both at the CNS and peripheral level.

## Tachykinin NK<sub>1</sub> receptor agonists

Until the mid-1990s, the order of potency of mammalian tachykinins in the stimulation of human NK<sub>1</sub> receptors was believed to be SP>NKA=NKB.<sup>21</sup>

Table 1. Amino acid sequences of mammalian tachykinins

<b>Substance P (SP)</b>	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-MetNH <sub>2</sub>
<b>Mouse Hemokinin-1 (mHK-1)</b>	Arg-Ser-Arg-Thr-Arg-Gln-Phe-Tyr-Gly-Leu-MetNH <sub>2</sub>
<b>Human Hemokinin-1 (hHK-1)</b>	Thr-Gly-Lys-Ala-Ser-Gln-Phe-Phe-Gly-Leu-MetNH <sub>2</sub>
<b>Neurokinin A (NKA)</b>	His-Lys-Thr-Asp-Ser-Val-Phe-Gly-Leu-MetNH <sub>2</sub>
<b>Neurokinin B (NKB)</b>	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-MetNH <sub>2</sub>
<b>Neuropeptide gamma (NP-gamma)</b>	Asp-Ala-Gly-His-Gly-Gln-Ile-Ser-His-Lys-Arg-Lys-Asp-Ser-Val-Phe-Gly-Leu-MetNH <sub>2</sub>
<b>Neuropeptide kappa (NP-kappa)</b>	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-Gly-Gln-Ile-Ser-His-Lys-Arg-Lys-Asp-Ser-Val-Phe-Gly-Leu-MetNH <sub>2</sub>

(Bold text denotes compounds available from Tocris)

Neurochemicals ● Pharmacological Probes ● Peptides  
Signal Transduction Agents ● Biochemicals ● Radioligands  
**Advancing Research for the Life Scientist**

Tocris Cookson Ltd., UK  
Tel: + 44 (0)117 916 3333  
Fax: + 44 (0)117 916 3344  
customerservice@tocris.co.uk  
technicalsupport@tocris.co.uk

www.tocris.com

**TOCRIS**<sup>™</sup>

Tocris Cookson Inc., USA  
Tel: (800) 421-3701  
Fax: (800) 483-1993  
customerservice@tocrisusa.com  
technicalsupport@tocrisusa.com

**Table 2. Mammalian tachykinin peptides, mRNAs and genes<sup>11,12,13,14</sup>**

DNA (gene)	mRNAs	Peptide
Preprotachykinin-A (TAC1 or PPT-A)	AlphaTAC1 (alphaPPT-A) BetaTAC1 (betaPPT-A) GammaTAC1 (gammaPPT-A) DeltaTAC1 (deltaPPT-A)	SP SP, NKA, Neuropeptide kappa* SP, NKA, Neuropeptide gamma* SP
Preprotachykinin-B (TAC3 or PPT-B)	AlphaTAC3 (alphaPPT-B) BetaTAC3 (betaPPT-B)	NKB NKB
Preprotachykinin-C (TAC4 or PPT-C)	AlphaTAC4 BetaTAC4 GammaTAC4 DeltaTAC4	hHK-1, EKA*, EKC* hHK-1, EKB*, EKD* hHK-1, EKB* hHK-1, EKB*

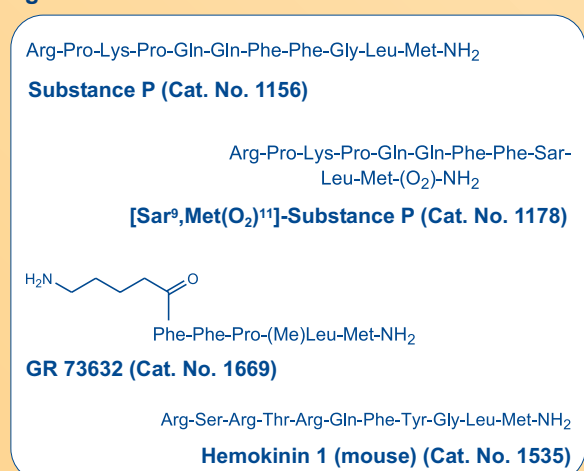
\*Neuropeptide gamma and neuropeptide kappa are elongated forms of NKA. EKA and EKB (endokinin A and B) are elongated forms of human hemokinin-1 (hHK-1), EKC and EKD are non-tachykinin peptides coded by the recently discovered TAC4 gene.<sup>20,38,221</sup>

This order of potency was assessed in the classic *in vitro* isolated organ bioassays which were considered monoreceptorial, such as the dog carotid artery, but also other preparations such as guinea-pig vas deferens, rabbit jugular vein, rabbit vena cava, mouse bronchus, and guinea-pig urethra have been used.<sup>21</sup> Following the assessment of species-related differences in receptorial pharmacology, the gold standard system for screening has become Chinese Hamster Ovary (CHO) cells transfected with human NK<sub>1</sub> receptors. In these bioassays, all tachykinins acted as full agonists, albeit with different potency.<sup>21</sup> However, when comparing the affinity of selective ((Pro<sup>9</sup>)-SP sulphone, SP methylester, septide) and non-selective (SP) tachykinin NK<sub>1</sub> receptor agonists, as estimated in the contraction of guinea-pig ileum and in the displacement of [<sup>3</sup>H]-(Pro<sup>9</sup>)-SP and [<sup>3</sup>H]-SP in the same organ, a clear discrepancy was observed. All the agonists were equipotent in inducing contraction, but septide was clearly less potent than (Pro<sup>9</sup>)-SP sulphone in displacing [<sup>3</sup>H]-(Pro<sup>9</sup>)-SP or [<sup>3</sup>H]-SP.<sup>22</sup> This and similar findings through the use of tachykinin agonists, including NKA<sup>23</sup> (but also with selective antagonists, see later on), led to the postulation of the existence of a "septide-sensitive NK<sub>1</sub> receptor" as opposed to the classic NK<sub>1</sub> receptor recognised by SP or by other selective NK<sub>1</sub> receptor agonists such as (Pro<sup>9</sup>)-SP or (Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>)-SP (Table 3, Figure 1). Further investigation on this topic concluded that these putative receptors correspond indeed to different conformers. This implies different ways of binding and activation of an unique NK<sub>1</sub> receptor,<sup>24</sup> where epitopes involved in the binding of SP on the one hand, and septide and NKA on the other, only partially overlaps.<sup>25</sup> The concept that different agonists for the same GPCR can activate it in different manners has been generalised to other

neurotransmitter systems and has led to the hypothesis that a given receptor can assume an almost infinite number of conformers.<sup>26</sup> Therefore, it is possible to figure out that the interaction of each conformer of the receptor with each G-protein is governed by the mass law equations, thus by different affinity constants, one for each pair of receptor conformer and G-protein conformer considered.<sup>27</sup> These different modes of receptor activation have functional consequences at the cellular<sup>28</sup> and tissue levels,<sup>29</sup> and also *in vivo*.<sup>30</sup> These different modes of receptor activation also have physiological relevance since it has been shown that endogenously released SP and NKA can both activate NK<sub>1</sub> receptors, and a selective antagonist blocks the effects induced by SP or NKA with different potencies.<sup>31,32</sup>

In CHO cells transfected with human NK<sub>1</sub> receptors, SP, (Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>)-SP, septide and NKA displayed a similar potency in inducing phosphatidylinositol hydrolysis, but the two latter agonists were markedly less potent than SP and (Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>)-SP in eliciting cAMP formation.<sup>28</sup> Likewise, in the isolated rat urinary bladder SP, septide, and NKA are almost equipotent in the induction of inositol phosphates accumulation, but the maximal effect induced by both septide and NKA exceeds that of SP.<sup>33</sup> Furthermore, in the guinea-pig trachea SP and (Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>)-SP can induce nitric oxide production which relaxes smooth muscle, whereas septide is not active in this respect.<sup>29</sup> In the guinea-pig ileum, the contraction induced by C-terminal truncated NK<sub>1</sub> receptor agonists (septide or GR-73632), but not that induced by full length agonists including SP and (Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>)-SP, are increased by tetrodotoxin.<sup>34,35</sup> *In vivo*, a "septide-sensitive" and "-insensitive" NK<sub>1</sub> receptor-mediated response has been respectively detected in the rat skin<sup>30</sup> by means of selective antagonists (see below), and in the guinea-pig airways by means of nitric oxide synthase inhibitor sensitivity.<sup>36</sup> Therefore, following these findings, the order of potency of tachykinins for the stimulation of NK<sub>1</sub> receptors has been revised as SP=NKA>NKB.

Recently, following the discovery of mouse HK-1 (mHK-1),<sup>20</sup> it has been found that mHK-1 promotes the survival (anti-apoptotic effect) of a bone marrow mouse B cell lineage (B220<sup>+</sup>), both in culture and *in vivo*. mHK-1 also stimulates the proliferation of interleukin-7-primed B cell precursors, whereas it blocks the endotoxin-induced differentiation of pre-B cells in culture. All the above mentioned effects of mHK-1 on B cells were blocked by selective tachykinin NK<sub>1</sub> receptor antagonists (L-732,138 or L-733,060). Moreover, the chronic *in vivo* treatment with L-732,138 or L-733,060 (but not the less active enantiomer of the latter compound on NK<sub>1</sub> receptors, L-733,061) significantly reduced the relative number of B220<sup>+</sup> cells in the femoral bone marrow of mice.<sup>20</sup>

**Figure 1. Structures of selected NK<sub>1</sub> receptor agonists**

(Bold text denotes compounds available from Tocris)

**Table 3. Order of potency of human tachykinins at NK<sub>1</sub>, NK<sub>2</sub>, and NK<sub>3</sub> receptors, and examples of selective agonists, antagonists and radioligands**

Receptor	NK <sub>1</sub>	NK <sub>2</sub>	NK <sub>3</sub>
Natural agonists	SP=hHK-1=NKA>NKB	NKA>NKB>SP=hHK-1	NKB>NKA>SP=hHK-1
Selective agonists	(Sar <sup>9</sup> ,Met(O <sub>2</sub> ) <sup>11</sup> )-SP Septide	(βAla <sup>9</sup> )NKA(4-10) (Lys <sup>5</sup> ,MeLeu <sup>9</sup> ,Nle <sup>10</sup> )NKA(4-10)	Senktide (Me-Phe <sup>7</sup> )NKB
Selective antagonists	<b>L-732,138</b> <b>L-733,060</b> SR-140333 GR 82334	<b>GR 159897</b> <b>MDL 29,913</b> SR-48968 MEN-11420	<b>SB 218795</b> <b>SB 222200</b> SR-142801 R-820
Radioligands	[ <sup>3</sup> H](Sar <sup>9</sup> ,Met(O <sub>2</sub> ) <sup>11</sup> )-SP [ <sup>125</sup> I]L-703606	[ <sup>125</sup> I](Lys <sup>5</sup> ,Tyr(I <sub>2</sub> ) <sup>7</sup> ,MeLeu <sup>9</sup> ,Nle <sup>10</sup> )-NKA(4-10) [ <sup>3</sup> H]SR-48968	[ <sup>125</sup> I]MePhe <sup>7</sup> -NKB [ <sup>3</sup> H]SB 222200

(Bold text denotes compounds available from Tocris)

An extensive pharmacological characterisation indicated that, according to the similarity in the sequences, this new tachykinin has a profile similar to that of SP both *in vitro* and *in vivo*.<sup>37</sup> More recently, the human form of HK-1 (hHK-1) has been isolated and characterised.<sup>38</sup> It has been established that both mHK-1 and hHK-1 are equipotent to SP (and about twice as potent as NKA) in stimulating calcium mobilisation and inositol phosphates accumulation in CHO cells expressing human NK<sub>1</sub> receptors. However, the affinity of hHK-1 (but not that of mHK-1) for displacing the binding of (<sup>125</sup>I)-SP is about ten times lower than that of SP itself.<sup>38</sup> Therefore, unlike mHK-1, it is possible that hHK-1 could act as “septide-like” agonist, as does NKA on NK<sub>1</sub> receptors. Species-related differences in the pharmacology of tachykinin receptors are commonly found with antagonists (see below) and seldom with agonists. An obvious explanation resides in the fact that receptor epitopes interacting with natural agonists are well-conserved along the species, whereas antagonists (especially non-peptide antagonists) can interact in other more variable regions of the receptor. To date, the order of potency of tachykinins for the stimulation of human NK<sub>1</sub> receptors has been established as SP=hHK-1=NKA>NKB (Table 3).

As we have seen, the induction of different receptor conformers by different receptor agonists can have macroscopically functional consequences; receptor desensitisation is another complex process made up by a series of independent events, which can affect the functional response induced by receptor stimulation. Interestingly, desensitisation of SP-mediated inositol phosphates accumulation in CHO cells expressing rat NK<sub>1</sub> receptors was regulated by the N-terminal sequence of SP. NKA and septide were less effective than SP in inducing receptor desensitisation but all these agonists were equipotent in evoking receptor phosphorylation.<sup>39</sup> The structure-activity of SP and other tachykinins in inducing receptor activation are clearly different from that governing receptor desensitisation.<sup>39,40</sup> N-terminal fragments of SP elicit tachykinin-receptor independent biological effects, which have, however, a close relationship with tachykinin NK<sub>1</sub> receptor physiology, including the regulation of desensitisation of SP-induced effects<sup>41</sup> and the regulation of NK<sub>1</sub> receptor expression.<sup>42</sup> However, in CHO cells N-terminal fragments of SP did not interfere themselves with the ability of other tachykinins or SP itself to induce desensitisation of NK<sub>1</sub> receptor-mediated inositol phosphates accumulation. Another important process in the NK<sub>1</sub> receptor physiology, which can take part in response desensitisation, is agonist-induced receptor internalisation.<sup>43</sup> This process, which requires receptor stimulation, is mediated by G-protein-coupled receptor kinases and beta-arrestins,<sup>44</sup> and it is important for limiting SP-mediated effects *in vivo*.<sup>45</sup>

The visualisation of NK<sub>1</sub> receptor endocytosis has allowed the monitoring of the precise sites and the time-course of receptor stimulation by endogenous tachykinins in the spinal cord during application of noxious stimuli<sup>32</sup> or in the periphery following visceral inflammation.<sup>45</sup> Interestingly, it has been established that both SP and septide induce a similar degree of NK<sub>1</sub> receptor endocytosis in both intestinal neurons and transfected CHO cells,<sup>39,46</sup> further suggesting that desensitisation of inositol phosphates accumulation and that induced by receptor internalisation are distinct phenomena.<sup>47</sup> In KRNK cells expressing rat NK<sub>1</sub> receptors, SP-induced Ca<sup>2+</sup> mobilisation was abolished following protein kinase C activation, indicating that this enzyme regulates desensitisation.<sup>48</sup> Importantly, this inhibition did not occur when these cells expressed a short form of NK<sub>1</sub> receptor, lacking the carboxyterminal intracellular domain of the receptor.<sup>48</sup> This short form of NK<sub>1</sub> receptor originates from an alternative splicing of both rat and human NK<sub>1</sub> receptor mRNA<sup>49</sup> and might have a different capability to transduce intracellular signals such as the activation of an anion inward current,<sup>50</sup> or Ca<sup>2+</sup> mobilisation. These differences seem related to a minor desensitisation rate affecting the short form, as compared to the long form,<sup>51</sup> and this short form could be considered as a true NK<sub>1</sub> receptor subtype. However, no evidence has been provided to indicate that this subtype can be differentiated from the long form through pharmacological means.

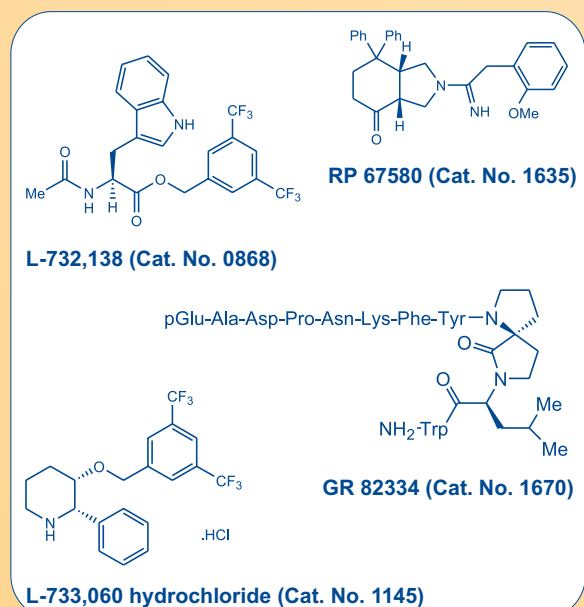
As introduced before, there are several cellular responses which are directly or indirectly activated through NK<sub>1</sub> receptors. In CHO cells transfected with the human NK<sub>1</sub> receptor, SP induces inositol phosphate accumulation through coupling to the alpha subunit of G<sub>q/11</sub>, increases cAMP levels through interaction with G<sub>s</sub>, and mobilises arachidonic acid through a pertussis toxin-sensitive G<sub>o</sub>.<sup>52</sup> Interestingly, in CHO cells, SP-induced Ca<sup>2+</sup> mobilisation is partly due to extracellular Ca<sup>2+</sup> entry, and to the release from intracellular stores; however, in both cases this mobilisation seems to depend on phospholipase C activity, since it is blocked by the inhibitor U-73122.<sup>51</sup> Furthermore, native NK<sub>1</sub> receptors expressed in CaCo-2 (colon adenocarcinoma cell line),<sup>53</sup> tracheal smooth muscle,<sup>54</sup> U373 MG (astrocytoma),<sup>55</sup> and peritoneal mast cells<sup>56</sup> are coupled to the activation of mitogen-activated kinases (MAPKs). The activation of this pathway by SP appears to be indirect since it is inhibited by pertussis toxin, genistein, staurosporine and U-73122.<sup>54</sup> The activation of several G-protein-coupled receptor kinases by SP also has been reported.<sup>57,58</sup> In endothelial,<sup>59</sup> epithelial,<sup>29</sup> and neuronal cells<sup>60</sup> the activation of NK<sub>1</sub> receptors induces nitric oxide and prostanoid production. This is possibly mediated by Ca<sup>2+</sup> mobilisation, whereas the production of superoxide anions occurs in neutrophils through a similar mechanism.<sup>61</sup> The stimulation of

phospholipase D activity by NK<sub>1</sub> receptor agonists has been reported in transfected CHO and astrocytoma cells,<sup>62</sup> and in monocytes,<sup>63</sup> probably as a consequence of protein kinase C activation.<sup>62</sup> Finally, tachykinins could activate rho kinase through the stimulation of NK<sub>1</sub> receptors.<sup>64,65</sup> The possibility of subtle pharmacological differences in the activation of these cellular pathways by different agonists is likely.<sup>53</sup> However, an extensive pharmacological characterisation of different NK<sub>1</sub> receptor-triggered transduction mechanisms has not yet been performed.

#### Tachykinin NK<sub>1</sub> receptor antagonists

As introduced in the above paragraph, different NK<sub>1</sub> receptor agonists can induce different cellular responses depending on their ability to induce and stabilise different receptor conformers, which in turn interact in a different manner with several G-proteins. If receptor antagonists could be capable of discriminating such conformers in terms of affinity and intrinsic activity, the pharmacological profile of receptor antagonists would be absolutely heterogeneous depending on the agonist used to elicit a response, the cellular system used, and the transduction pathway considered. This is indeed what is actually emerging in the characterisation of prototype NK<sub>1</sub> receptor antagonists structurally related to tachykinins, a phenomenon which has been defined as biased agonism. In fact, the first generation tachykinin antagonist (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP<sup>66</sup> behaved as a full potent agonist in the MAPK pathway in CaCo-2 cells<sup>53</sup> whereas it antagonised tachykinin-induced accumulation of inositol phosphate and contraction in the rabbit iris sphincter muscle<sup>67</sup> and in guinea-pig taenia coli.<sup>68</sup> Interestingly, the non-peptide NK<sub>1</sub> receptor antagonist CP-96345 inhibited both (D-Pro<sup>2</sup>, D-Trp<sup>7,9</sup>)-SP - and SP-induced MAPK activation.<sup>53</sup> Obviously, the larger the structural difference between the agonist and the antagonist, the more likely the possibility that the antagonist behaves as a “pure” antagonist. There are, however, several examples of non-peptide agonists for other kinds of peptide receptors (opioid, bradykinin, cholecystokinin) which differ from natural agonists in the modality of receptor activation. Following the tachykinin sequence-based peptide antagonists, which possessed several drawbacks (low potency, poor selectivity, residual agonist activity),<sup>21</sup> the

**Figure 2. Structures of selected NK<sub>1</sub> receptor antagonists**



(Bold text denotes compounds available from Tocris)

**Table 4. Affinity of agonists and antagonists as evaluated in the displacement of [<sup>125</sup>I]SP binding in human NK<sub>1</sub> receptor-transfected CHO cells<sup>38,72,73</sup>**

Ligand	IC <sub>50</sub> (nM)
<b>SP</b>	0.12
hHK-1	1.8
<b>mHK-1</b>	0.13
<b>NKA</b>	14
SR-140333	0.04
GR-203040	0.08
L-742694	0.09
LY-303870	0.4
FK-888	0.51
CP-99994	0.53
CGP-49823	1.0
RPR-100893	1.5
<b>L-732,138</b>	2.3

(Bold text denotes compounds available from Tocris)

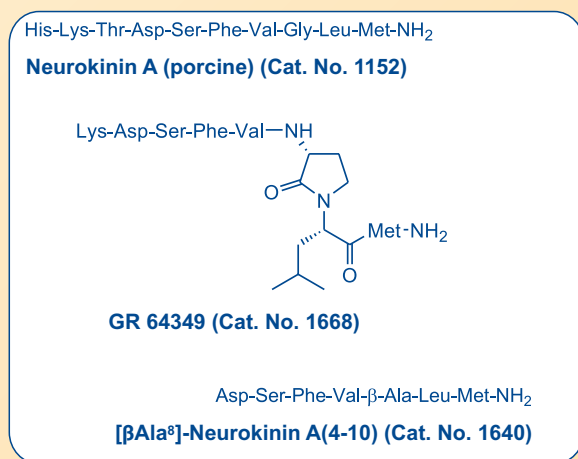
second generation of tachykinin antagonists, such as GR 82334<sup>69</sup> (Figure 2) proved to be good tools for receptor characterisation, especially *in vitro* (Table 3). The actual turning point in NK<sub>1</sub> receptor pharmacology was the discovery of potent and selective non-peptide antagonists, such as CP-96345<sup>70</sup> and RP 67580.<sup>71</sup> However, even these compounds showed unfavourable characteristics such as presence of non-specific effects and species-related variations in the affinity for NK<sub>1</sub> receptors.<sup>21</sup> In particular, the affinity of CP-96345 is high at the human, bovine, guinea-pig, and rabbit, but is markedly lower at the mouse and rat NK<sub>1</sub> receptors, whereas the opposite profile applies for RP 67580.<sup>21</sup> A large number of non-peptide NK<sub>1</sub> receptor antagonists have followed these first ones, most of them displaying a species selectivity similar to CP-96345, including the tryptophan and the piperidine derivative L-732,138 and L-733,060, respectively.<sup>72,73,74</sup> (Table 4, Figure 2). Interestingly, the apparent affinity of CP-96,345 and RP 67,580 estimated respectively in guinea-pig and rat NK<sub>1</sub> receptor bioassays, was higher against septide or NKA than SP or (Sar<sup>9</sup>, Met(O<sub>2</sub>)<sup>11</sup>)-SP. This led to the postulation of the existence of “septide-sensitive” receptors formerly, and of different receptor conformers later on, respectively induced by septide and SP (see above). This difference in the affinity of antagonists, when evaluated against SP or NKA, has physiopharmacological relevance, since the NK<sub>1</sub> receptor antagonist GR-205171 potently blocked spinal cord NK<sub>1</sub> receptor internalisation induced by NKA but was much less active against SP, either when the agonists were exogenously administered or when they were released from primary afferent neurons following noxious stimulation.<sup>32</sup> Among potent and selective NK<sub>1</sub> receptor antagonists, the quaternary ammonium SR-140333<sup>75</sup> has been particularly useful for the characterisation of peripheral effects mediated by NK<sub>1</sub> receptors, since its affinity minimally varied in different species and because of its poor blood-brain barrier penetration. In contrast, L-733,060, which antagonises SP-induced Ca<sup>2+</sup> mobilisation in transfected CHO cells with an estimated affinity of 0.8 nM,<sup>74</sup> possesses a good blood-brain barrier permeability and has been instrumental to the characterisation of CNS effects mediated by NK<sub>1</sub> receptors in guinea-pigs, gerbils, and mice.<sup>76,77</sup> Unlike CP-96345, L-733,060 did not produce unspecific cardiovascular effects in rats up to

1 mg/kg i.v.,<sup>74</sup> this despite the above-mentioned greater affinity for human, guinea-pig, and gerbil vs rat and mouse NK<sub>1</sub> receptors.

### Tachykinin NK<sub>2</sub> receptor agonists

All mammalian tachykinins act as full agonists at tachykinin NK<sub>2</sub> receptors in all species examined so far.<sup>21</sup> The order of potency at the human NK<sub>2</sub> receptor is NKA>NKB>SP=hHK<sup>121,38</sup> (Table 3). The prototypical monoreceptorial bioassay has been the rabbit pulmonary artery, although the rat vas deferens, hamster trachea, human urinary bladder, bronchus and colon have also been used for the characterisation of NK<sub>2</sub> receptor ligands.<sup>21</sup> Recent evidence indicates that a small component of the contractile response in human bronchus is mediated by tachykinin NK<sub>1</sub> receptors,<sup>78</sup> but their contribution to NKA-induced contractions is negligible. As for NK<sub>1</sub> receptors, the gold standard bioassay has become CHO cells transfected with human NK<sub>2</sub> receptors, because of the evidence of species-related differences in NK<sub>2</sub> receptor pharmacology, especially for those concerned with antagonist affinity. However, minor differences have been also observed with agonists, since the first selective NK<sub>2</sub> receptor agonist characterized, (βAla<sup>8</sup>)NKA(4-10)<sup>79</sup> (Figure 3), was

**Figure 3. Structures of selected NK<sub>2</sub> receptor agonists**



(Bold text denotes compounds available from Tocris)

almost inactive at the mouse NK<sub>2</sub> receptor, as determined in the Ca<sup>2+</sup> mobilisation response in murine neuroblastoma C1300 cells<sup>80</sup> or in inducing contraction in the isolated mouse urinary bladder.<sup>81</sup> (βAla<sup>8</sup>)NKA(4-10) maintains its selectivity up to micromolar concentrations,<sup>79</sup> then stimulation of NK<sub>1</sub> receptors can occur. Therefore, the use of a selective NK<sub>1</sub> receptor antagonist is recommended when estimating the apparent affinity of NK<sub>2</sub> receptor antagonists in preparations which also express NK<sub>1</sub> receptors coupled to contractile mechanisms. A water-soluble, selective NK<sub>2</sub> receptor agonist (Lys<sup>5</sup>,MeLeu<sup>9</sup>,Nle<sup>10</sup>)NKA(4-10) is also available<sup>82</sup> (Table 3).

Elegant works have analysed the relationships between agonist binding and second messenger responses in HEK 293 cells transfected with human NK<sub>2</sub> receptors. This has been achieved by labelling, with a fluorescence probe, both the receptors and the agonist, thus allowing the simultaneous detection of binding and Ca<sup>2+</sup> mobilisation. cAMP production also has been monitored.<sup>83,84,85</sup> Fluorescent NKA binds to the receptors in a biphasic manner since a fast (seconds) component is followed by a slower one (minutes); the fast component correlated with the

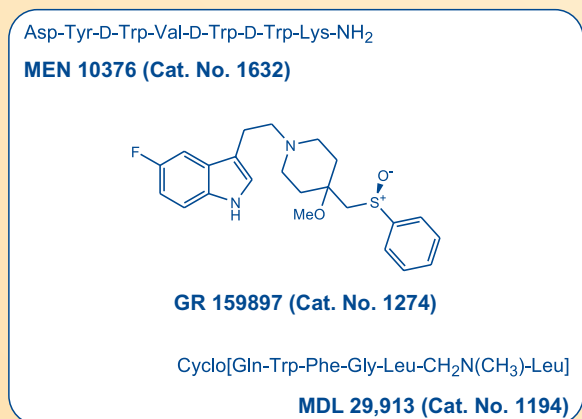
onset of Ca<sup>2+</sup> mobilisation whereas the slower component was associated to the phase of desensitisation of this response and the onset of cAMP production.<sup>85</sup> Interestingly, the truncated form, fluorescent NKA(4-10) possessed the fast component of binding only, and was ineffective on cAMP production; furthermore the Ca<sup>2+</sup> mobilisation induced by this agonist followed a pattern of bursts, whereas that induced by NKA was monophasic.<sup>85</sup> A previous study indicated that phosphorylation of the C-terminal intracellular domain of NK<sub>2</sub> receptors by protein kinase C was responsible for desensitisation of inositol phosphate accumulation, as assessed by the magnified and sustained response in cells expressing a C-terminal truncated form of NK<sub>2</sub> receptors.<sup>83</sup> Therefore, although the extent of desensitisation induced by protein kinase C activation is less than for NK<sub>1</sub> receptors, this enzyme also takes part in NK<sub>2</sub> receptor desensitisation.<sup>83,85</sup> Likewise, the C-terminal domain of the NK<sub>2</sub> receptor regulates the extent of MAPK activation, since this signal is sustained in the mutant, but transient in wild type receptors.<sup>86</sup> Similar to NK<sub>1</sub> receptors, the activation of human NK<sub>2</sub> receptors in CHO cells induced a pertussis toxin-sensitive (35% inhibition) arachidonic acid mobilisation and consequential PGE<sub>2</sub> production.<sup>87</sup> This response was dependent on Ca<sup>2+</sup> mobilisation, since it was abolished by the channel blocker SKF-96365 or EGTA but was phospholipase C-independent because it was unaffected by U-73122. On the other hand, SKF-96365 or EGTA had no effect on NKA-induced inositol triphosphate production.<sup>87</sup> These results indicate that, following NK<sub>2</sub> receptor stimulation by NKA, but also by the truncated agonist (βAla<sup>8</sup>)NKA(4-10), at least two independent transduction pathways are activated. One pathway mobilises intracellular calcium through the stimulation of inositol triphosphate receptors, the other one requires extracellular calcium entry which activates a phospholipase A<sub>2</sub> cascade. This latter pathway was activated by lower concentrations of agonists than the former, and this was consistent for all the agonists tested.<sup>87</sup> Finally, tachykinins could activate rho kinase in the rabbit penis and mouse urinary bladder but whether NK<sub>2</sub> receptors are involved in this effect is not yet known.<sup>64,65</sup>

A splice-variant of mRNA for human and rat NK<sub>2</sub> receptors has been recently identified.<sup>88</sup> However, it is unknown whether a receptor protein corresponds to this mRNA, and whether tachykinins can bind to this protein.

### Tachykinin NK<sub>2</sub> receptor antagonists

The extensive characterisation of first generation selective tachykinin NK<sub>2</sub> receptor antagonists, such as the peptides MEN 10376<sup>89</sup> and L-659877,<sup>90</sup> led to the detection of marked species-related differences in affinity of antagonists<sup>21</sup> (Table 5). In particular, the affinity of MEN 10376 was higher than that of L-659877 in preparations from rabbits, guinea-pigs, bovines, and humans, whereas the opposite occurred in hamster and rat preparations.<sup>21</sup> The cyclic peptide MDL 29,913<sup>91</sup> (Figure 4) possesses high affinity for rat (gastric fundus, 2 sites, high and low affinity, K<sub>i</sub> = 0.03 and 26 nM, respectively), and hamster (trachea pA<sub>2</sub> = 8.6) preparations and lower for dog (urinary bladder pA<sub>2</sub> = 6.4) rabbit (pulmonary artery pA<sub>2</sub> = 7.8), guinea-pig (bronchus pA<sub>2</sub> = 6.4) and human (inactive at 2 μM) preparations.<sup>21,91,92,93</sup> MDL 29,913 has been useful for the characterisation of NK<sub>2</sub> receptor-mediated effects, at the peripheral level.<sup>94</sup> However, the major drawback of this compound was the display of non-specific effects<sup>94</sup> and short duration of effect *in vivo*.<sup>89,94,95</sup> These problems have been overcome with the discovery of the bicyclic glycosylated peptide MEN-11420<sup>96</sup> and the non-

**Figure 4. Structures of selected NK<sub>2</sub> receptor antagonists**



(Bold text denotes compounds available from Tocris)

peptide NK<sub>2</sub> receptor antagonists SR-48968<sup>97</sup> and GR 159897.<sup>98</sup> The affinity of various NK<sub>2</sub> receptor antagonists, as evaluated through the inhibition of NK<sub>2</sub> receptor-mediated contraction in isolated preparations, is shown in Table 5. GR 159897 is a potent antagonist when evaluated in functional experiments in the guinea-pig trachea ( $pA_2 = 8.7$ ), or in binding studies performed in CHO cells transfected with human NK<sub>2</sub> receptors ( $pK_i = 9.5$ ), or in rat colon membranes ( $pK_i = 10$ ). Through the use of these antagonists, the pathophysiological role of NK<sub>2</sub> receptors has been thoroughly investigated in both the periphery and CNS.

**Table 5. Affinity (pK<sub>B</sub>) values of selective tachykinin NK<sub>2</sub> receptor antagonists evaluated against (βAla<sup>8</sup>)NKA(4-10)-induced contractions in hamster trachea (HT), rabbit pulmonary artery (RPA), rat vas deferens (RVD) or rat urinary bladder (RUB\*).**<sup>96,210</sup>

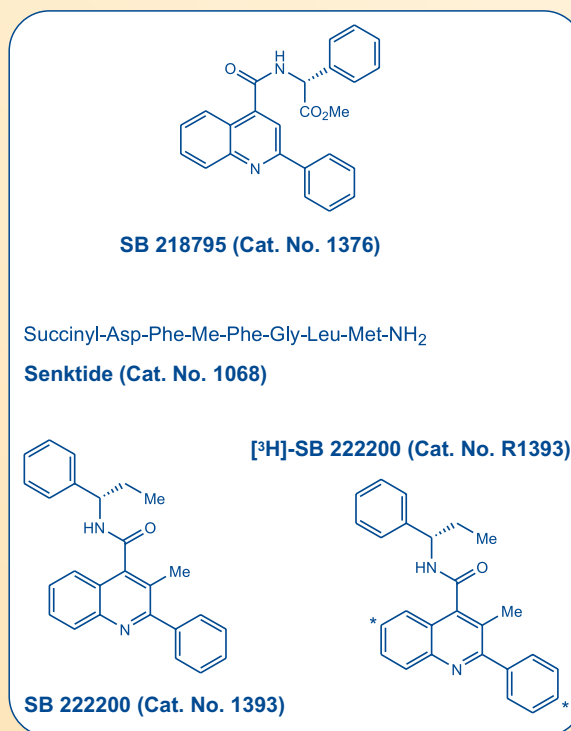
	HT	RPA	RVD or RUB*
<b>MEN 10376</b>	5.6	8.1	6.7
<b>MEN 10627</b>	10.2	8.6	9.0*
MEN-11420	10.1	8.1	8.8*
L-659877	7.9	6.7	7.9
<b>MDL 29,913</b>	8.6	7.6	8.5
SR-48968	8.5	9.6	8.3

(Bold text denotes compounds available from Tocris)

#### Tachykinin NK<sub>3</sub> receptor agonists

All mammalian tachykinins act as full agonists at tachykinin NK<sub>3</sub> receptors in all species examined so far.<sup>21</sup> The order of potency at the human NK<sub>3</sub> receptor is NKB>NKA>SP=hHK-1<sup>21,37,38</sup> (Table 3). The prototypical monoreceptorial bioassay has been the rat portal vein, although the guinea-pig ileum and the rabbit iris have been also used for the characterisation of these receptors.<sup>21,99</sup> NKB and the selective agonists senktide<sup>100</sup> (Figure 5) and (MePhe<sup>7</sup>)-NKB displayed higher affinity in binding experiments at murine as compared to human NK<sub>3</sub> receptors expressed in HEK 293 cells, and similar differences have been also detected in both binding and functional experiments with NKA and SP.<sup>101</sup> Although selective, (MePhe<sup>7</sup>)-NKB is capable of stimulating guinea-pig NK<sub>2</sub> receptors at micromolar concentrations.<sup>102</sup> In CHO cells transfected with human NK<sub>3</sub> receptors, senktide potently ( $pEC_{50} = 8.7$ ) induces extracellular medium acidification. This

**Figure 5. Structures of selected NK<sub>3</sub> receptor agonists and antagonists**



(Bold text denotes compounds available from Tocris)

response was inhibited by the protein kinase C inhibitor staurosporine, and by intracellular calcium depletion through thapsigargin. Therefore, the coupling of NK<sub>3</sub> receptors with G<sub>q/11</sub> and a consequent phospholipase C activation seem likely.<sup>103</sup> A similar transduction mechanism was also described in human cultured intestinal smooth muscle cells.<sup>104</sup> The possibility of the existence of a novel tachykinin receptor homologous to the NK<sub>3</sub> receptor, defined as NK<sub>4</sub> receptor,<sup>105</sup> has been recently excluded by pharmacological and molecular biology techniques.<sup>106,107</sup>

#### Tachykinin NK<sub>3</sub> receptor antagonists

The prototype peptide NK<sub>3</sub> receptor antagonist (Trp<sup>7</sup>,βAla<sup>8</sup>)NKA(4-10) possesses a relatively moderate affinity and a poor selectivity for NK<sub>3</sub> receptors.<sup>21</sup> However, its derivative R-820 shows a comparable affinity ( $pA_2 = 7.6$  on rat portal vein) but an increased selectivity over NK<sub>1</sub> and NK<sub>2</sub> receptors.<sup>108</sup> A turning point in NK<sub>3</sub> receptor pharmacology has been the discovery of SR-142801 (Table 3), which possesses high affinity for human and guinea-pig NK<sub>3</sub> receptors, but significantly lower for rodent NK<sub>3</sub> receptors, with the risk of non-specific effects at pharmacological concentrations.<sup>109</sup> Similar affinity profiles were described in binding experiments for the quinoline carboxamide derivatives SB 218795<sup>110</sup> and SB 222200<sup>111</sup> (Table 6, Figure 5). Affinities ( $pA_2$  values) estimated in rabbit iris through functional experiments (senktide as agonist) were 7.9 and 7.4 for SB 222200 and SB 218795, respectively.<sup>99</sup> Such antagonism was also observed *in vivo*, since miosis induced by senktide in rabbits was potently antagonised by SB 222200 (1 and 2 mg/kg i.v.), or SB 218795 (0.5 and 1 mg/kg i.v.).<sup>99</sup> Despite the lower affinity for rodent NK<sub>3</sub> receptors, SB 222200 is useful for the characterisation of the effects mediated by NK<sub>3</sub> receptors in CNS of both rats and mice since this compound possesses a good penetration of the blood brain barrier and, in this latter species at moderate (5 mg/kg) oral doses, it is capable of antagonising behavioural effects induced by the i.c.v. administration of senktide.<sup>106</sup>

**Table 6. Affinity ( $K_i$ , nM) of SB 218795 and SB 222200 for NK<sub>3</sub> receptors, as determined through binding experiments at human (hNK<sub>3</sub>) and mouse (mNK<sub>3</sub>) receptors, and in rat and guinea-pig<sup>110,111</sup>**

	SB 218795	SB 222200
CHO-hNK <sub>3</sub>	13	4
HEK 293 mNK <sub>3</sub>	n.e.	174
Rat brain NK <sub>3</sub>	n.e.	88
Guinea-pig brain	n.e.	3

n.e. = not evaluated

(Bold text denotes compounds available from Tocris)

### Effects mediated by tachykinin receptors in the central nervous system

#### Nociception

Since Lembeck's early discovery that SP was expressed by primary afferent neurons,<sup>112</sup> a large number of experimental works have substantiated, supported and expanded this concept. SP, released by primary afferent fibres upon noxious stimulation, depolarises spinal cord neurons projecting to supraspinal sites.<sup>113</sup> Despite this, the antinociceptive effect is not very broad and consistent across different antagonists in integrated animal models, and the analgesic effect of these drugs is very limited, if at all, in humans.<sup>114</sup> This may depend on the experimental conditions, the kind of model examined, the relative neuronal plasticity and species-related differences in the physiology of the tachykinin system, but also on the specific arrangement of neuronal circuitries activated by tachykinins.<sup>115,116,117</sup> Mice lacking the preprotachykinin-A gene display analgesia to moderate-to-intense thermal, mechanical and chemical stimulation, supporting the concept that SP and NKA are key mediators and, together with excitatory amino acids, are released by primary afferent neurons following noxious stimulation.<sup>18</sup> Despite this, acute thermal and mechanical nociceptive thresholds were not changed in NK<sub>1</sub> receptor knock-out mice, and the only deficit in sensory transmission shown by these animals was the absence of wind-up in spinal cord neurons in response to repetitive noxious stimulation.<sup>19</sup> However, NK<sub>1</sub> receptor knock-out mice displayed another important deficit that is related to the emotional component in the response to noxious and stressful stimulation, i.e., the absence of stress-induced analgesia. Importantly, such an effect was reproduced by the selective NK<sub>1</sub> receptor antagonist RP 67580.<sup>19</sup> The possibility that NK<sub>1</sub> receptors are important elements in the activation of supraspinal inhibitory control on spinal cord afferent input has been further supported by studies with NK<sub>1</sub> receptor antagonists (RP 67580) and NK<sub>1</sub> receptor knock-out mice. Following these manipulations, c-fos expression induced by noxious thermal stimulation of the paws was reduced in the spinal cord but increased at supraspinal sites.<sup>118</sup> The testing of selective NK<sub>1</sub> receptor antagonists such as RP 67580 (0.2-6 mg/kg), CP-96345 (0.5-8 mg/kg), or L-733,060 (3-15 mg/kg) in dopamine beta-hydroxylase knock-out mice, has allowed the establishment that stimulation of NK<sub>1</sub> receptors contributes to the activation of noradrenergic descending inhibitory systems.<sup>119</sup> In fact, mice lacking noradrenaline were hyperalgesic to thermal stimuli and this hyperalgesia was reversed by the above-mentioned NK<sub>1</sub> receptor antagonists. Importantly, these mice were less sensitive to the antinociceptive effects of morphine, and this effect was fully reversed by L-733,060 (15 mg/kg).<sup>119</sup> From

these findings, beyond the involvement of tachykinins and NK<sub>1</sub> receptors in emotional behaviour (see below), it could be deduced that the analgesic effect of NK<sub>1</sub> antagonists is more evident in those paradigms depending on spinal reflexes, and in experimental conditions where the descending inhibitory control has been minimised. Furthermore, since tachykinins are more abundant in visceral than in somatic primary afferent neurons, tachykinin antagonists are more effective in models of visceral hyperalgesia. Therefore, NK<sub>1</sub> receptor knock-out mice have a decreased nocifensor response to intracolonic acetic acid, capsaicin or cyclophosphamide-induced cystitis<sup>120</sup> and a reduced response to intradermal capsaicin.<sup>121</sup> This finding has been reproduced through the use of the selective tachykinin NK<sub>1</sub> receptor antagonist L-703,606, which also reduced ATP-induced nociception but not the early phase of formalin-induced nociception.<sup>122</sup> In contrast, the late phase of the formalin test was inhibited by several NK<sub>1</sub> receptor antagonists,<sup>71,123</sup> including L-733,060.<sup>124</sup> Evidence has been also provided for the involvement of NK<sub>1</sub> receptors in models of neuropathic pain, since NK<sub>1</sub> receptor knock-out mice do not develop mechanical hyperalgesia following nerve injury.<sup>125</sup> Furthermore, intrathecal administration of RP 67580, CP-96345, SR-140,333, or L-732,138 or systemic administration of CI-1021 or GR205171 reduce mechanical hyperalgesia induced by sciatic nerve ligation or diabetes.<sup>126,127,128,129</sup>

Tachykinin NK<sub>3</sub> receptor antagonists have anti-hyperalgesic effects following spinal cord sensitisation in various models. For instance, SB-223412 (50 mg/kg p.o.) antagonised thermal hyperalgesia induced by complete Freund's adjuvant-induced monoarthritis in rats<sup>130</sup> whereas SR-142801 reduces neuronal hyperexcitability induced by stimulation of the sural nerve in rabbits.<sup>131</sup> SR-142801 also reduces the sensitisation of visceromotor response induced by rectal distension in zymosan-treated rats.<sup>132</sup> There are a few reports describing centrally-mediated analgesic effects induced by NK<sub>2</sub> receptor antagonists. SR-48968 was active in the late phase of the formalin test in mice, in a model of neuropathic pain, or reflex facilitation following sural nerve stimulation.<sup>123,126,131</sup> In this latter model, however, the effect of SR-48968 has been attributed to the interaction with NK<sub>3</sub> receptors.<sup>131</sup>

#### Psychopharmacology

Following the discovery of the antidepressant effect exerted by MK-869 (aprepitant) in humans,<sup>133</sup> several studies have investigated the psychopharmacological profile of tachykinin receptor antagonists. As recently pointed out,<sup>134</sup> the antidepressant profile of NK<sub>1</sub> receptor antagonists in animal models is rather atypical, since most of them are also active in models of anxiety, whereas tricyclic antidepressants or serotonin reuptake inhibitors are not. The first report of anxiolytic effect of an NK<sub>1</sub> receptor antagonist (FK-888, administered i.c.v.) was on the elevated plus maze test in mice,<sup>135</sup> and this effect was later on confirmed in gerbils through the use of MK-869, L-742649, L-733,060, CP-99994, and CP-122721.<sup>136</sup> In the same species L-760735, another NK<sub>1</sub> receptor antagonist, was active in the social interaction test, and against conditioned fear.<sup>137,138</sup> Likewise, NPK608 (an NK<sub>1</sub> receptor antagonist) was active in the social interaction test in rats and blocked stress-induced hyperthermia in mice.<sup>139,140</sup> In this latter paradigm, it is known that hyperthermia is associated with tachycardia and an increase in arterial blood pressure due to sympathetic activation. Interestingly, the i.c.v. administration of CP-96345 or RP 67580 reduced the cardiovascular and behavioural activation induced by a stressful noxious stimulation in rats,<sup>141</sup> and the i.c.v.

administration of tachykinins mimicked both cardiovascular and behavioural effect induced by stress.<sup>142</sup> These results are consistent with the important role of NK<sub>1</sub> receptors in the activation of supraspinal catecholaminergic system and in the triggering of autonomic responses to stress.<sup>119</sup>

NK<sub>2</sub> receptor antagonists also display anxiolytic activity in animal models. GR-100679, GR 159897 and SR-48968 increased the time spent in the light in the mouse light-dark box paradigm, and decreased the fear of marmosets exposed to a threat.<sup>143,144</sup> SR-48968 was also active in the plus maze test in mice.<sup>135</sup>

Selective NK<sub>3</sub> receptor agonists show a potential anxiolytic activity in the elevated plus maze test in mice,<sup>135,145</sup> whereas the antagonist R-820, administered i.c.v., displays an anxiogenic profile.<sup>145</sup>

Several NK<sub>1</sub> receptor antagonists have been shown to possess a potential antidepressant activity in animal models such as chronic mild stress, learned helplessness, forced swimming test, resident intruder test, and inhibition of neonatal vocalisation induced by maternal separation.<sup>134</sup> In this latter paradigm GR-205171 and L-733,060 were active in mice and guinea-pigs, respectively.<sup>146</sup> Recently, MK-869, L-742694, L-733,060, CP-99994, and CP-122721 have been shown to reduce the immobility time in the tail suspension test adapted for gerbils, without affecting spontaneous locomotor activity.<sup>147</sup>

There is evidence for antidepressant activity exerted by the NK<sub>2</sub> receptor antagonist SR-48968 in the forced swimming test and in neonatal vocalisation induced by maternal separation; this effect was associated with a reduction of locus coeruleus firing induced by stress or by administration of corticotropin releasing factor.<sup>148</sup> Furthermore, the chronic administration of SR-48968, or established antidepressant drugs, or treatment (electroconvulsive shock), increased the PRAX1 protein in the CA1 pyramidal cell layer of the hippocampus.<sup>149</sup>

Recently, a selective NK<sub>3</sub> receptor agonist has been found active in the forced swimming test, this effect being associated with the release of opioids, since it was absent in opioid-deficient mice, and antagonised by naltrexone in normal mice.<sup>150</sup>

A link between tachykinins and opioids has been shown to occur in mechanisms regulating locomotor activity and reward. In NK<sub>1</sub> receptor knock-out mice, morphine did not induce hyperlocomotion or conditioned place preference, and the behavioural response induced by naloxone in morphine-treated mice was altered (i.e., jumping was absent).<sup>151</sup> A reduction of naloxone-induced withdrawal signs in morphine-dependent rats was also observed following the intrathecal administration of the NK<sub>1</sub> antagonist SR-140333.<sup>152</sup> Although cocaine-induced place preference was not inhibited in NK<sub>1</sub> receptor knock-out mice, L-733,060 significantly reduced cocaine-induced dopamine overflow in the striatum,<sup>153</sup> an effect that could account for the neuroprotective effect of L-733,060 against methamphetamine-induced neurotoxicity in striatal dopaminergic neurons.<sup>154</sup> Tachykinin NK<sub>3</sub> receptors are also involved in rewarding properties of opioids since the selective agonist aminosenkide induced conditioned place preference in a naloxone-sensitive manner.<sup>155</sup>

### Epilepsy and brain ischemia

Tachykinin-mediated neurotransmission is intimately related to excitatory amino acid function in both CNS and sensory neurons. In particular, tachykinins are involved in acute neuronal hyperexcitability in various

brain areas. Therefore, intrahippocampal injection of SP induced status epilepticus in rats by enhancing glutamate release, an effect blocked by selective NK<sub>1</sub> receptor antagonists.<sup>156</sup> Furthermore, preprotachykinin-A knock-out mice (lacking SP and NKA) were protected by kainic acid- or metrazole-induced hippocampal excitotoxicity,<sup>157</sup> and, in normal animals, a selective NK<sub>1</sub> receptor antagonist reduced kainic acid-induced seizures.<sup>158</sup> Interestingly, the tachykinin receptors involved in cytotoxicity vary according to the area examined: in cerebellar granule cells, the NK<sub>2</sub> or NK<sub>3</sub> receptor antagonists (MEN 10627 or R-820, respectively) reduced the sensitising effects of glutamate, whereas NK<sub>1</sub> receptors did not seem to be involved.<sup>159</sup>

Interactions between NK<sub>1</sub> and excitatory amino acids are also involved in ischemia-induced excitotoxicity, since cerebral ischemia up-regulates NK<sub>1</sub> receptors in a population of glutamergic pyramidal cells.<sup>160</sup> In addition, the i.c.v. administration of SR-140333 markedly reduced the infarct volume 24 h following focal cerebral ischemia.<sup>161</sup>

### Regulation of autonomic functions

Anti-emetic activity is the first therapeutical indication established for tachykinin antagonists. In particular, blood-brain penetrant NK<sub>1</sub> receptor antagonists (CP-99994, GR203040, L-742694 and RPR-100893) inhibited retching and vomiting induced by a large variety of stimuli, whereas antagonists acting mainly at the periphery were inactive.<sup>162,163</sup>

As mentioned before, tachykinins increase blood pressure and heart rate following i.c.v. injection, possibly because of an altered emotional status (e.g. anxiety, alertness). However, it has been recently shown that the intranigral injection of R-820 reduced the blood pressure in spontaneously hypertensive rats but not in normal counterparts. These results indicate that nigral NK<sub>3</sub> receptors could be directly involved in the CNS regulation of an altered cardiovascular function.<sup>164</sup> Interestingly, high levels of circulating NKB have been associated with the development of hypertension during pre-eclampsia.<sup>165</sup> However, it is unknown whether the effect of NKB is exerted at the peripheral or CNS level. Theoretically, since NKB can stimulate NK<sub>1</sub> receptors and permeabilise the blood-brain barrier through this mechanism,<sup>166</sup> NK<sub>3</sub> receptors located at the CNS level could be involved in hypertension associated with pre-eclampsia.

Tachykinin receptors have a role in the regulation of respiratory functions. Administration of selective NK<sub>1</sub> or NK<sub>3</sub> receptor agonists in the nucleus of solitary tract increased tidal volume in anaesthetised rats, whereas NK<sub>1</sub>, NK<sub>2</sub>, and NK<sub>3</sub> agonists produced a transient apnoea.<sup>167</sup> It seems likely that these effects are related to an interference with cardiorespiratory reflexes, since NK<sub>1</sub> receptor knock-out mice have enhanced bradycardic response following the pressure response induced by phenylephrine.<sup>168</sup> On the other hand, these mice have a weaker ventilatory response after short-lasting hypoxia.<sup>169</sup>

CNS tachykinin NK<sub>1</sub> and NK<sub>2</sub> receptors are also involved in the triggering of cough reflex in both cats and guinea-pigs since capsaicin-induced cough was reduced by the i.c.v. injection of CP-99994 or SR48968 in both species.<sup>170</sup>

Tachykinin receptors modulate the micturition reflex at both spinal and supraspinal level. RP 67580 or SR-48968 (NK<sub>1</sub> or NK<sub>2</sub> antagonist, respectively) reduced L-dopa-induced bladder hyperactivity and also increased bladder capacity per sé.<sup>171</sup> At the spinal level, the intrathecal administration of NK<sub>1</sub> (but not NK<sub>2</sub>) receptor antagonists increased the bladder

capacity while having no effect on the amplitude of micturition contractions in anaesthetised rats. Since this effect was absent in capsaicin-pretreated animals, it has been suggested that NK<sub>1</sub> receptors located on dorsal horn or lumbosacral parasympathetic nucleus cells receive a direct input from bladder afferent neurons.<sup>172</sup> This hypothesis has been supported by the results obtained with TAK-637 (NK<sub>1</sub> antagonist) in both cats and guinea-pigs.<sup>172,173</sup> Tachykinin NK<sub>1</sub> receptor-mediated afferent input from the bladder is enhanced in models of experimental cystitis because the increase in bladder capacity induced by NK<sub>1</sub> antagonists is more prominent following bladder inflammation than in control conditions,<sup>172</sup> and because the same antagonists inhibit spinal cord c-fos expression induced by noxious stimulation of the viscus.<sup>174</sup> Tachykinin NK<sub>3</sub> receptors are involved in the regulation of the urethral function at both spinal and supraspinal level, although with an opposite role. The i.c.v. administration of senktide in anaesthetised rats enhances urethral activity, whereas the intrathecal administration of the same agonist inhibits urethral opening during cystometries.<sup>172</sup>

### Effects mediated by tachykinin receptors at the peripheral level

At the peripheral level, tachykinin receptors mediate a variety of effects on airway, gastrointestinal and urinary functions. These effects, which are often referred as neurogenic inflammation, include smooth muscle contraction, glandular or epithelial secretions, pro-inflammatory effects on vasculature, modulation of neuronal excitability, immunomodulation and regulation of cell proliferation. Tachykinins and their receptors are also important at the somatic level where they contribute, together with glutamate, to inflammatory hyperalgesia. For instance, the local administration of GR 82334 or GR 94800 (peptide NK<sub>1</sub> and NK<sub>2</sub> antagonists, respectively) reduces hyperalgesia to mechanical stimuli induced by a spider venom injected in the rat paw.<sup>175</sup> A similar anti-hyperalgesic effect was exerted by RP 67580 (NK<sub>1</sub> antagonist) in a model of chemical nociception induced by intradermal injection of capsaicin in prostaglandin E<sub>2</sub>-pretreated animals.<sup>176</sup> Likewise, there is evidence for the involvement of tachykinins in visceral hyperalgesia, especially at the intestinal and urinary level.<sup>177</sup>

### Respiratory tract

In the airways there is evidence that tachykinins are involved in smooth muscle contractions, glandular secretions, cellular proliferation, blood flow regulation, recruitment and activation of immune cells and regulation of neuronal excitability, either at efferent or afferent level.

Peripherally-acting tachykinin receptor antagonists, such as the peptide FK-888 (NK<sub>1</sub>), MEN 10627 (NK<sub>2</sub>), or the non-peptide SB-235375 (NK<sub>3</sub>), inhibit irritant-induced cough.<sup>177,178</sup> This effect could be related to the excitability of sensory neurons, since it has been shown that, following inflammatory stimuli, tachykinins depolarise isolated vagal neurons through the stimulation of NK<sub>2</sub> receptors.<sup>179</sup>

In guinea-pigs, the stimulation of tachykinin NK<sub>2</sub> receptors induces a prominent airway smooth muscle contraction, and this effect is antagonised in a potent and long-lasting manner by GR 159897.<sup>98</sup> This mechanism is also activated by endogenous tachykinins, since the peptide NK<sub>2</sub> antagonist MDL 29,913 delayed and reduced capsaicin-induced dyspnea.<sup>94</sup> NK<sub>2</sub> receptors play an important role in allergic bronchoconstriction and airway

hyperreactivity induced by allergic or non-allergic stimuli.<sup>180,181,182</sup> In guinea-pigs, NK<sub>1</sub> receptors also play a role in airway smooth muscle contraction but their role is less important in other species. Rather, NK<sub>1</sub> receptors play an important role in inflammation and in mucus secretion.<sup>183,184</sup> Antigen-induced plasma protein extravasation depends on NK<sub>1</sub> receptor stimulation,<sup>185</sup> likewise the tachykinin NK<sub>1</sub> antagonist MEN-11467 reduces allergen-induced mucus secretion in sensitised guinea-pigs.<sup>186</sup> NK<sub>1</sub> receptors are also involved in the exaggerated neurogenic inflammatory response which develops following chronic smoking, or exposure to endotoxin, viral infections and other stimuli.<sup>177,183</sup> A role for NK<sub>3</sub> receptors in hyperresponsiveness to inflammatory and bronchoconstrictor stimuli has been assessed in guinea-pig airways. Selective NK<sub>3</sub> receptor antagonists, such as SR-142801 or SB-235375, prevented airway hyperreactivity to the bronchomotor effects of acetylcholine and decreased the enhancement of histamine-induced plasma protein extravasation following citric acid inhalation.<sup>178,187</sup> Interestingly, this effect occurred despite the fact that selective NK<sub>3</sub> receptor agonists (senktide, (MePhe<sup>7</sup>)NKB) had no intrinsic effects on airway motility or inflammation.<sup>188</sup>

### Gastrointestinal tract

In the gastrointestinal tract, tachykinins regulate a number of physiological functions either by directly acting on effector cells, or by stimulating neurons or nerve fibres. These functions include motility.

Tachykinins, acting mainly at NK<sub>1</sub> and/or NK<sub>2</sub> receptors are potent spasmogens of both circular and longitudinal smooth muscle layers in various segments of the gastrointestinal tract.<sup>189</sup> However, NK<sub>1</sub>, NK<sub>2</sub>, and NK<sub>3</sub> receptors are also expressed in myenteric and submucous plexus neurons, and some of these neurons can drive inhibitory inputs to the smooth muscle layers. As a consequence of this arrangement, the effects induced by selective tachykinin receptor agonists in integrated *in vitro* or *in vivo* models can be excitatory, inhibitory or both, depending on the doses used, the characteristics of the model, or the segment investigated.<sup>189,190</sup> In the isolated guinea-pig small intestine, selective NK<sub>1</sub> receptor stimulation inhibits peristalsis through nitric oxide release,<sup>191,192</sup> an effect that is evident also *in vivo*.<sup>193</sup> This NK<sub>1</sub> receptor-mediated inhibitory function is tonically activated in physiological conditions since the administration of selective antagonists stimulated small or large intestine *in vitro* peristalsis in guinea-pigs and rabbits, respectively.<sup>191,194</sup> In the guinea-pig isolated small or large intestine the stimulation of NK<sub>2</sub> receptors accelerated peristalsis,<sup>191</sup> possibly through the enhancement of cholinergic transmission, as determined by the interaction of selective antagonists MEN 10376 or MEN-11420 with atropine.<sup>195,196</sup> In rats, this mechanism is not operant *in vivo*, since the NK<sub>2</sub> receptor antagonists (MEN 10627 or SR-48968) do not inhibit intestinal transit at doses which inhibit the increase of intestinal transit induced by selective NK<sub>2</sub> receptor agonists, or other stimuli (e.g. endotoxin).<sup>197,198</sup> In rabbits, NK<sub>2</sub> receptor antagonists (MEN 10627, SR-48968) induce a biphasic effect on distal colon propulsion *in vitro*: excitatory and inhibitory effect at low and high concentrations, respectively.<sup>199</sup> The excitatory effect was due to the inhibition of neuronal receptors located on inhibitory nitrergic neurons, whereas the inhibitory effect could be due to the blockade of receptors on smooth muscle or motor neuron nerve terminals.<sup>199</sup> Tachykinin NK<sub>3</sub> receptors are mainly located on intestinal neurons, although recent studies have provided evidence for their expression on both interstitial cells of Cajal and smooth muscle.<sup>200</sup> The

inhibition of these receptors does not affect peristalsis in guinea-pig isolated small or large intestine.<sup>196,201</sup> However, in the rabbit isolated distal colon, the selective NK<sub>3</sub> receptor agonist senktide induces a biphasic effect on propulsion: a nitric oxide-mediated inhibitory effect at low concentrations and excitatory effect at high concentrations, respectively.<sup>202</sup> In the same model, NK<sub>3</sub> receptor antagonists, such as SR-142801 or SB 222200, displayed prokinetic effect at low concentrations and an inhibitory effect at high concentrations. These antagonists also enhanced the inhibitory effect exerted by hexamethonium.<sup>202</sup> A nitric oxide-dependent, NK<sub>3</sub> receptor-triggered inhibitory effect was also evident in rat colonic motility *in vivo*.<sup>203</sup> It has been proposed that the excitatory effect induced by NK<sub>3</sub> receptor antagonists on rat colonic motility could be due to the blockade of receptors located on smooth muscle (or interstitial cells of Cajal) because this effect was still present following tetrodotoxin pretreatment.<sup>204</sup>

As observed for motor effect, marked species-related differences exist in tachykinin receptors involved in regulation of water movements across the intestinal wall. As recently reviewed, the activation of NK<sub>1</sub> receptors induces water secretion in the intestinal lumen of all species examined so far, NK<sub>2</sub> receptors stimulate secretion in rats and human but not guinea-pigs, whereas the role of NK<sub>3</sub> receptors seems restricted to guinea-pigs.<sup>177,190</sup> In rats, the secretory effect induced by colonic overdistension, or inflammatory stimuli, is blocked by selective NK<sub>1</sub> or NK<sub>2</sub> receptor antagonists.<sup>205,206</sup>

Tachykinins are important mediators of the inflammatory response at the intestinal level. Abundant evidence indicates that NK<sub>1</sub> receptors play a pivotal role in this response, although the contribution of NK<sub>2</sub> receptors can be also appreciated in models of colitis or ileitis.<sup>207</sup> The role of tachykinin NK<sub>1</sub> receptors is not limited to the induction of plasma protein extravasation since the stimulation of these receptors produces tissue injury. This has been established in the pancreas, where the severity of experimental pancreatitis and associated lethality are decreased in NK<sub>1</sub> receptor knock out mice.<sup>208</sup> Tissue injury has also been shown in the liver where NK<sub>1</sub> selective antagonists (CP-96345 or L-733,060) protected tissue injury induced by the combined administration of antigen and endotoxin in sensitised mice.<sup>209</sup>

## Urinary tract

Except for rats and guinea-pigs, where there is an important contribution by NK<sub>1</sub> receptors in tachykinin-induced bladder smooth muscle contractions, in the other species investigated so far, including humans, tachykinin NK<sub>2</sub> receptors play a prominent role in stimulating bladder motility.<sup>172</sup> The pathophysiological role of bladder NK<sub>2</sub> receptors has been investigated through the use of selective NK<sub>2</sub> receptor antagonists such as MEN-11420, SR-48968 or GR 159897, which warrant potent and long lasting inhibition of (βAla<sup>8</sup>)NKA(4-10)-induced rat bladder contractions.<sup>296,210,211</sup> The role of NK<sub>2</sub> receptors is evident in models of acute bladder irritation<sup>172</sup> or in neurogenic bladder hyperreflexia that follows spinal cord transection.<sup>212</sup> It is not so evident during physiological micturition, since NK<sub>2</sub> receptor antagonists reduce the frequency and the amplitude of hyperreflexic rat bladder contractions<sup>212</sup> but do not alter urodynamic parameters during cystometries in rats, guinea-pigs or hamsters.<sup>172,177</sup> Although NK<sub>1</sub> receptors may also play a role in bladder hypermotility induced by exogenous administration of SP, NKA or NKB,<sup>213,214,215</sup> or by endogenous tachykinins released during bladder hyperreflexia in spinal cord transected rats,<sup>212</sup> these receptors have a key role in allergic or non-allergic models of bladder inflammation.<sup>172</sup> Antigen- or cyclophosphamide-induced plasma protein extravasation and tissue damage are reduced by selective NK<sub>1</sub> receptor antagonists<sup>216,217</sup> or in NK<sub>1</sub> receptor knock out mice,<sup>120,218</sup> and these effects can involve NK<sub>1</sub> receptors located on post-capillary venules, neutrophils, and/or urothelium.<sup>213,219,220</sup>

## Conclusions

Pharmacological investigation on tachykinin receptors has allowed the delineation of basic processes governing ligand-receptor-G protein physiology at the molecular and cellular level. Likewise, an intense *in vitro* and *in vivo* investigation with selective agonists and antagonists has highlighted the multiple pathophysiological roles that these receptors play in the CNS and the periphery, emphasising the importance of tachykinins in the bi-directional communication between neurons and the immune system. However, the list of these roles has not been completed yet, and further exciting discoveries in basic and applied science are foreseen in this field.

## References

- Von Euler and Gaddum (1931) *J.Physiol.* **72** 577.
- Erspamer and Falconieri Erspamer (1962) *Br.J.Pharmacol.* **19** 337.
- Severini *et al* (2002) *Pharmacol.Rev.* **54** 285.
- Lembeck and Starke (1968) *Naunyn-Schmied.Arch.Pharmacol.* **259** 375.
- Konishi and Otsuka (1974) *Brain Res.* **65** 397.
- Hokfelt *et al* (1975) *Science* **190** 889.
- Chang and Leeman (1970) *J.Biol.Chem.* **245** 4784.
- Kangawa *et al* (1983) *Biochem.Biophys.Res.Comm.* **114** 533.
- Kanazawa *et al* (1984) *Neurosci.Res.* **2** 111.
- Regoli *et al* (1987) *Life Sci.* **40** 109.
- Nawa *et al* (1983) *Nature* **306** 32.
- Kotani *et al* (1986) *Proc.Natl.Acad.Sci.USA* **83** 7074.
- Tatemoto *et al* (1985) *Biochem.Biophys.Res.Comm.* **128** 947.
- Kage *et al* (1988) *J.Neurochem.* **50** 1412.
- Masu *et al* (1987) *Nature* **329** 836.
- Yokota *et al* (1989) *J.Biol.Chem.* **264** 17649.
- Ingi *et al* (1991) *J.Pharmacol.Exp.Ther.* **259** 968.
- Cao *et al* (1998) *Nature* **392** 390.
- De Felipe (1998) *Nature* **392** 394.
- Zhang *et al* (2000) *Nature Immunol.* **1** 392.
- Maggi *et al* (1993) *J.Auton.Pharmacol.* **13** 23.
- Petit *et al* (1992) *Peptides* **13** 383.
- Meini *et al* (1994) *Br.J.Pharmacol.* **111** 739.
- Harstrup and Schwartz (1996) *FEBS Lett.* **399** 264.
- Wijkhuisen *et al* (1999) *FEBS Lett.* **447** 155.
- Kenakin and Onaran (2002) *Trends Pharmacol.Sci.* **23** 275.
- Holst *et al* (2001) *J.Biol.Chem.* **276** 19793.
- Sagan *et al* (1996) *J.Pharmacol.Exp.Ther.* **276** 1039.
- Figini *et al* (1996) *Br.J.Pharmacol.* **117** 1270.
- Ahluwalia *et al* (1995) *Br.J.Pharmacol.* **116** 2170.
- Maggi and Schwartz (1997) *TIPS.* **18** 351.
- Trafton *et al* (2001) *J.Neurosci.* **21** 3656.
- Torrens (1997) *Neuropeptides* **31** 243.
- Burcher and Stamatakos (1994) *Eur.J.Pharmacol.* **258** R9.
- Shahbazian and Holzer (2000) *Neurogastroenterol.Mot.* **12** 197.
- Ricciardolo *et al* (2000) *Br.J.Pharmacol.* **129** 915.
- Bellucci *et al* (2002) *Br.J.Pharmacol.* **135** 266.
- Kurtz *et al* (2002) *Gene* **296** 205.
- Vigna (2001) *Neuropeptides* **35** 24.
- Vigna (2003) *Neuropeptides* **37** 30.
- Igwe *et al* (1990) *Peptides* **11** 817.
- Velasquez (2002) *Eur.J.Neurosci.* **16** 229.
- Bowden *et al* (1994) *Proc.Natl.Acad.Sci.USA* **91** 8694.
- McConalogue *et al* (1998) *Mol.Biol.Cell* **9** 2305.
- Maa *et al* (2000) *Am.J.Physiol.Gastrintest.Liver Physiol.* **279** G726.
- Jenkinson *et al* (1999) *Br.J.Pharmacol.* **126** 131.
- Sanders and Levine (1996) *J.Neurochem.* **67** 2362.
- Dery *et al* (2001) *Am.J.Physiol.Cell.Physiol.* **280** C1097.
- Kage *et al* (1993) *J.Neurochem.* **60** 347.
- Fong *et al* (1992) *Mol.Pharmacol.* **41** 24.
- Li *et al* (1997) *Proc.Natl.Acad.Sci.USA* **19** 9475.
- Rush and Kwatra (1998) *FEBS Lett.* **428** 291.

53. Bockmann (2002) Peptides **23** 1783.
54. Yang *et al* (2002) Cell Signal. **14** 913.
55. Castagliuolo *et al* (2000) J.Biol.Chem. **275** 26545.
56. Azzolina *et al* (2002) Cytokine **18** 72.
57. Nishimura *et al* (1998) Biochemistry **37** 1192.
58. Warabi *et al* (2002) FEBS Lett. **521** 140.
59. Mechiche *et al* (2003) J.Cardiovasc.Pharmacol. **41** 343.
60. Lomax *et al* (1998) Cell Tissue Res. **294** 27.
61. Tanabe *et al* (1996) Eur.J.Pharmacol. **299** 187.
62. Torrens *et al* (1998) J.Neurochem. **70** 2091.
63. Kavelaars *et al* (1994) J.Immunol. **153** 3691.
64. Takahashi *et al* (2002) Br.J.Pharmacol. **137** 845.
65. Wibberley *et al* (2003) Br.J.Pharmacol. **138** 757.
66. Leander *et al* (1981) Nature **294** 467.
67. Taniguchi *et al* (1992) Jap.J.Pharmacol. **59** 213.
68. Shuttleworth *et al* (1991) Neuropeptides **19** 23.
69. Hagan *et al* (1991) Br.J.Pharmacol. **102** 168P.
70. Snider *et al* (1991) Science **251** 435.
71. Garret *et al* (1991) Proc.Natl.Acad.Sci.USA **88** 10208.
72. Cascieri *et al* (1994) J.Biol.Chem. **269** 6587.
73. Rupniak *et al* (1997) Eur.J.Pharmacol. **326** 201.
74. Seabrook *et al* (1996) Eur.J.Pharmacol. **317** 129.
75. Emonds-Alt *et al* (1993) Eur.J.Pharmacol. **250** 403.
76. Rupniak *et al* (2000) Neuropharmacology **39** 1413.
77. Rupniak *et al* (1996) Pain **67** 189.
78. Amadesi *et al* (2001) Am.J.Respir.Crit.Care.Med. **163** 1206.
79. Rovero *et al* (1989) Peptides **10** 593.
80. Fukuhara *et al* (1995) Peptides **16** 211.
81. Nsa Allogho *et al* (1997) Can.J.Physiol.Pharmacol. **75** 552.
82. Burcher *et al* (1993) Eur.J.Pharmacol. **233** 201.
83. Alblas *et al* (1995) J.Biol.Chem. **270** 8944.
84. Volmer *et al* (1999) J.Biol.Chem. **274** 37915.
85. Palanche *et al* (2001) J.Biol.Chem. **276** 34853.
86. Alblas *et al* (1996) EMBO J. **15** 3351.
87. Catalioto *et al* (1998) Naunyn-Schmied.Arch.Pharmacol. **358** 395.
88. Luz Candenias *et al* (2002) Life Sci. **72** 269.
89. Maggi *et al* (1991) J.Pharmacol.Exp.Ther. **257** 1152.
90. McKnight *et al* (1991) Br.J.Pharmacol. **104** 335.
91. Burcher *et al* (1991) Neuropeptides **20** 79.
92. Mussap *et al* (1996) J.Pharmacol.Exp.Ther. **279** 423.
93. Burcher *et al* (1995) Can.J.Physiol.Pharmacol. **73** 915.
94. Kudlacz *et al* (1993) Eur.J.Pharmacol. **241** 17.
95. Yuan *et al* (1996) Clin.Exp.Pharmacol.Physiol. **23** 119.
96. Catalioto *et al* (1998) Br.J.Pharmacol. **123** 81.
97. Emonds-Alt *et al* (1992) Life Sci. **50** PL101.
98. Beresford *et al* (1995) Eur.J.Pharmacol. **272** 241.
99. Medhurst *et al* (1997) Br.J.Pharmacol. **122** 469.
100. Wormser *et al* (1986) EMBO J. **5** 2805.
101. Sarau *et al* (2001) Eur.J.Pharmacol. **413** 143.
102. Patacchini and Maggi (1992) J.Pharmacol.Exp.Ther. **261** 191.
103. Jordan *et al* (1998) Br.J.Pharmacol. **125** 761.
104. Hellstrom (1993) J.Pharmacol.Exp.Ther. **270** 236.
105. Xie *et al* (1992) Proc.Natl.Acad.Sci.USA **89** 4124.
106. Sarau *et al* (2000) Mol.Pharmacol. **58** 552.
107. Page and Bell (2002) Eur.J.Pharmacol. **437** 27.
108. Regoli *et al* (1994) Life Sci. **54** 2035.
109. Emonds-Alt (1995) Life Sci. **56** PL27.
110. Giardina *et al* (1997) J.Med.Chem. **40** 1794.
111. Sarau *et al* (2000) J.Pharmacol.Exp.Ther. **295** 373.
112. Lembeck (1953) Naunyn-Schmied.Arch.Pharmacol. **219** 197.
113. Otsuka and Yoshioka (1993) Physiol.Rev. **73** 229.
114. Hill (2000) TIPS **21** 244.
115. Urban and Fox (2000) TIPS **21** 462.
116. Hockfelt *et al* (2001) J.Int.Med. **249** 27.
117. Hill (2002) Proc.Natl.Acad.Sci.USA **99** 549.
118. Bester *et al* (2001) J.Neurosci. **21** 1039.
119. Jasmin *et al* (2002) Proc.Natl.Acad.Sci.USA **99** 1029.
120. Laird *et al* (2000) Neuroscience **98** 345.
121. Laird *et al* (2001) Pain **90** 97.
122. Wismer *et al* (2003) Brain Res. **965** 187.
123. Seguin *et al* (1995) Pain **61** 325.
124. Rupniak *et al* (1996) Pain **67** 189.
125. Mansikka *et al* (2000) Exp.Neurol. **162** 343.
126. Coudouré-Civiale *et al* (1998) Eur.J.Pharmacol. **361** 175.
127. Field *et al* (1998) J.Pharmacol.Exp.Ther. **285** 1226.
128. Cumberbatch *et al* (1998) Neuropharmacology **37** 1535.
129. Cahill and Coderre (2002) Pain **95** 277.
130. Zarrantin *et al* (2000) Neuropharmacology **39** 141.
131. Houghton *et al* (2000) Neuropharmacology **39** 133.
132. Kamp *et al* (2001) J.Pharmacol.Exp.Ther. **285** 1226.
133. Kramer *et al* (1998) Science **281** 1640.
134. Rupniak (2002) Curr.Opin.Invest.Drugs **3** 257.
135. Teixeira *et al* (1996) Eur.J.Pharmacol. **311** 7.
136. Varty *et al* (2002) Neuropsychopharmacology **27** 371.
137. Cheeta *et al* (2001) Brain Res. **915** 170.
138. Rupniak *et al* (2003) Neuropharmacology **44** 516.
139. Vassout *et al* (2000) Regul.Peptides **96** 7.
140. Spooren *et al* (2002) Eur.J.Pharmacol. **435** 161.
141. Culman *et al* (1997) J.Pharmacol.Exp.Ther. **280** 238.
142. Couture *et al* (1995) Can.J.Physiol.Pharmacol. **73** 892.
143. Stratton *et al* (1993) Eur.J.Pharmacol. **250** R11.
144. Walsh *et al* (1995) Psychopharmacology **121** 186.
145. Ribeiro *et al* (1999) Neuropeptides **33** 181.
146. Rupniak *et al* (2000) Neuropharmacology **36** 1413.
147. Varty *et al* (2003) Behav.Pharmacol. **14** 87.
148. Steinberg *et al* (2001) J.Pharmacol.Exp.Ther. **299** 449.
149. Chardenot *et al* (2002) Mol.Pharmacol. **62** 1314.
150. Panocka *et al* (2001) Peptides **22** 1037.
151. Mutra *et al* (2000) Nature **405** 180.
152. Trang *et al* (2002) Br.J.Pharmacol. **136** 37.
153. Noailles and Angulo (2002) Ann.N.Y.Acad.Sci. **965** 267.
154. Yu *et al* (2002) J.Neurochem. **83** 613.
155. Massi *et al* (2000) Peptides **21** 1597.
156. Liu *et al* (1999) Proc.Natl.Acad.Sci.USA **96** 5286.
157. Liu *et al* (1999) Proc.Natl.Acad.Sci.USA **96** 12096.
158. Zachrisson *et al* (1998) Brain Res.Mol.Brain Res. **160** 291.
159. Severini *et al* (2003) Neuropharmacology **44** 117.
160. Stumm *et al* (2001) J.Neurosci. **21** 798.
161. Yu *et al* (1997) Neuroreport **8** 2117.
162. Bountra *et al* (1993) Eur.J.Pharmacol. **249** R3.
163. Rupniak *et al* (1997) Eur.J.Pharmacol. **326** 201.
164. Lessard *et al* (2003) Br.J.Pharmacol. **138** 554.
165. Page *et al* (2000) Nature **405** 797.
166. Hu and Fraser (1999) Regul.Peptides **80** 115.
167. Mazzone and Geraghty (2000) Br.J.Pharmacol. **129** 1121.
168. Butcher *et al* (1998) J.Auton.Nerv.Sys. **69** 89.
169. Ptak *et al* (2002) Eur.J.Neurosci. **16** 2245.
170. Bolser *et al* (1997) Br.J.Pharmacol. **121** 165.
171. Ishizuka *et al* (2000) NeuroUrol.Urodyn. **19** 101.
172. Lecci and Maggi (2001) Regul.Peptides **101** 1.
173. Kamo and Doi (2001) Jap.J.Pharmacol. **86** 165.
174. Mitsui *et al* (2002) Am.J.Physiol.Regul.Integr.Comp.Physiol. **283** R576.
175. Zanchet and Cury (2003) Eur.J.Pharmacol. **467** 111.
176. Holzer-Petsche and Rodorf-Nikolic (1995) Br.J.Pharmacol. **115** 486.
177. Lecci and Maggi (2003) Expert Opin.Ther.Targets **7** 1.
178. Hay *et al* (2002) J.Pharmacol.Exp.Ther. **300** 314.
179. Moore *et al* (1999) J.Physiol. **514** 111.
180. Schuiling *et al* (1999) Br.J.Pharmacol. **127** 1030.
181. Maghni *et al* (2000) Am.J.Respir.Crit.Care Med. **162** 1068.
182. Tramontana *et al* (2002) Eur.J.Pharmacol. **439** 149.
183. Joos *et al* (2001) Eur.J.Pharmacol. **429** 239.
184. Rogers (2001) Expert Opin.Ther.Patents **11** 1097.
185. Bertrand *et al* (1993) J.Immunol. **150** 1479.
186. Khan *et al* (2001) Br.J.Pharmacol. **132** 189.
187. Daoui *et al* (1998) Am.J.Respir.Crit.Care Med. **158** 42.
188. Daoui *et al* (2000) Br.J.Pharmacol. **130** 49.
189. Holzer and Holzer-Petsche (1997) Pharmacol.Ther. **73** 173.
190. Lecci *et al* (2002) Curr.Opin.Invest.Drugs **3** 589.
191. Holzer *et al* (1995) J.Pharmacol.Exp.Ther. **274** 322.
192. Holzer (1997) Neuroreport **8** 2857.
193. Lecci *et al* (1999) Neuropeptides **33** 91.
194. Onori *et al* (2003) Am.J.Physiol.Gastrointest.Liver Physiol., in press.
195. Holzer and Maggi (1994) Naunyn-Schmied.Arch.Pharmacol. **349** 194.
196. Tonini *et al* (2001) Gastroenterology **120** 938.
197. Tramontana *et al* (1994) Jap.J.Pharmacol. **65** 281.
198. Croci *et al* (1994) J.Pharm.Pharmacol. **46** 383.
199. Onori *et al* (2000) Am.J.Physiol.Gastrointest.Liver Physiol. **278** G137.
200. Lecci *et al* (2002) Curr.Opin.Pharmacol. **2** 630.
201. Holzer *et al* (1998) Neuropharmacology **37** 131.
202. Onori *et al* (2001) Neurogastroent.Mot. **13** 211.
203. Lecci *et al* (1996) Naunyn-Schmied.Arch.Pharmacol. **353** 671.
204. Gonzales and Sarna (2001) Am.J.Physiol.Gastrointest.Liver Physiol. **281** G275.
205. Eutamene *et al* (1995) Gastroenterology **109** 483.
206. Eutamene *et al* (1997) Gastroenterology **112** 1595.
207. Evangelista (2001) Curr.Pharm.Des. **7** 19.
208. Bathia *et al* (1998) Proc.Natl.Acad.Sci.USA **95** 4760.
209. Bang *et al* (2003) J.Pharmacol.Exp.Ther. **305** 31.
210. Maggi *et al* (1993) Eur.J.Pharmacol. **234** 83.
211. Choppin *et al* (2002) Pharmacology **65** 96.
212. Abdel-Gawad *et al* (2001) J.Urol. **165** 1739.
213. Chien *et al* (2003) Am.J.Physiol.Renal Physiol. **284** F840.
214. Ishizuka *et al* (1995) J.Urol. **154** 257.
215. Lecci *et al* (1994) Neuropeptides **27** 53.
216. Ahluwalia *et al* (1998) Br.J.Pharmacol. **124** 190.
217. Aifieri and Cubeddu (2000) J.Pharmacol.Exp.Ther. **295** 824.
218. Saban *et al* (2000) Am.J.Pathol. **156** 775.
219. Ahluwalia *et al* (1998) Br.J.Pharmacol. **124** 1013.
220. Hammond *et al* (2000) Am.J.Physiol.Renal Physiol. **278** F440.
221. Page *et al* (2003) Proc.Natl.Acad.Sci.USA **100** 6245.

# Tachykinin Receptor Compounds available from Tocris

## NK<sub>1</sub> Receptor Compounds

1669	GR 73632	Potent, selective NK <sub>1</sub> agonist
1670	GR 82334	Tachykinin NK <sub>1</sub> receptor antagonist
1535	Hemokinin 1 (mouse)	Endogenous, high affinity, selective NK <sub>1</sub> agonist
0868	L-732,138	Potent, selective NK <sub>1</sub> antagonist
1145	L-733,060	Potent NK <sub>1</sub> antagonist
1635	RP 67580	Potent and selective NK <sub>1</sub> antagonist
1784	Spantide I	Selective NK <sub>1</sub> antagonist
1178	[Sar <sup>9</sup> ,Met(O <sub>2</sub> ) <sup>11</sup> ]-Substance P	Potent, selective NK <sub>1</sub> agonist

## NK<sub>2</sub> Receptor Compounds

1668	GR 64349	Potent, selective NK <sub>2</sub> agonist
1667	GR 94800	Potent, selective NK <sub>2</sub> antagonist
1274	GR 159897	Non-peptide, potent NK <sub>2</sub> antagonist
1194	MDL 29,913	Selective NK <sub>2</sub> antagonist
1632	MEN 10376	Potent, selective NK <sub>2</sub> antagonist
1633	MEN 10627	Potent, selective NK <sub>2</sub> antagonist
1640	[βAla <sup>8</sup> ]-Neurokinin A(4-10)	NK <sub>2</sub> agonist

## NK<sub>3</sub> Receptor Compounds

1376	SB 218795	Potent, selective non-peptide NK <sub>3</sub> antagonist
1393	SB 222200	Potent, selective non-peptide NK <sub>3</sub> antagonist. Brain penetrant
R1393	[ <sup>3</sup> H]-SB 222200	Radiolabelled form of (1393)
1068	Senktide	Tachykinin NK <sub>3</sub> agonist

## Other Tachykinin Receptor Compounds

1152	Neurokinin A (porcine)	Endogenous tachykinin peptide
1582	Neurokinin B (human, porcine)	Endogenous tachykinin peptide
1156	Substance P	Sensory neuropeptide, inflammatory mediator

Ligands also available in the following peptide receptor areas:

### Amyloid β-Peptides

### Angiotensin Receptor Compounds

### Bombesin-Related Peptides

### Bradykinin Receptor Compounds

### Calcitonin Gene-Related Peptide Receptor Compounds

### Cholecystokinin Receptor Compounds

### Corticotropin Releasing Factor Receptor Compounds

### Endothelin Receptor Compounds

### Galanin Receptor Compounds

### Growth Hormone Receptor Compounds

### Neuropeptide Y Receptor Compounds

### Orexin Receptor Compounds

### Pituitary Adenylate-Cyclase Activating Peptide Receptor Compounds

### Proteinase-Activated Receptor Compounds

### Somatostatin Receptor Compounds

### Urotensin II Receptor Compounds

### Vasoactive Intestinal Peptide Receptor Compounds

For details contact your local Tocris Cookson office or distributor, or visit our website, [www.tocris.com](http://www.tocris.com).

*Pharmacology and Function of Tachykinin Receptors,*  
Tocris Reviews No. 24, September 2003

©2003 Tocris Cookson  
Published and distributed by Tocris Cookson, Bristol, UK

Editors: Samantha Manley, Ph.D., Natalie Barker, B.Sc.  
Design and Production: Jane Champness

Pep[Rev](0903)

Tocris Cookson Ltd.  
Northpoint Fourth Way  
Avonmouth BS11 8TA UK  
Tel: + 44 (0)117 916 3333  
Fax: + 44 (0)117 916 3344  
[customerservice@tocris.co.uk](mailto:customerservice@tocris.co.uk)  
[technicalsupport@tocris.co.uk](mailto:technicalsupport@tocris.co.uk)

[www.tocris.com](http://www.tocris.com)

Tocris Cookson Inc.  
16144 Westwoods Business Park  
Ellisville MO 63021 USA  
Tel: (800) 421-3701  
Fax: (800) 483-1993  
[customerservice@tocrisusa.com](mailto:customerservice@tocrisusa.com)  
[technicalsupport@tocrisusa.com](mailto:technicalsupport@tocrisusa.com)