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ORIGINAL ARTICLE

Involvement of substance P and the NK-1 receptor in human pathology

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Abstract The peptide substance P (SP) shows a widespread distribution in both the central and peripheral nervous systems, but it is also present in cells not belonging to the nervous system (immune cells, liver, lung, placenta, etc.). SP is located in all body fluids, such as blood, cerebrospinal fluid, breast milk, etc. i.e. it is ubiquitous in human body. After binding to the neurokinin-1 (NK-1) receptor, SP regulates many pathophysiological functions in the central nervous system, such as emotional behavior, stress, depression, anxiety, emesis, vomiting, migraine, alcohol addiction, seizures and neurodegeneration. SP has been also implicated in pain, inflammation, hepatitis, hepatotoxicity, cholestasis, pruritus, myocarditis, bronchiolitis, abortus, bacteria and viral infection (e.g., HIV infection) and it plays an important role in cancer (e.g., tumor cell proliferation, antiapoptotic effects in tumor cells, angiogenesis, migration of tumor cells for invasion, infiltration and metastasis). This means that the SP/NK-1 receptor system is involved in the molecular bases of many human pathologies. Thus, knowledge of this system is the key for a better understanding and hence a better management of many human diseases. In this review, we update the involvement of the SP/NK-1 receptor system in the physiopathology of the above-mentioned pathologies and we suggest valuable future therapeutic interventions involving the use of NK-1 receptor antagonists, particularly in the treatment of emesis, depression, cancer, neural degeneration, inflammatory bowel disease, viral infection and pruritus, in which that system is upregulated.

Keywords Substance P · NK-1 receptor · NK-1 receptor antagonists · Molecular bases · Human pathology

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Introduction

Currently, there continue to exist many unknowns in our knowledge of many human pathologies. A deeper knowledge of the mechanisms underlying human diseases could not only improve our understanding of them, but could also improve the specific treatment of the diseases as well as their prognosis and evolution. Thus, a huge effort is necessary to improve our view of the underlying mechanisms in human diseases.

Substance P (SP) is an undecapeptide that belongs to the tachykinin family of peptides (this family also includes peptides, such as neurokinin A, neurokinin B, kassinin, ranakinin, eledoisin, neuropeptide K and neuropeptide Gamma). SP is derived from the preprotachykinin-A gene and it acts as a neurotransmitter or neuromodulator in the nervous system. The biological actions of tachykinins are mediated by three receptors, designated neurokinin (NK)-1, NK-2 and NK-3 (see Ebner and Singewald 2006 and Ebner et al. 2009 for review). The biological action of SP is mainly mediated by the NK-1 receptor, since SP is the natural ligand with the highest affinity for the NK-1 receptor (see Muñoz and Coveñas 2013a). There are many

studies reporting the distribution of SP in the mammalian central and peripheral nervous systems (Cuello and Kanazawa 1978; Hökfelt et al. 1978; Mai et al. 1986), and all of them have shown that this neuropeptide has a widespread distribution in both systems. Likewise, many studies have also revealed a widespread distribution of the NK-1 receptor in the mammalian central nervous system and also in peripheral tissues, vascular endothelial cells, muscle cells, gastrointestinal and genitourinary tracts, lung, thyroid gland, and in immune cells (Wolf et al. 1985; Saffroy et al. 1988; Maeno et al. 1993; Nakaya et al. 1994; Ebner and Singewald 2006; Ebner et al. 2009; Muñoz and Coveñas 2011).

After binding to the NK-1 receptor, SP regulates many biological functions (physiological and pathophysiological) (Fig. 1). SP has been implicated in the regulation of the cardiovascular system, in the dilatation of the arterial system, in neuronal survival and degeneration, in sensory perception, in the regulation of respiratory mechanisms, in movement control, in micturition, in gastric motility, in inflammation, in pain, in cancer, in salivation and in depression (Unger et al. 1981; Kramer et al. 1998; Quartara and Maggi 1998; Samsam et al. 2000; Bang et al. 2003; Ebner and Singewald 2006; Ebner et al. 2009; Muñoz and Coveñas 2013a). Moreover, SP is an important regulator of motility in several cells: it mediates the chemotaxis of human peripheral blood leukocytes and the carboxyl-terminal sequence of SP induces the chemotaxis of human monocytes (Ruff et al. 1985; Schratzberger et al. 1997). The peptide has also chemotactic effects on eosinophils and stimulates the migration of natural killer cells in a dose-dependent manner (Dunzendorfer et al. 1998; Feistritzer et al. 2003). Moreover, SP induces a rapid cellular shape change, including blebbing (Meshki et al. 2009); membrane blebbing is important in cell movement, cell spreading, and cancer cell invasion (Fackler and Grosse 2008). It is also known that both SP and hemokinin-1 exert an antiapoptotic effect on bone-marrow-derived dendritic cells (which according to immunotherapy protocols are the preferred targets). This effect enhances the survival of dendritic cells both in vitro and in vivo, and when induced in such cells it exerts a potent immune-stimulatory activation, which promotes a robust cellular immunity (Janelsins et al. 2009). Moreover, it is known that SP stimulates platelet aggregation, that platelets express both SP and NK-1 receptors, that SP is secreted from platelets upon activation, and that NK-1 receptor-blocking antibodies inhibit platelet aggregation. These data imply that SP regulates platelet function and that NK-1 receptor antagonists could inhibit platelet aggregation (Graham et al. 2004). It is also important to note that SP acts as a mediator of the crosstalk between the nervous and the immune systems (Fig. 2); that the peptide also acts independently on other cells in a paracrine and/or autocrine

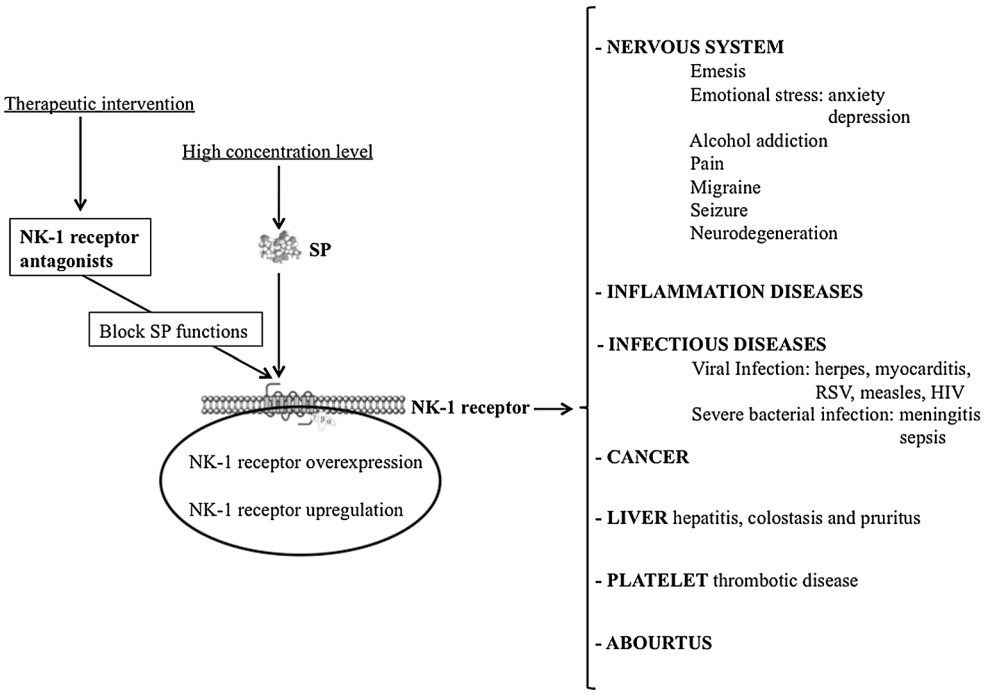

Fig. 1 Involvement of the substance P/neurokinin-1 receptor system in human pathology. In many diseases, such system is altered and hence NK-1 receptor antagonists could block the pathophysiological actions mediated by substance P. In cancer cells, an overexpression of the NK-1 receptor occurs and, for example, high levels of substance P were reported in human immunodeficiency virus-positive patients

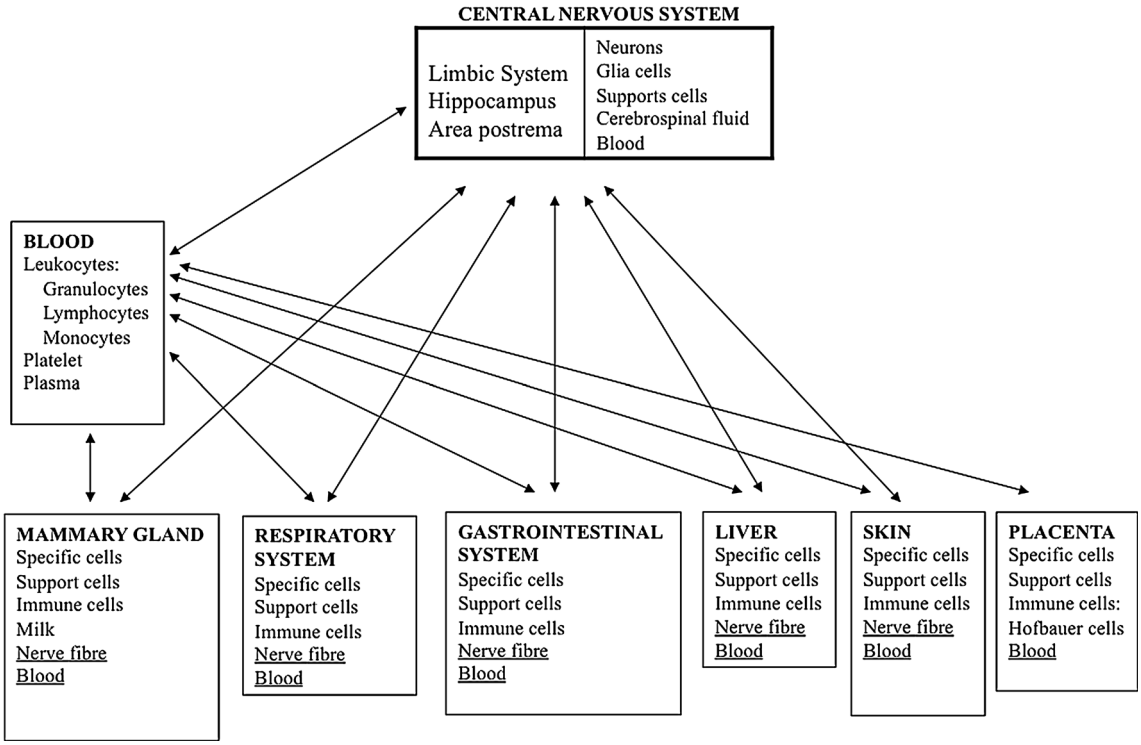

Fig. 2 Crosstalk between the CNS and other systems in which the SP/NK-1 receptor is upregulated under pathological conditions. SP binds to the NK-1 receptor by autocrine (SP is secreted from cells), paracrine (SP exerts a biological action in endothelial cells and in other cells) and/or endocrine (SP is secreted from the different organs into the blood vessels) mechanisms. SP is also released from nerve terminals and/or the peptide reaches the whole body through the bloodstream (this is regulated by the limbic system)

fashion, and that SP is also located in the body fluids, such as blood, cerebrospinal fluid, breast milk, etc. That is, SP is ubiquitous throughout human body. This means that SP can regulate cell function by autocrine, paracrine, endocrine, and/or neuroendocrine mechanisms (see Muñoz and Coveñas 2013a).

Peptides may be preferentially released, at least in some systems (e.g., SP/NK-1 receptor), when neurons or non-neuron cells are strongly activated and/or under pathological conditions (see Hökfelt et al. 2000). Thus, peptide antagonists (e.g., non-peptide NK-1 receptor antagonists) normally have no effect and will only act on deranged systems with increased peptide (e.g., SP) release (Hökfelt et al. 2000). This occurs in emesis, depression, neural degeneration, inflammatory bowel disease, pruritus, cancer and in herpes simplex virus-1, encephalomyocarditis virus and human immunodeficiency virus infection. Moreover, the NK-1 receptor is upregulated when an infection due to respiratory syncytial virus occurs. Thus, in these diseases, an upregulation of the SP/NK-1 receptor system occurs and the NK-1 receptor can, therefore, be considered as an important target for the treatment of the above diseases. The aim of this review is to update the findings that support the involvement of the SP/NK-1 receptor system in human pathology (Fig. 1).

Nervous system

Emesis

SP is present in the nucleus tractus solitarius and in the area postrema, both of them involved in the control of emesis (Figs. 1, 2; Table 1); when they are activated, this results in a vomiting reflex, which is mediated by SP (Armstrong et al. 1981). Currently, aprepitant (Emend, MK-869, L-754,030) (oral) and fosaprepitant (a prodrug of aprepitant, intravenous) are the only NK-1 receptor antagonists available in clinical practice (Table 1). Fosaprepitant is rapidly converted to aprepitant via the action of ubiquitous phosphatases (Saito et al. 2013). Both NK-1 receptor antagonists are used for the prevention of chemotherapy-induced nausea and vomiting (CINV) and post-operative nausea and vomiting (PONV) (see Muñoz and Coveñas 2013b). Chemotherapy induces the release of SP and aprepitant blocks the unwanted actions exerted by SP in the central nervous system (CNS). Many clinical human trials have reported the efficacy and safety of aprepitant when it is used for the treatment of acute (<24 h post-chemotherapy) and delayed (>24 h post-chemotherapy) emesis (Abidi et al. 2012; see Muñoz and Coveñas 2013b for review). However, there is a relative lack of efficacy in

Table 1 NK-1 receptor antagonists in emesis, emotional stress, alcohol addiction, seizures and neurodegeneration

Nervous system					
EMESIS					
Aprepitant drug	CINV/PONV Clinical Assay	Human	125, 80,80 mg/day 3 days p.o.	Human use approved (drug)	
Casopitant	CINV Phase III trial	Human	150 mg day 1; 50 mg days 2–3 p.o.	Discontinued Additional safety data required	Herrstedt et al. (2009)
CJ-11,974 (ezlopitant)	CINV Phase II trial	Human	100 mg p.o.	Antiemetic effect	Hesketh et al. (1999)
CP-99,994	Antiemetic Preclinical assay	Ferret	3 mg/kg p.o.	Antiemetic effect	Bountra et al. (1993)
Fosaprepitant drug	CINV/PONV Clinical assay	Human	150 mg/day 30 min before chemotherapy i.v.	Human use approved (drug)	
Emotional stress (anxiety and depression)					
Aprepitant drug	Antidepressant Phase II trial	Human	300 mg/day p.o. for 4 weeks	Antidepressant Well tolerated	Kramer et al. (1998)
	Antidepressant Phase III trial	Human	160 mg/day p.o. for 8 weeks	No effect	Keller et al. (2006)
	Preclinical assay	Gerbil	0.1–30 mg/kg p.o.	Anxiolytic effect	Wallace-Boone et al. (2008)
CP-122,721	Preclinical assay	Gerbil	0.3–30 mg/kg p.o.	Anxiolytic effect	Wallace-Boone et al. (2008)
GR-205,171 (vofopitant)	Social anxiety disorder Phase II trial	Human	5 mg/day p.o. for 6 weeks	Alleviated social anxiety	Furmark et al. (2005)
L-733,060	Attenuates neonatal vocalization Preclinical assay	Guinea-pig and mouse	0.1–10 mg/kg i.c.v.	May have clinical utility in the treatment of a range of anxiety and mood disorders.	Rupniak et al. (2000)
	Stress-induced vocalization Preclinical assay	Guinea-pig	3 mg/kg i.p.	Anxiolytic effect	Kramer et al. (1998)
	Preclinical assay	Gerbil	10–30 mg/kg p.o.	Anxiolytic effect	Wallace-Boone et al. (2008)
L-759,274	Depression Clinical assay	Human	40 mg/day for 6 weeks p.o.	Antidepressant and anxiolytic effect. Well tolerated	Kramer et al. (2004)
L-822,429	Elevated plus-maze. Preclinical assay	Rat	100 pmol, 1 nmol i.c.	Anxiolytic	Ebner et al. (2004)
WIN-51,708	Elevated plus-maze model of anxiety by SP Preclinical assay	Rat	10–20 mg/kg i.p.	Anxiolytic effect in a dose-dependent manner	Nikolaus et al. (1999a)
	Elevated plus-maze. Preclinical assay	Rat	10 and 20 mg/kg i.p.	No effect (but blocked SP-induced anxiolytic effect)	Nikolaus et al. (1999b)
Alcohol addiction					
L-703,606	Alcohol consumption and alcohol reward Preclinical assay	Mouse	3–10 mg/kg i.p.	Dose-dependently suppressed alcohol intake	Thorsell et al. (2010)
LY-686,017	Randomized controlled trial	Human	1–100 mg/day p.o.	Suppressed alcohol craving and beneficial effect on global measures of wellbeing	George et al. (2008)
Pain					
Aprepitant	Postoperative dental pain Clinical trial	Human	300 mg p.o. 2 h prior surgery	No effect	Reinhardt (1998)
CP-99,994	Subjects undergoing third molar extraction Clinical assay	Human	750 microg/kg i.v. over 5 h	Reduction in postoperative pain	Dionne et al. (1998)
Lanepitant (LY-303,870)	Neurophatic pain Clinical trial	Human	50, 100, 400 mg, 7 days p.o.	No effect	Goldstein et al. (2001)
L-733,060	Formalin paw late phase Preclinical assay	Gerbil	0.17 mg/kg i.v.	May be of therapeutic use as centrally acting analgesic	Rupniak et al. (1996)

Table 1 continued

Nervous system					
Migraine					
GR-205,171 (vofopitant)	Migraine Clinical trial	Human	25 mg i.v.	No effect	Connor (1998)
Lanepitant (LY-303,870)	Migraine Clinical trial	Human	30,80, 240 mg p.o.	No effect	Goldstein et al. (2001)
L-758,298	(fosaprepitant) Migraine Clinical trial	Human	20, 40, 60 mg i.v.	No effect	
Norman (1998)					
RPR-100,893	Migraine Clinical trial	Human	1, 5, 20 mg p.o.	No effect	Diener (2003)
SR-140,333B	Hyperalgesia Preclinical assay	Rat	3 mg/kg i.p.	Reduced orofacial heat hyperalgesia	Teodoro et al. (2013)
Seizures					
Aprepitant	Seizures in neurocysticercosis Preclinical assay	Rat	1 μg i.c.	Prevented seizure activity	Robinson et al. (2012)
CP-122,721-1	Kainic acid-induced seizure activity Preclinical assay	Rat	0.3 mg/kg i.c.	Anticonvulsant	Zachrisson et al. (1998)
GR-205,171 (vofopitant)	Generalized seizure induced by electroshock. Preclinical assay	Rat	1, 3, 10, 30 mg/kg s.c.	No effect In combination therapy with lamotrigine enhanced anticonvulsant effect	Kalinichev et al. (2010)
SP antagonist analogs	Preclinical assay	Rat	7–12 nmol. Intranigral administration	Attenuated convulsions	Garant et al. (1986)
Neurodegeneration					
L-732,138	SP induces neurodegeneration in a concentration-dependent manner Preclinical assay	Neurons primary cultures	5 nM treated with SP	Completely inhibited SP-induced neuron death	Castro-Obregón et al. (2002)
L-733,060	SP induces neurodegeneration in a concentration-dependent manner Preclinical assay	Neurons primary cultures	1 nM treated with SP	Completely inhibited SP-induced neuron death	Castro-Obregón et al. (2002)
	Methamphetamine-induced striatal dopaminergic neurotoxicity in the murine brain Preclinical assay	Mouse	0.5, 1, 2 mg/kg i.p.	Abrogated methamphetamine-induced striatal dopaminergic neurotoxicity	Yu et al. (2002)
	Intrastriatal 6-hydroxydopamine model of early Parkinson's disease Preclinical assay	Rat	2 μL at 100 nM i.c. after the neurotoxin	Neuroprotective	Thornton and Vink (2012)
WIN-51,708	Methamphetamine-induced striatal dopaminergic neurotoxicity in the murine brain Preclinical assay	Mouse	2.5, 5, 10, 20, 30 mg/kg i.p.	Abrogated methamphetamine-induced striatal dopaminergic neurotoxicity	Yu et al. (2002)

i.c. Intracerebral, *i.c.v.* intracerebroventricular, *i.p.* intraperitoneal, *i.v.* intravenous, *p.o.* per os, *s.c.* subcutaneous

controlling nausea compared with vomiting (Olver et al. 2013).

The efficacy and safety of the NK-1 receptor antagonist CJ-11,974 (ezlopitant) (Table 1) in the control of acute cisplatin-induced emesis have also been reported (Hesketh et al. 1999). Moreover, the NK-1 receptor antagonist CP-99,994 (Table 1) abolishes both acute and delayed emesis and exhibits a broad-spectrum activity against peripheral and centrally acting emetogens (Bountra et al. 1993). This antagonist prevents the emesis induced by a wide range of emetic stimuli, such as apomorphine, morphine, nicotine, copper sulfate, ipecacuanha, radiation, cyclophosphamide, cisplatin, and motion. This broad spectrum of antiemetic activity is not shared by serotonin and dopamine receptor antagonists and this suggests that SP exerts a critical role in the emetic reflex pathway (Hesketh et al. 1999) and that the NK-1 receptor may be an appropriate target for therapeutic intervention.

Finally, despite the positive effects of the NK-1 receptor antagonist casopitant (Table 1) in controlling CINV in

clinical trials (Herrstedt et al. 2009), the use of this NK-1 receptor antagonist has been discontinued because further safety studies have been demanded (see Muñoz and Coveñas 2013b).

Emotional stress (anxiety and depression)

It has been reported that the undecapeptide SP is involved in the integration of emotional responses to stress (Fig. 1), suggesting that the pathogenesis of depression is due to an alteration of the SP/NK-1 receptor system (Kramer et al. 1998). In fact, in depression an increase in the production of SP has been observed (Kramer et al. 1998). In this sense, NK-1 receptor antagonists (Table 1) have been suggested as possible therapeutic agents for affective disorders, because a central injection of SP or related peptide agonists exerts an anxiogenic effect, whereas the genetic deletion of the NK-1 receptor induces an anxiolytic and an antidepressant effect (Rupniak et al. 2000, 2001). In fact, administration of the NK-1 receptor antagonist L-822,429 (Table 1) prevented the anxiogenic effect mediated by SP when the peptide was injected into the medial amygdala (Ebner et al. 2004).

Other studies have reported that the administration of SP agonists into the amygdala, the periaqueductal gray or the lateral ventricle produced anxiogenic effects, but that the administration of SP antagonists (Table 1) attenuated anxiolytic-like signs (Nikolaus et al. 1999a, b, 2000) and that SP regulates states of anxiety acting in the human amygdala (Carletti et al. 2005). Numerous studies have reported the presence of neuropeptides belonging to the tachykinin family of peptides (SP, neurokinin A, neurokinin B) as well as the presence of their receptors (NK-1, NK-2 and NK-3) in the regions of the CNS that are critical for the regulation of affective behavior and neurochemical responses to stress (see Ebner and Singewald 2006 for review; Ebner et al. 2009). In these regions, these neuropeptides act as neurotransmitters and/or neuromodulators, interacting with classical neurotransmitters, such as serotonin, dopamine and noradrenalin (Ebner and Singewald 2006; Ebner et al. 2009). Some noradrenalin and serotonin-containing cell bodies also coexpress SP, presenting opportunities for more direct neuronal modulation. The potential for such functional interactions in vivo is supported by the observation that repeated administration of antidepressant drugs causes a down-regulation of SP biosynthesis in discrete brain regions (Kramer et al. 1998). In this sense, chronic treatment with antidepressant drugs produces a decrease in the concentrations of SP in the striatum, the substantia nigra and the amygdala. These findings suggest that a reduction in SP levels in certain brain regions could contribute to a common therapeutic effect of the antidepressant drugs in affective disorders (Shirayama et al. 1996). However, another study has shown that the chronic administration of several antidepressants does not cause significant changes in the expression of the NK-1 receptor located in the rat brain (Sartori et al. 2004). Many other data from animal and human studies have suggested that tachykinin receptors play an important role as therapeutic targets in stress-related disorders (see Ebner and Singewald 2006 and Ebner et al. 2009 for review).

In a recent study carried out in monocytes from patients with recurrent major depressive disorder and treated with an antidepressant therapy, it has been reported that the NK receptor system is altered (Bardelli et al. 2013). Thus, in comparison with healthy subjects, depressive patients showed a down-regulation of the NK-1 receptor expression and an upregulation of the NK-2 receptor expression. In addition, SP, neurokinin A and NK-1 and NK-2 agonists stimulated a higher release of tumor necrosis factor-alpha (TNFα) from monocytes in depressive patients than in healthy subjects and they also induced the activation of NF-κB, which was reversed after using NK-1/NK-2 receptor antagonists (Bardelli et al. 2013). All these data indicate that the tachykinin/NK receptor system plays an important role in major depression. The upregulation of the tachykinin transmission in patients suffering from stress-related disorders suggests that the use of tachykinin receptor antagonists is a promising strategy for the treatment of these patients (Ebner and Singewald 2006; Ebner et al. 2009). In fact, in clinical assay, it has been established that the drug aprepitant (an NK-1 receptor antagonist) (Table 1) exerts an antidepressant effect similar to that reported for the selective serotonin re-uptake inhibitor paroxetine (Kramer et al. 1998). Moreover, in patients suffering major depression it has been demonstrated that the NK-1 receptor antagonist L-759,274 (Table 1) exerts an antidepressant and anxiolytic effect (Kramer et al. 2004). In both studies, aprepitant and L-759,274 were well tolerated (Kramer et al. 1998, 2004). However, in another clinical trial, a lack of efficacy of aprepitant (Table 1) has been reported in the treatment of major depression (Keller et al. 2006). It is also known that oral administration of NK-1 receptor antagonists (aprepitant, L-733,060, CP-122,721) (Table 1) produces anxiolytic-like effects in the gerbil elevated plus-maze, and NK-1 antagonists reduce immobility in the gerbil forced-swim test without affecting locomotor activity (Wallace-Boone et al. 2008). This is important, since it is known that the gerbil NK-1 receptor is similar in homology to the human NK-1 receptor. Moreover, in numerous experimental animal models, it has been reported that other NK-1 receptor antagonists (e.g., CP-96,345, CP-99,994, etc.) exert anxiolytic and antidepressant actions (see Ebner and Singewald 2006 for review). All the above data suggest that the SP/NK-1 receptor system is involved in emotional stress.

Alcohol addiction

Understanding the pathophysiology of addictive disorders is critical for the development of new treatments. Animals genetically deficient of NK-1 receptors showed a decrease in alcohol consumption (Fig. 1) and an increased sensitivity to the sedative effects of alcohol (George et al. 2008). Genetic and pharmacological studies have shown that the binding of antagonists to the NK-1 receptor decreases anxiety-related behaviors, the self administration of alcohol in experimental animals and craving for alcohol in humans (see Rodríguez and Coveñas 2011 for review). Studies carried out with NK-1 receptor knock-out mice show that the blockade decreases voluntary alcohol intake (Ripley et al. 2002) and intensifies the sensitivity to the sedative effects of alcohol (George et al. 2008). Moreover, in NK-1 receptor knock-out animals, the decrease in alcohol consumption was accompanied by an absence of escalation of alcohol intake (Thorsell et al. 2010). This means that NK-1 receptor antagonists mimic the effects of NK-1 receptor gene deletion on alcohol consumption and that the NK-1 receptor is involved in the regulation of alcohol intake because it modulates two key components of addiction: alcohol reward and escalation (Thorsell et al. 2010). It has also been reported that the blockade of NK-1 receptors with an NK-1 receptor antagonist (L-703,606) (Table 1) suppressed alcohol intake dose-dependently in the control group, but was ineffective in NK-1 receptor-deficient animals (Thorsell et al. 2010). This means that the antagonist specifically suppresses alcohol drinking by acting at the target receptors.

The deletion of the *TACR1* gene, which encodes the NK-1 receptor, blocks opiate reward (Murtra et al. 2000). Because endogenous opioids in part mediate alcohol reward, modulation of the opioid mechanism by NK-1 receptors could represent an additional mechanism through which NK-1 receptor antagonists contribute to altering alcohol reward (Thorsell et al. 2010). The genetic deletion of the NK-1 receptor suppresses alcohol intake and NK-1 receptor antagonists suppress alcohol intake in a manner that mimics the effects of genetically inactivating the NK-1 receptor (Rodríguez and Coveñas 2011). In a human randomized controlled trial, it has also been reported that the NK-1 receptor antagonist LY-686,017 (Table 1) suppresses spontaneous alcohol cravings and exerts a beneficial effect on global measures of wellbeing (George et al. 2008). Also, this antagonist reduces both the subjective craving response to the combined challenge and the concomitant cortisol response (George et al. 2008; see Muñoz and Coveñas 2011).

It is also known that artificial microRNA-based NK-1 receptor gene silencing reduces alcohol consumption (Baek et al. 2010). The data reported above indicate that the SP/NK-1 receptor system is involved in the control of alcohol intake and that NK-1 receptor antagonists could have a therapeutic niche in alcoholism.

Pain

SP and NK-1 receptors are present in the dorsal horn of the spinal cord and in nociception spinal SP and neurokinin A play an important role (Samsam et al. 2001). Nerve fibers also transmit afferent signals to the CNS in response to inflammation (SP contributes to pain transmission in the CNS in inflammatory processes) (Samsam et al. 2001). In this sense, neuropeptides released from the peripheral terminals of the primary afferents play an important role in mechanical hyperalgesia after peripheral nerve injury, and peripherally released neuropeptides contribute to the generation of neuropathic pain and, in this case, SP is involved in the induction phase (Jang et al. 2004). It has also been reported that intravenous administration of the NK-1 receptor antagonist L-733,060 (Table 1) to gerbils before an intraplantar injection of formalin causes a dose-dependent and complete inhibition of the late, but not early, nociceptive response phase (paw licking) (Rupniak et al. 1996). In contrast, the non-brain penetrant quaternary ketone NK-1 receptor antagonist L-743,310 did not attenuate the response to formalin, indicating that the antinociceptive effect due to the blockade of NK-1 receptors by L-733,060 is centrally mediated (Rupniak et al. 1996).

However, despite the data reported from preclinical studies showing that NK-1 receptor antagonists exert an analgesic effect, clinical studies in humans have shown that these antagonists are generally ineffective for the treatment of pain (see Borsook et al. 2012 for review). Currently, the analgesic action of the NK-1 receptor antagonists (Table 1) aprepitant, lanepitant (LY-303,870), AV-608 and CJ-11,974 has been tested in human trials and in all the cases the drug was ineffective in relieving pain (e.g., neuropathic pain, visceral pain, postoperative dental pain, osteoarthritis, fibromyalgia) (see Borsook et al. 2012). However, the NK-1 receptor antagonist CP-99,994 (Table 1) decreased postoperative dental pain (Dionne et al. 1998). These are the first data acquired in humans supporting the notion that the SP/NK-1 receptor system plays an important role in pain. In the case of aprepitant (Table 1), this NK-1 receptor antagonist did not block postoperative dental pain (see Borsook et al. 2012). It has been suggested that the ineffective analgesic action of some NK-1 receptor antagonists in human trials would be due to the poor brain penetration of these compounds at the doses tested (Hill 2000), although this was not the case for aprepitant (see Borsook et al. 2012). These latter authors, using pharmacological/functional magnetic resonance imaging of fosaprepitant in humans, have suggested that this drug has a low probability

of success as an analgesic according to the modest effects that fosaprepitant has on the pain-processing regions of the brain (Borsook et al. 2012).

Migraine

Migraine is a chronic neurological disorder characterized by recurrent moderate to severe headaches. It is known that the primary trigeminal neurons of the trigeminal ganglion innervate major parts of the face and head, including the dura mater and the caudal trigeminal nucleus. In fact, the release of SP from primary trigeminal sensory terminals has been demonstrated in this nucleus after electrical stimulation of the trigeminal ganglion (Samsam et al. 2000, 2001). It has been suggested that SP might act as a transmitter/modulator at the first central synapses of the trigeminal sensory pathway. In addition, it has been proposed that SP may activate the bilateral trigeminal nociceptive pathways, leading to the perception of a poorly localized/generalized pain or headache rather than a unilateral one (Samsam et al. 2001). By contrast, it is known that during migraine attacks treatment with NK-1 receptor antagonists (Table 1) blocks protein plasma leakage and blood flood induced by SP (Bolay et al. 2002).

In experimental animals, it has been reported that SP induces orofacial heat hyperalgesia, which was reduced by the administration of the NK-1 receptor antagonist SR-140,333B (Table 1) (Teodoro et al. 2013). Moreover, heat, but not cold or mechanical, hyperalgesia induced by constriction of the infraorbital nerve, a model of trigeminal neuropathic pain, was abolished by pretreatment with SR-140,333B. Considering that in these experiments SP was injected peripherally and that the NK-1 receptor antagonist used lacks the ability to cross the blood–brain barrier, the results suggest that the peripheral SP/NK-1 receptor system participates in heat hyperalgesia and in persistent pain in the orofacial region (Teodoro et al. 2013).

However, in spite of a study reporting the increased plasma SP levels (Fig. 1) (Fusayasu et al. 2007), several clinical studies failed to find SP in the blood of migraine sufferers (Goadsby et al. 1990; Gallai et al. 1995) during headache, and it has also been reported that NK-1 receptor antagonists fail to alleviate the pain of migraine patients (Goldstein et al. 1997; Diener 2003), although this might be due to the poor bioavailability of these drugs during such attacks. For example, lanepitant (Table 1) was ineffective in migraine prevention and acute migraine; RPR-100,893 (Table 1) had no effects on migraine attacks; L-758,298, a pro-drug of aprepitant, failed to abort migraine attacks (Table 1), and GR-205,171 was ineffective against the treatment of migraine (Table 1) (see Borsook et al. 2012 for review). Moreover, the data suggest that the neurogenic vasodilatation mediated by neuropeptides (e.g., calcitonin gene-related peptide) is the dominant mechanism rather than the inflammation and plasma protein extravasation mediated by SP in human meningeal vessels during migraine.

Seizure

There are many data demonstrating that SP induces seizures. Kainic acid (KA) induces limbic seizure, resulting in a decreased SP-immunoreactivity level in the frontal cortex and hippocampus (Sperk et al. 1986). In addition, in the rat intranigral microinfusion of substance P antagonist analogs (NK-1 receptor antagonists) significantly attenuated the convulsions induced by maximal electroshock or intravenous bicuculline (Garant et al. 1986) (Table 1). Moreover, SP is elevated in children with seizure disorders (Fig. 1), both in serum and in the cerebrospinal fluid (Ko et al. 1991). It has been also reported in primary rat astrocytes and human astrocyoma cells that valproic acid downregulates the expression of NK-1 receptors (Lieb et al. 2003). Patients with medically intractable temporal lobe epilepsy are subjected to medial temporal lobectomy with hippocampectomy. Analysis of the hippocampus of these patients reveals that in the subgranular region of the hilus a selective loss of SP-immunoreactive interneurons occurs (de Lanerolle et al. 1992). It has also been reported that KA caused seizures and neuronal toxicity, as indicated by a reduction in the number of neurons located in the hippocampal CA1 subregion (Zachrisson et al. 1998). In other experiment, KA was also administered but after pretreatment with the NK-1 receptor antagonist CP-122,721-1 (Table 1) (Zachrisson et al. 1998). The pretreatment decreased seizure activity and a correlation was found between seizure activity and the survival of CA1 neurons. Conclusively, treatment with CP-122,721-1 has an anticonvulsant effect and may possibly counteract KA-induced nerve cell death in the CA1 subregion (Zachrisson et al. 1998). In fact, mice with disruption of the preprotachykinin-A gene, which encodes SP and neurokinin A, are resistant to KA excitotoxicity (Liu et al. 1999). The mice show a reduction in the duration and severity of seizures induced by KA or pentylenetetrazole, and both necrosis and apoptosis of hippocampal neurons are prevented. Although KA induces the expression of bax and caspase 3 in the hippocampus of wild-type mice, these critical intracellular mediators of cell death pathways are not altered by KA injection in mutant mice, indicating that the reduction in seizure activity and the neuroprotection observed in preprotachykinin-A null mice are caused by the extinction of an SP/neurokinin A-mediated signaling pathway that is activated by seizures. The data suggest that these tachykinins are critical for the control of the excitability, the seizures, and the vulnerability of the

hippocampus (Liu et al. 1999). Furthermore, in a rodent model of neurocysticercosis (NCC), it was reported that seizures were induced after intrahippocampal injection of SP alone or extracts of cysticercosis granuloma obtained from infected wild type (WT), but not from infected SP precursor-deficient mice (Robinson et al. 2012). Seizure activity was correlated with SP levels within WT granuloma extracts and was prevented by intrahippocampal preinjection of NK-1 receptor antagonists (aprepitant) (Table 1). Furthermore, extracts of granuloma from WT mice cause seizures when injected into the hippocampus of WT mice, but not when injected into NK-1 receptor-deficient mice (Robinson et al. 2012). Moreover, brain biopsies from NCC patients contain SP, but the peptide is not found in uninfected brains (Robinson et al. 2012).

All these data indicate that SP causes seizures and suggest that seizures may be prevented and/or treated with NK-1 receptor antagonists. However, this potential has not been fully explored in animals or humans. A recent study carried out in an animal experimental model has evaluated the efficacy of the NK-1 receptor antagonist, vofopitant (GR-205,171) (Table 1), both alone and in combination with different anticonvulsant drugs, because it is known that many patients with epilepsy are refractory to anticonvulsant drugs or do not tolerate side effects associated with the high doses required to fully prevent seizures (Kalinichev et al. 2010). The authors found that vofopitant had no anticonvulsant efficacy by itself, but could enhance the anticonvulsant efficacy of lamotrigine and other sodium channel blockers. This effect was mediated by NK-1 receptors (Kalinichev et al. 2010). Moreover, at the doses tested vofopitant did not produce CNS side effects, and it did not enhance side effects induced by high doses of lamotrigine. Analysis of the dose–effect relationship for GR-205,171 indicated that a high (>99 %) occupancy of NK-1 receptors is required for an effect to be observed, consistent with previous behavioral and human clinical studies with this pharmacologic class (Kalinichev et al. 2010). The authors concluded that in patients with refractory epilepsy some benefit could be derived from adding treatment with a suitable NK-1 receptor antagonist to treatment with a sodium channel blocker.

Neurodegeneration

Neurodegenerative diseases are a severe health problem that has not yet been resolved. Among these diseases, Parkinson's, Alzheimer's, multiple sclerosis, amyotrophic lateral sclerosis and Huntington's chorea are the most representative. SP and NK-1 receptors are located in the regions of the CNS involved in these diseases. For example, neuropeptides such as SP, neurokinin A and enkephalins are intimately involved in the postsynaptic actions of dopamine in the nigrostriatal system (Angulo and McEwen 1994) and the administration of SP/neurokinin A to either the substantia nigra or striatum increases both striatal dopamine and glutamate release; in contrast, this release can be blocked by NK-1 receptor antagonists (Fig. 1) (Reid et al. 1990a, b). Methamphetamine (METH) causes extensive neural degeneration (the apoptosis pathway included) in the CNS (Yu et al. 2002). Because METH increases striatal SP levels (Fig. 1), it has been speculated that this neuropeptide plays a role in METH-induced toxicity and neural damage in the striatum through the NK-1 receptor. In this region, the administration of NK-1 receptor antagonists (WIN-51,708 or L-733,060) (Table 1) prevents the loss of dopamine transporters, the loss of tissue dopamine, and the loss of tyrosine hydroxylase (Yu et al. 2002). Moreover, SP induces programmed cell death in primary cultures of hippocampal, striatal, and cortical neurons; this cell death requires NK-1 receptor gene expression (Table 1) (Castro-Obregón et al. 2002). In fact, hippocampal, striatal, and cortical neurons die at micromolar concentrations of SP 48 h after exposure and to nanomolar concentrations 7 days after exposure. Despite this, it has been reported that at nanomolar concentrations SP induces proliferation in both normal and tumor cells (see Muñoz and Coveñas 2010 for a review). The paradoxical effect on neurons might be related with the aborted proliferation capacity of these cells because they are very specialized cells, and SP stimuli could induce cell death in a concentration- or time-dependent manner. However, SP induces fibroblast and tumor cell proliferation, because the proliferation mechanism is normal in these cells (Muñoz and Coveñas 2010). In contrast, NK-1 receptor antagonists (L-732,138, L-733,060) completely inhibit SP-induced cell death in striatal cells (Table 1), confirming that SP-induced neurotoxicity is mediated by NK-1 receptors (Castro-Obregón et al. 2002). Thus, the use of NK-1 receptor antagonists could improve the outlook in these neurodegenerative diseases by blocking SP-induced neuron death. These findings could lead to new knowledge and therapeutic strategies in the treatment of neurodegenerative diseases.

Moreover, it is known that SP is an important mediator of both neuroinflammation and blood–brain barrier dysfunction through its NK-1 receptor (Thornton and Vink 2012). In an experimental animal model, these authors demonstrated that intrastriatal 6-hydroxydopamine lesioning produced an increase in the ipsilateral nigral SP content, along with a breakdown of the blood–brain barrier and activation of microglia and astrocytes. Further exacerbation of SP levels accelerated disease progression, whereas treatment with the NK-1 receptor antagonist L-733,060 (Table 1) protected dopaminergic neurons, preserved barrier integrity, reduced neuroinflammation and significantly

improved motor function (Thornton and Vink 2012). The authors concluded that NK-1 receptor antagonists may represent a novel neuroprotective therapy.

In sum, many human clinical trials have reported the efficacy of aprepitant and fosaprepitant for the prevention of CINV and PONV and, in fact, they are the only NK-1 receptor antagonists currently available in clinical practice. Other NK-1 receptor antagonists (e.g., ezlopitant, casopitant) have shown an antiemetic effect in phase II/III trials, but currently they are not used in clinical practice since in the case of casopitant, for example, further safety studies have been required. In stress-related disorders, an increase in the production of SP has been reported and in numerous experimental animal models it has been demonstrated that NK-1 receptor antagonists exert anxiolytic and antidepressant actions. However, in human clinical trials, the antidepressive action of aprepitant is controversial and further in-depth studies are required to fully demonstrate its antidepressive action. Another NK-1 receptor antagonists, L-759,274, showed no antidepressive effect in human clinical trials. Regarding alcohol addiction, the results obtained in animals are quite promising since it seems that the SP/NK-1 receptor system is involved in the control of alcohol intake. However, clinical trials are scarce and in fact in human it has only been reported that LY-686,017 suppresses alcohol craving. Preclinical studies have shown that NK-1 receptor antagonists exert an analgesic effect, but in human clinical trials, these antagonists were unsuccessful for the treatment of pain (including migraine), except in the case of CP-99,994, which decreased postoperative dental pain. Finally, although preclinical studies have reported that NK-1 receptor antagonists exert anticonvulsive and neuroprotective effects; no human clinical trial testing of these effects has yet been developed.

Inflammation diseases

It is known that both SP and the NK-1 receptor are upregulated during the inflammation processes and that, in rats, NK-1 receptor antagonists exert an antiinflammatory action (see Muñoz and Coveñas 2013a for review) (Fig. 1). Capsaicin-sensitive primary afferent neurons are responsible for neurogenic inflammation in peripheral organs (Holzer 1988) and SP is considered a key mediator in neurogenic inflammation (Harrison and Geppetti 2001). SP contributes to pain transmission in the CNS in inflammatory processes (Holzer 1988). The hallmarks of neurogenic inflammation are an increase in vascular permeability, plasma extravasation, edema formation, and leukocyte infiltration (Holzer 1988; Harrison and Geppetti 2001). SP contributes to leukocyte recruitment in inflammatory processes (Rittner et al. 2007). This recruitment involves the upregulation of adhesion molecule expression, through NK-1 receptors, in endothelial cells and augmented chemokine production or chemotaxis, through NK-1 receptors, in leukocytes. In inflammation, leukocytes can trigger endogenous antinociception through the release of opioid peptides and the activation of opioid receptors on peripheral sensory neurons (Rittner et al. 2007). Moreover, these authors report that systemically and peripherally selective, but not intrathecally, NK-1 receptor blockade by NK-1 receptor antagonists (L-733,060, SR-140,333) (Table 2) reduces stress-induced antinociception without affecting baseline hyperalgesia. In parallel, the local recruitment of opioid-containing leukocytes is decreased (Rittner et al. 2007). Peripheral NK-1 receptor blockade does not alter the endothelial expression of intercellular adhesion molecule-1 or local chemokine or cytokine production, but decreases polymorphonuclear cell and macrophage recruitment (Rittner et al. 2007). Thus, it seems that NK-1 receptor antagonists impair the recruitment of opioid-containing leukocytes and stress-induced antinociception, since endogenous inhibition of inflammatory pain is dependent on the NK-1 receptor-mediated recruitment of opioid-containing leukocytes (Rittner et al. 2007).

SP causes vasodilatation by acting directly on smooth-muscle cells and indirectly by stimulating histamine release from mast cells. It is known that both the N-terminal residue and the hydrophobic C-terminal of SP play an important role in the mechanism of histamine release (Shibata et al. 1985). Increased microvascular permeability, edema formation, and subsequent plasma protein extravasation are prominent peripheral effects of tachykinins, underlying their powerful proinflammatory properties. The edema induced by SP is primarily due to increased vascular permeability, mediated through its action on NK-1 receptors situated in post-capillary venule endothelial cells (Lembeck et al. 1992). The SP-induced contraction of endothelial cells and subsequent plasma extravasation allow the bradykinin and the histamine to gain access to the site of injury and to afferent nerve terminals.

SP is not only synthesized and secreted by nerve cells; monocytes, macrophages, dendritic cells, eosinophils, lymphocytes, and mast cells also synthesize this peptide. Human T-lymphocytes contain preprotachykinin mRNA, encoding SP, and produce endogenous SP (Lai et al. 1998), whereas in vitro activation with lipopolysaccharide produces a marked increase in SP expression by mononuclear phagocytes and dendritic cells (Lambrecht et al. 1999). SP induces T-lymphocyte proliferation (Scicchinato et al. 1988; Nio et al. 1993) and most of the immune cells producing SP also express NK-1 receptors (Lai et al. 1998). SP acts not only as a mediator of the crosstalk between the nervous and the immune systems but also acts

Table 2 NK-1 receptor antagonists in inflammation diseases and in viral and bacterial infection

Inflammation diseases					
CJ-12,255	Model of obesity and asthma Preclinical assay	Mouse	300 μg i.p.	Improved both obesity and asthma	Ramalho et al. (2013)
CP-122,721	Neurogenic inflammation Preclinical assay	Rat	10 mg/kg s.c.	Antiinflammatory	King et al. (2001)
CP-96,345	Toxic hepatitis Preclinical assay	Mouse	10 mg/kg i.p. 30 min after injury	Antiinflammatory	Bang et al. (2003, 2004)
L-733,060	Leukocytes recruitment and stress-induced antinociception	Rat	20 mg/kg i.p.	Impaired leukocyte recruitment and stress-induced antinociception	Rittner et al. (2007)
	Toxic hepatitis Preclinical assay	Mouse	20 mg/kg i.p. 30 min after injury	Antiinflammatory	Bang et al. (2003, 2004)
SR-140,333	Leukocytes recruitment and stress-induced antinociception	Rat	10 mg/kg i.p.	Impaired leukocyte recruitment and stress-induced antinociception	Rittner et al. (2007)
Viral infection					
Aprepitant drug	Anti-HIV Phase II trial	Human	125 or 250 mg/day p.o. daily, for 14 days	Showed biological activity, but no significant antiviral activity	Tebas et al. (2011)
	Measles virus infection	Mouse	80 mg/kg p.o.	Less viral infection, which seemed to be more focally restricted within the parenchyma	Makhortova et al. (2007)
	Mononuclear infection with HIV	Mononuclear cells	IC_{50}: 5.4 μM	High anti-HIV-1 activity	Manak et al. (2010)
CP-96,345	Mononuclear infection with HIV	Mononuclear cells	IC_{50}: 15.2 μM	Anti-HIV-1 activity	Manak et al. (2010)
L-733,060	Mononuclear infection with HIV	Mononuclear cells	IC_{50}: 5.6 μM	Anti-HIV-1 activity	Manak et al. (2010)
RP-67,580	Mononuclear infection with HIV	Mononuclear cells	IC_{50}: 11 μM	Anti-HIV-1 activity	Manak et al. (2010)
Spantide	Herpetic stromal keratitis lesion Preclinical assay	Mouse	36 μg per eye Subconjunctival	Significant reduction in corneal opacity and angiogenesis	Twardy et al. (2011)
	Brain endothelium culture	Rat	10^{-9}–10^{-5} M	Completely neutralized the effect of gp120 on brain endothelium permeability	Annunziata et al. (1998)
	Measles virus infection	Neurons measles virus spread	200 μM	Inhibitor of measles virus fusion; prevented both infection and spread in primary neurons	Makhortova et al. (2007)
Z-D-Phe-L-Phe-Gly oligopeptide	Measles virus–SP receptor interaction Preclinical assay	Human IM-9 lymphoblast	10^{-7} M	Inhibited measles virus fusion with target cells	Harrowe et al. (1990)
	Measles virus infection	Neurons measles virus spread	200 μM	Inhibitor of measles virus fusion; prevented both infection and spread in primary neurons	Makhortova et al. (2007)

Table 2 continued

Bacterial infection					
L-703,606	Experimental meningitis to: Neisseria meningitidis and Borrelia burgdorferi	Mouse	5 mg/kg s.c.	Reduced inflammatory cytokine production	Chauhan et al. (2008)
	Preclinical assay				
	Pneumococcal meningitis	Mouse	5 mg/kg s.c. daily during 3 days, postinfection	Antiinflammatory	Chauhan et al. (2011)
	Preclinical assay				
Spantide	Pneumococcal meningitis	Rat	10^{-7} M	Attenuated arteriolar vasodilatation	Pfister et al. (1995)
	Preclinical assay				
SR-140,333	Lung injury in polymicrobial sepsis	Mouse	1 mg/kg, s.c. 30 min before or 1 h after infection	Antiinflammatory	Hegde et al. (2007, 2010a, b)
	Preclinical assay				

i.p. Intraperitoneal, *p.o.* per os, *s.c.* subcutaneous

independently on sensory nerves in a paracrine and/or autocrine fashion (Fig. 2). Moreover, SP has been implicated in inflammatory processes in the respiratory, gastrointestinal, and musculoskeletal systems (O'Connor et al. 2004). Many substances induce neuropeptide release from sensory nerves in the lung, including allergens, histamine, prostaglandins, and leukotrienes. Patients with asthma are hyperresponsive to SP, and NK-1 receptor expression is increased in their bronchi (O'Connor et al. 2004) (Fig. 1). Elevated levels of SP and upregulated NK-1 receptor expression have also been reported in the rectum and colon of patients with inflammatory bowel disease (IBD), increased levels of SP are found in the synovial fluid and serum of patients with rheumatoid arthritis (RA), and the NK-1 receptor mRNA is upregulated in RA synoviocytes (O'Connor et al. 2004). Glucocorticoids may attenuate neurogenic inflammation by decreasing NK-1 receptor expression in epithelial and inflammatory cells and by increasing the production of neutral endopeptidase, an enzyme that degrades SP (O'Connor et al. 2004). Thus, the prevention of the proinflammatory effects of SP, using NK-1 receptor antagonists, may have therapeutic potential in inflammatory diseases, such as asthma, sarcoidosis, chronic bronchitis, IBD, RA, and indeed in all the inflammatory diseases (see O'Connor et al. 2004).

In a recent study, the authors explored the effect of the NK-1 receptor antagonist CJ-12,255 (Table 2) in a mouse model of diet-induced obesity and asthma (Ramalho et al. 2013). The authors found that CJ-12,255 improved both obesity and asthma. This means that SP must be involved in bronchial neurogenic inflammation by acting through the NK-1 receptor.

Viral infection

There are data demonstrating the involvement of the SP/NK-1 receptor system in viral infection, replication and proliferation (Fig. 1).

Herpes virus

In a mouse model, SP has been involved in the severity of herpetic stromal keratitis (HSK) caused by herpes simplex virus-1 (Twardy et al. 2011). A significantly higher level of SP was observed in corneas with severe HSK lesions in comparison with mild lesions (Fig. 1). The corneas also exhibited higher amounts of proinflammatory cytokines (IL-6, IFNγ) and chemokines (CCL3, CXCL2) when compared with corneas with a lower level of SP (Twardy et al. 2011). Moreover, subconjunctival inoculation of the NK-1 receptor antagonist spantide during the clinical phase of HSK resulted in a significant reduction in

corneal opacity and in angiogenesis (Twardy et al. 2011) (Table 2).

Measles virus

Measles virus (MV) encodes the fusion protein (F), which mediates cell fusion and the intercellular spread of the virus and is homologous to the carboxy terminus of the SP (Harrowe et al. 1990). In addition, the oligopeptide Z-D-Phe-L-Phe-Gly (Table 2), a homologous antagonist (peptide NK-1 receptor antagonist) to F and SP, inhibits MV fusion with target cells. These observations raise the question of whether MV uses the NK-1 receptor during a specific phase of its infective cycle. Moreover, bound MV and SP have been shown to displace each other from target cells reciprocally (Harrowe et al. 1990). In addition, anti-SP antisera inhibit the cell-to-cell spread of MV, blocking MV fusion with target cells. These results indicate the presence of MV-NK-1 receptor interactions during viral fusion, and suggest possible mechanisms for viral entry into cells by the NK-1 receptor mimicking the SP function (Harrowe et al. 1990). Moreover, MV can invade and persist within the human CNS, leading to progressive and even fatal neurological diseases, including subacute sclerosing panencephalitis (Makhortova et al. 2007). It has been reported that MV is transmitted trans-synaptically, that SP blocks neuronal MV spreading, and that the genetic deletion or pharmacological inhibition of the NK-1 receptor (Table 2) reduces infection by MV (Makhortova et al. 2007).

Myocarditis

Myocarditis is an inflammatory disorder of the heart that causes degeneration of the myocardium and it is an important cause of heart failure. Myocarditis is most commonly caused by viral infections, such as coxsackie virus, echovirus, adenovirus and picornovirus, and also appears as a complication of bacterial or parasitic infections. Viral myocarditis is characterized by cardiac inflammation and cardiomyocyte necrosis. The molecular pathogenesis of viral myocarditis is incompletely understood and no specific therapies are available. It is known that SP stimulates the production of proinflammatory cytokines and contributes to the pathogenesis of several viral and parasitic infections in mice and humans and that NK-1 receptors are expressed at the surface of cardiomyocytes, endothelial cells and immunocytes, including lymphocytes and macrophages (see Muñoz and Coveñas 2011). Murine myocarditis caused by infection with encephalomyocarditis virus (EMCV) is a commonly used experimental model to study viral myocarditis. Proinflammatory cytokines such as IL-1β, TNFα and IL-6 have been implicated in the pathogenesis of myocarditis caused by EMCV infection (Robinson et al. 2009). It has been reported that SP levels are increased 61-fold in EMCV-infected wild-type mice (Fig. 1). EMCV infection results in 51 % mortality and a 1.56-fold increase in the heart-to-body weight ratio, accompanied by cardiac inflammation and necrosis along with cardiomyocyte apoptosis and hypertrophy of surviving cells (Robinson et al. 2009). In contrast, SP precursor knockout mice are completely protected from EMCV mortality, cardiomegaly, cardiac inflammation and necrosis as well as from cardiomyocyte apoptosis and hypertrophy (Robinson et al. 2009). These results indicate that SP is essential for the pathogenesis of EMCV myocarditis. Thus, NK-1 receptor antagonists (blocking the SP pathophysiological functions) could improve cardiomegaly, cardiac inflammation and necrosis as well as cardiomyocyte apoptosis and hypertrophy.

Respiratory syncytial virus

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infections in infants. Bronchiolitis is an inflammation of the bronchioles usually caused by viruses, most commonly being due to RSV. RSV infection causes exaggerated inflammation after intrapulmonary sensory nerve stimulation (see Muñoz and Coveñas 2011). Moreover, the level of mRNA encoding the NK-1 receptor is increased fourfold in RSV-infected lungs (Fig. 1), whereas mRNA encoding the vasoactive intestinal peptide receptor (VIPR)-1 for the anti-inflammatory VIP increases to a much lesser extent (King et al. 2001). mRNAs encoding NK-2 and VIPR-2 receptors are not affected by the virus. Selective inhibition of the NK-1 receptor abolishes neurogenic inflammation in RSV-infected intrapulmonary airways (King et al. 2001) (Table 2). Moreover, neurogenic inflammation and NK-1 receptor upregulation in infected lungs are inhibited by prophylaxis after using a monoclonal antibody against RSV (King et al. 2001). Likewise, it has been reported that SP/NK-1 receptor mRNA levels increase several times in RSV-infected lung, and that the number of SP-binding sites in the bronchial mucosa increases threefold (Piedimonte et al. 1999). RSV renders the airways abnormally susceptible to the proinflammatory effects of SP by upregulating NK-1 receptor gene expression, thereby increasing the density of these receptors on target cells (Piedimonte et al. 1999). This effect may contribute to the inflammatory reaction to the virus and could be a target for the therapy (using NK-1 receptor antagonists) of RSV disease and its possible sequelae (recurrent wheezing and childhood asthma).

Human immunodeficiency virus

SP is an immunomodulator that, in particular, regulates the immune function of mononuclear phagocytes. SP specifically activates NF-kB, a transcription factor involved in the control of cytokine expression (Lieb et al. 1997; Marriott et al. 2000), and stimulates human peripheral blood monocytes to produce inflammatory cytokines, including IL-1, IL-6, IL-12, and TNFα (Lotz et al. 1988). These cytokines alter human immunodeficiency virus (HIV) expression in T cells and monocytes (Rosenberg and Fauci 1990, 1991). SP is secreted by human monocyte-derived macrophages (MDM) and macrophages and the peptide participates in immunoregulation processes in an autocrine manner (Pascual and Bost 1990). Thus, SP plays an important role in the pathogenesis of immune-mediated diseases, including neuroimmunological diseases and HIV/AIDS. HIV-positive children have higher plasma levels of SP compared with HIV-negative children (Azzari et al. 1992) (Fig. 1). Moreover, SP plays a critical role in HIV gp120-induced increases in the permeability of brain endothelium cultures, this effect being abrogated by NK-1 receptor antagonists (spantide) (Annunziata et al. 1998) (Table 2). It has also been reported that SP modulates HIV replication in human MDM, that SP enhances HIV replication in mononuclear phagocytes isolated from human blood, and that SP is involved in HIV infection of human immune cells, such as MDM (Ho et al. 1996). Thus, by blocking the action of SP, NK-1 receptor antagonists could inhibit HIV infection of MDM.

It has been reported that in HIV-infected adults not receiving antiretroviral therapy, the administration of aprepitant was found to be safe (Tebas et al. 2011) (Table 2). In these patients, the concentration of SP in plasma decreased. It has also been reported that aprepitant inhibits HIV infection and can enhance the anti-HIV activity of certain antiretrovirals (Manak et al. 2010) (Table 2). In fact, aprepitant exerts the greatest inhibitory effect in comparison with other NK-1 receptor antagonists, such as L-733,060, CP-96,345, CJ-12,255 and RP-67,580 (Manak et al. 2010; see Muñoz and Coveñas 2011) (Table 2).

Severe bacterial infection

Sepsis

Sepsis is a systemic inflammatory response syndrome caused by severe bacterial infection. The SP/NK-1 receptor system has been implicated in severe bacterial infection. Preprotachykinin-A (PPT-A), the SP-producing gene, has been described to play an important role in neurogenic inflammation (Puneet et al. 2006). Several data from a model of gene-deficient mice (PPT-A (−/−)) in which cecal ligation and puncture induced sepsis was carried out have been reported. PPT-A gene deletion protects significantly against mortality, delays the onset of lethality, and improves long-term survival. PPT-A (−/−) mice had significantly attenuated inflammation and damage in the lungs (Puneet et al. 2006). The data suggest a role for SP in inducing lung injury in sepsis (Fig. 1). Moreover, in a model of mice sepsis, a significant increase in lung levels of chemokines, cytokines and adhesion molecules (MIP-2, MCP-1, IL-1beta, IL-6, ICAM-1, E- and P-selectin) has been reported, together with an increase in myeloperoxidase (MPO) activity in comparison with control mice (Hegde et al. 2007). In addition, the NK-1 receptor antagonist SR-140,333 (Table 2) attenuates the increased lung MPO activity and the levels of MIP-2, MCP-1, IL-1beta, IL-6, ICAM-1, and E- and P-selectin in comparison with control mice. Histological evaluation of the lung further supports the beneficial effect of NK-1 receptor antagonists on lung inflammation (Hegde et al. 2007). Thus, NK-1 receptor antagonists can exert a potential therapeutic effect in sepsis, and this effect is brought about via a reduction in leukocyte recruitment. Additionally, in sepsis, the gene expression profiles in mouse lung tissue and the effect of PPT-A gene deletion have been described (Hegde et al. 2010a). In a comparison of wild type and PPT-A-knockout septic mice, a whole range of genes were differentially expressed (more than twofold). Genetic deletion of SP resulted in a significantly different expression profile of the genes (e.g., CCL-2, CCL-3, CCL-4, CCL-9, CXCL-1, CXCL-2, CXCL-10, IL-6β) involved in inflammation and immunomodulation after the induction of sepsis as compared with wild-type mice (Hegde et al. 2010a). Apart from the various proinflammatory mediators, the antiinflammatory cytokine IL-1 receptor antagonist gene is also much more elevated in PPT-A(−/−) septic mice (Hegde et al. 2010a). The elevated levels of inflammatory gene expression in the early stages of sepsis in PPT-A-knockout mice are possibly aimed to resolve the infection without excessive immunosuppression. In a mouse model of cecal ligation and puncture (CLP)-induced sepsis, the action of the NK-1 receptor antagonist SR-140,333 (Table 2) has been studied (Hegde et al. 2010b). Lung tissue was collected and analyzed. CLP alone caused a significant increase in the activation of the transcription factors, protein kinase C-α, extracellular signal-regulated kinases, NK-1 receptors, and SP levels in lung when compared to sham-operated mice. The NK-1 receptor antagonist SR-140,333 injected pre- and post-surgery significantly attenuates the activation of transcription factors and protein kinase C-α and the plasma levels of SP as compared to CLP-operated mice injected with vehicle (Hegde et al. 2010b). The data suggest that in sepsis SP acts through NK-1 receptors, initiating a signaling cascade

mediated mainly by protein kinase C-α, leading to NF-κB and activator protein-1 activation, and further modulates proinflammatory mediators.

Meningitis

The involvement of the SP/NK-1 receptor system in bacterial meningitis has been demonstrated. In the rat, SP induces pia mater arteriolar vasodilatation during pneumococcal meningitis because treatment with the NK-1 receptor antagonist Spantide (Table 2) significantly attenuates arteriolar vasodilatation in this type of experimental meningitis (Pfister et al. 1995). Moreover, the expression of NK-1 receptors in microglia has been reported and it has been shown that SP can significantly elevate bacterially induced inflammatory prostanoid production by isolated cultures of these cells (Chauhan et al. 2008). The SP/NK-1 receptor interaction is an essential component in the initiation and/or progression of CNS inflammation in vivo following exposure to two clinically relevant Gram-negative bacterial CNS pathogens: *Neisseria meningitidis* and *Borrelia burgdorferi.* In vivo, both the elevation in inflammatory cytokine production and the decrease in the production of an immunosuppressive cytokine are markedly attenuated in mice that are genetically deficient in the expression of the NK-1 receptor or in mice treated with an NK-1 receptor antagonist (Chauhan et al. 2008) (Table 2). In addition, SP can augment inflammatory cytokine production by microglia after exposure to either of these bacterial pathogens (Chauhan et al. 2008). It has also been demonstrated that in an in vivo model of pneumococcal meningitis that SP similarly enhances the inflammatory glial responses to Gram-positive *Streptococcus pneumoniae*, the causative agent of bacterial meningitis, and that the SP/NK-1 receptor interaction plays a critical role in the development of CNS inflammation (Chauhan et al. 2011). Moreover, targeting of the NK-1 receptor (Table 2) not only prevents the development of damaging inflammation when administered prophylactically, but it can also limit or reverse the neuroinflammation associated with an established pneumococcal CNS infection when delivered therapeutically (Chauhan et al. 2011). NK-1 receptor antagonists attenuate the increase in CNS inflammatory cytokine levels and decrease the immunosuppressive cytokine production associated with an ongoing *S. pneumoniae* infection. Furthermore, therapeutic intervention with NK-1 receptor antagonists reverses infection-associated gliosis and demyelization in the absence of changes in the CNS bacterial burden (Chauhan et al. 2011). The data suggest that targeting the SP/NK-1 receptor interaction could be an excellent strategy for the treatment of microbially induced neuroinflammation.

In sum, regarding inflammatory diseases (e.g. inflammatory bowel disease) and viral (herpes virus, measles virus, encephalomyocarditis virus, respiratory syncytial virus) and bacterial infections, the results found in preclinical assays suggest the involvement of the SP/NK-1 receptor system in these processes. However, to date no human clinical assays using NK-1 receptor antagonists against the above pathologies have been developed. Many studies have demonstrated the relationship between SP and the HIV. In this case, the use of aprepitant as anti-HIV therapy has been tested in human clinical trials, although this NK-1 receptor antagonist showed no significant antiviral activity. NK-1 receptor antagonists merit further investigation as potential therapeutic antiviral, anti-bacterial and anti-inflammatory agents.

Cancer

It known that SP is expressed in keratocystic odontogenic tumors, oral squamous cell carcinoma, larynx carcinoma, blast cells, melanoma, glioma, retinoblastoma, neuroblastoma and lung cancer (González-Moles et al. 2008; Brener et al. 2009; Esteban et al. 2009; Muñoz and Coveñas 2010; Muñoz et al. 2010). SP has been located in the cytoplasm and in the nucleus of tumor cells (González-Moles et al. 2008; Brener et al. 2009; Esteban et al. 2009; Muñoz et al. 2012). Moreover, the PPT1 gene is expressed more in breast cancer than in epithelial breast cells (Singh et al. 2000). NK-1 receptors have also been demonstrated in human cancer cell lines and/or in primary tumors [e.g., glioma, astrocytoma, retinoblastoma, ganglioneuroblastoma, leukemia, neuroblastoma, carcinomas (pancreatic, larynx, gastric, colon, medullary thyroid, breast, oral)] (Friess et al. 2003; González-Moles et al. 2008; Brener et al. 2009; Esteban et al. 2009; Muñoz et al. 2007; Muñoz and Coveñas 2010). It is also known that tumor cells overexpress NK-1 receptors, in comparison with non-tumor cells (Singh et al. 2000; Friess et al. 2003; Muñoz and Coveñas 2010; Muñoz et al. 2012) (Fig. 1) and that SP, in a concentration-dependent manner, induces tumor cell proliferation in many different human tumor cell lines (for a review, see Muñoz and Coveñas 2010). By contrast, the use of NK-1 receptor antagonists (aprepitant, fosaprepitant, L-732,138, L-733,060) elicits antitumor action, inhibiting tumor cell proliferation, and tumor cells die by apoptosis (Muñoz et al. 2007, 2012, 2014; Muñoz and Coveñas 2010, 2013a; Berger et al. 2014) (Table 3). Moreover, in vivo it has been demonstrated that aprepitant exerts an inhibitory effect on the expression of the truncated-NK-1 receptor (Berger et al. 2014). Neoangiogenesis, a hallmark of tumor development, is stimulated by SP (the peptide induces the proliferation of endothelial cells) (Ziche et al. 1990). In most tumors investigated, both SP and NK-1 receptors are found in intra- and peri-tumoral blood vessels, and in fact

Table 3 NK-1 receptor antagonists in cancer, hepatitis, pruritus, abortus and micturition

Cancer					
Aprepitant drug	Hepatoblastoma Preclinical assay	Mouse	80 mg/kg/day p.o. 4 weeks	Antitumor action Reduced tumor volume	Berger et al. (2014)
	Antitumor action in vitro Preclinical assay	Neuroblastoma, glioma, retinoblastoma, melanoma, osteosarcoma, acute lymphoblastic leukemia, hepatoblastoma, larynx, pancreatic, gastric, colon, lung carcinoma cell lines	IC_{50}: 19–33 μM	Tumor cell proliferation inhibition Apoptosis	See for review Muñoz and Coveñas (2013a)
[D-Arg1,D Trp5,7,9,Leu11] SP	Pancreatic cancer xenograft Preclinical assay	Mouse	35 μg/g/day for 10 days Peritumoral administration	Reduced tumor volume Antiangiogenic	Guha et al. (2005)
Fosaprepitant drug	Osteosarcoma Preclinical assay	Mouse	80 mg/kg/day s.c. for 28 days	Antitumor action Reduced tumor volume	Muñoz et al. (2014)
L-732,138	Antitumor action in vitro Preclinical assay	Neuroblastoma, glioma, retinoblastoma, melanoma, osteosarcoma, acute lymphoblastic leukemia, hepatoblastoma, larynx, lung carcinoma cell lines	IC_{50}: 38–140 μM	Tumor cell proliferation inhibition Apoptosis	Berger et al. (2014) see for review Muñoz and Coveñas (2013a)
L-733,060	Migration of breast cancer cells model Preclinical assay	Breast cancer cells	1 μM	Inhibition of migration of tumor cells	Lang et al. (2004)
	Antitumor action in vitro Preclinical assay	Neuroblastoma, glioma, retinoblastoma, melanoma, osteosarcoma, acute lymphoblastic leukemia, hepatoblastoma, larynx, pancreatic, gastric, colon, lung carcinoma cell lines	IC_{50}: 11–22 μM	Tumor cell proliferation inhibition Apoptosis	Berger et al. (2014) see for review Muñoz and Coveñas (2013a)
Hepatitis					
CP-96,345	Toxic hepatitis Preclinical assay	Mouse	10 mg/kg i.p. 30 min after injury	Hepatoprotector, antiapoptotic, antinecrosis, antiinflammatory	Bang et al. (2003, 2004)
L-733,060	Toxic hepatitis Preclinical assay	Mouse	20 mg/kg i.p. 30 min after injury	Hepatoprotector, antiapoptotic, antinecrosis, antiinflammatory	Bang et al. (2003, 2004)
Pruritus					
Aprepitant drug	Patients with chronic pruritus Clinical assay	Human	80 mg/day p.o. 1 week	Antipruritus effect Side effects were mild and only occurred in a few patients	Ständer et al. (2010)
	Severe pruritus related to biological cancer treatment Phase II trial	Human	125 mg on day 1; 80 mg on day 3; 80 mg on day 5 p.o.	Decreased severe pruritus No side effects	Santini et al. (2012)
Abortus					
CP-96,345	Experimental stress-induced abortus. Preclinical assay	Mouse	200 μM for 2 days and 400 μM the last day	Stress failed to increase the abortion. The increased levels of TNFα, observed in stressed animals were completely abrogated	Arck et al. (1995)
Micturition					
Aprepitant drug	Overactive bladder with urge urinary incontinence Phase II trial	Human	160 mg/day for 8 days. p.o.	Reduced urinary incontinence Well tolerated Adverse events generally mild	Green et al. (2006)

i.p. Intraperitoneal, *p.o.* per os, *s.c.* subcutaneous

during neoangiogenesis both tissue innervation and the expression of NK-1 receptors are increased (see Muñoz and Coveñas 2010). By contrast, it has been reported that NK-1 receptor antagonists inhibit tumor neoangiogenesis (Guha et al. 2005) (Table 3). Moreover, the migration of tumor cells is a crucial requirement for the development of metastasis and the progression of cancer. 90 % of cancer deaths are derived not from the primary tumor but from the development of metastases (Sporn 1996). It is known that tumor cell migration is induced by classical neurotransmitters (dopamine, noradrenalin), as well as by SP and that such migration is inhibited after the administration of D_2 receptor, adrenoceptor or NK-1 receptor antagonists (Lang et al. 2004) (Table 3). It is also known that after binding to the NK-1 receptor SP induces a rapid change in cellular shape (including blebbing) and that membrane blebbing is important in cell movement, cell spreading, and cancer cell infiltration (Fackler and Grosse 2008; Meshki et al. 2009). All these data indicate that NK-1 receptor antagonists could be a new tool in the treatment of cancer.

Hepatitis

It has been demonstrated that primary afferents from sensory neurons are necessary for disease activity in T cell-mediated immune hepatitis, and the involvement of SP in liver inflammation has been suggested (Bang et al. 2003). The depletion of primary afferent nerve fibers by neonatal capsaicin treatment or pretreatment with NK-1 receptor antagonists (CP-96,345, L-733,060) protects against liver injury in a dose-dependent manner (Bang et al. 2003) (Tables 2, 3). NK-1 receptor antagonists reduce inflammatory liver damage (e.g., edema formation, neutrophil infiltration, hepatocyte apoptosis, and necrosis) and inhibit the production of TNFα and IFNγ, whereas the synthesis of the hepatoprotective cytokines IL-6 and IL-10 is increased. NK-1 receptor antagonists prevent hepatocyte apoptosis, meaning that by binding to NK-1 receptors in hepatocytes SP might aggravate apoptotic signals in these cells (Bang et al. 2003). Because NK-1 receptor antagonists not only suppress the proinflammatory cytokine response in the liver but also prevent hepatocyte apoptosis, they might be considered potent drugs for the treatment of inflammatory liver disease, most likely through an inhibition of the effects of SP (Bang et al. 2003, 2004).

Colostasis and pruritus

Chronic pruritus is a global clinical problem with a high impact on the quality of life but no specific therapies. Pruritus, a complication of cholestasis, is a nociceptive stimulus. SP is a major mediator of pruritus and it has been suggested that cholestasis is associated with an increased neurotransmission via SP, as partly evidenced by the increased serum concentrations of this peptide in patients with pruritus secondary to cholestasis (Fig. 1). Thus, the serum SP concentrations of patients with chronic liver disease (CLD) and pruritus are significantly higher than those of patients with CLD but no pruritus, and than those of control groups (Trivedi and Bergasa 2010). Since SP regulates functions after binding to the NK-1 receptor, the use of NK-1 receptor antagonists in cholestasis pruritus could improve both the pruritus and the cholestasis. Patients with chronic pruritus treated with the NK-1 receptor antagonist aprepitant underwent a considerable reduction in itch intensity (Ständer et al. 2010) (Table 3). Probably, the SP/NK-1 receptor system is involved in the pruritus of uncurable renal, liver, skin, neurological and psychiatric diseases. Patients with dermatological disease (e.g. atopic diathesis, prurigo nodularis) benefited best from the treatment with NK-1 receptor antagonists (Ständer et al. 2010). These results are promising enough to warrant studies aimed at confirming the efficacy of NK-1 receptor antagonists in a randomized controlled clinical trial.

Platelet

SP stimulates platelet aggregation, and underlying this is the intracellular mobilization of calcium and degranulation (Graham et al. 2004). Platelets also express NK-1 and NK-3 receptors and there is evidence of the involvement of the NK-1 receptor in SP-mediated platelet aggregation (Graham et al. 2004) (Fig. 1). Platelets show SP-immunoreactivity and the peptide is secreted upon activation, suggesting the involvement of SP in the autocrine/paracrine regulation of these cells (Graham et al. 2004). Moreover, blockade of NK-1 receptors with antibodies inhibits platelet aggregation. It is of great interest to known the physiological role that SP and/or endokinin A (EKA) and B may play in homeostasis. The observation that SP and, potentially, EKA and B are released from platelets following activation, and their potential role in a positive feedback mechanism indicates that SP and the NK-1 receptor in platelets may play an important role in the regulation of thrombus formation (Graham et al. 2004). These findings increase our understanding of thrombotic diseases and suggest possible therapeutic interventions using NK-1 receptor antagonists to improve the treatment of this disease.

Abortus

Stress is known to induce abortions, but the underlying mechanisms are currently unknown. In stressed mice,

increased levels of the abortogenic cytokine TNFα have been associated with decreased levels of pregnancy-protective transforming growth factor beta 2-related suppressive activity in uterine decidua (Arck et al. 1995). The production of TNFα may be stimulated by SP; after the administration of an SP receptor antagonist or an SP-antibody, stress failed to increase the abortion rate above the background level (Arck et al. 1995) (Table 3). The increased levels of TNFα observed in stressed animals were completely abrogated in the animals that had received an NK-1 receptor antagonist; stress also failed to decrease pregnancy-protective suppressive activity in the decidua of these animals (Arck et al. 1995). The data suggest that stress may inhibit protective suppressor mechanisms and promote the secretion of abortogenic cytokines, such as TNFα, via SP.

In sum, preclinical studies have reported the advantage to use NK-1 receptor antagonists for the treatment of cancer, liver inflammation, pruritus and for preventing abortus. Human clinical trials have been developed to test the action of aprepitant in the treatment of patients suffering pruritus. These trials are quite promising. Moreover, the in vitro and in vivo preclinical data showing the antitumor action of NK-1 receptor antagonists are also quite encouraging and hence the antitumor action of NK-1 receptor antagonists already available in clinical practice for the treatment of emesis (aprepitant, fosaprepitant) should be tested in human clinical trials.

Therapy with NK-1 receptor antagonists in human pathology

There are two groups of NK-1 receptor antagonists: peptide NK-1 receptor antagonists (also called SP antagonists, SP analog antagonists and SP receptor antagonists) and non-peptide NK-1 receptor antagonists (L-733,060, L-741,671, L-742,694 (benzylether piperidines); RP-67,580, RP-73,467, RPR-100,893 (perhydroisoindolones); WIN-51,708 (steroid); L-732,138 (tryptophan-based); CP-99,994, CP-122,721, GR-203,040, GR-205,171 (benzylamino piperidines); CP-96,345, L-709,210 (benzylamino and benzylether quinuclidine)) (for a review, see Muñoz and Coveñas 2010). Most work carried out on the design and preparation of peptide NK-1 receptor antagonists has focused on the introduction of D-amino acids. However, the lower affinity of these antagonists than that of natural agonists, the metabolic instability of the peptides and their inability to gain access to the CNS through the blood–brain barrier limits their usefulness for in vivo studies. In addition, after administration in the CNS, these substances induce neurotoxicity (see Muñoz and Coveñas 2010). Although there are more than 300 NK-1 receptor antagonists, currently aprepitant and fosaprepitant (aprepitant dimeglumine, for intravenous use) are the only NK-1 receptor antagonists available for clinical use. These antagonists are indicated for the treatment of acute and delayed CINV. The binding sites for non-peptide NK-1 receptor antagonists, SP and peptide NK-1 receptor antagonists are different. SP and peptide NK-1 receptor antagonists bind at the extracellular ends of the transmembrane helices and especially at the extracellular loops of the receptor, whereas non-peptide NK-1 receptor antagonists bind more deeply between the transmembrane segments (Hökfelt et al. 2001). After binding to NK1 receptor, NK-1 receptor antagonists block the pathophysiological functions mediated by SP (Fig. 1). NK-1 receptor antagonists act in a concentration-dependent manner. These antagonists show different chemical compositions, but their activity is linked to stereochemical features, i.e. their affinity for the NK-1 receptor. Thus, NK-1 receptor antagonists could be used as a therapeutic tool in several human pathologies in which the SP/NK-1 receptor system is involved (Fig. 1).

According to the data reported in the previous sections, the SP/NK-1 receptor system is upregulated in many human pathologies (depression, cancer, neural degeneration, inflammatory bowel disease, viral infection and pruritus), suggesting that the administration of NK-1 receptor antagonists is an excellent strategy for the treatment of these diseases. In addition, fewer side effects should be expected after the administration of these drugs to patients, since for example in the possible treatment of cancer the drug used (NK-1 receptor antagonist) would be specific for a given upregulated target (NK-1 receptor). This contrasts with the action exerted by cytostatic drugs, since these are not specific against tumor cells and, therefore, elicit very severe side effects. In addition, peptide antagonists normally have no effect and only act on deranged systems with increased peptide release (Hökfelt et al. 2000). Many clinical trials have also reported the absence of serious side effects when NK-1 receptor antagonists have been administered to humans (see Muñoz and Coveñas 2013b for review), even when the NK-1 receptor antagonist was administered at high doses (300 mg/day) (Kramer et al. 1998). Accordingly, novel possibilities for translational research are emerging for improving the treatment of diseases in which the SP/NK-1 receptor system is upregulated. However, it should be noted that many preclinical studies have reported the beneficial effects of NK-1 receptor antagonists in the treatment of certain diseases, although these beneficial effects were often not found in human clinical trials. This could be explained in part by the species used in those studies. In this sense, it has been reported that several species show changes in the amino acid sequence of the NK-1 receptor and hence these variations

could regulate the intensity of the action of the NK-1 receptor antagonists studied (Fong et al. 1992; Sachais et al. 1993; Ebner and Singewald 2006).

Another explanation could be the clinical criteria chosen for the selection of patients enrolled in human trials (see Ebner and Singewald 2006). For example, the use of aprepitant as an antidepressant drug is debatable (Kramer et al. 1998; Keller et al. 2006). This drug (160 mg) was administered to patients suffering a major depressive disorder (Keller et al. 2006). Paroxetine was included in the trial. No statistically significant differences with placebo effects on the Hamilton rating scale for depression (HAM-D17) were observed for aprepitant, whereas paroxetine was significantly more effective than the placebo. Thus, this study showed the antidepressant efficacy of paroxetine and the absence of this effect for aprepitant (Keller et al. 2006). Thus, the findings of this study and those of Kramer et al. (1998) are contradictory. In the study carried out by Kramer et al. (1998), the HAM-D21 score at 6 weeks indicated that the effect of aprepitant was similar to that of paroxetine, while in the study carried out by Keller et al. (2006) the HAM-D17 score, at 8 weeks, indicated no antidepressant effect of the NK-1 receptor antagonist, although the effect was found with paroxetine. Nevertheless, there is an important point to note: the doses of aprepitant used in both studies: 300 mg/day (Kramer et al. 1998) and 160 mg/day (Keller et al. 2006). It seems that the different doses used could be responsible for the contradictory results. Despite initial findings in support of the antidepressant activity of NK-1 receptor antagonists in humans, the clinical efficacy of these indications has not been appropriately checked, and development has largely been discontinued. Further studies must be carried out to check the possible antidepressant action of aprepitant.

In human trials, aprepitant has failed to exert a beneficial effect in the treatment of dental pain and neuropathic pain (see Borsook et al. 2012). However, the efficacy and safety of NK-1 receptor antagonists (e.g., aprepitant) have not fully tested in other diseases (e.g., cancer) in which the SP/NK-1 receptor system is involved. Thus, the antitumor action of NK-1 receptor antagonists should be addressed (see Muñoz and Coveñas 2013a). In fact, according to the data obtained from preclinical studies (see Muñoz and Coveñas 2013a for review), the use of these antagonists in oncology therapy is quite promising. It is known that (1) NK-1 receptor antagonists (e.g., aprepitant) exert an antitumor action against human cancer cells. This action depends on the concentration used; (2) These antagonists induce apoptosis in tumor cells; (3) NK-1 receptor antagonists inhibit the migratory activity of tumor cells; (4) NK-1 receptor antagonists exert antiangiogenic properties (see Muñoz and Coveñas 2013a for review). Moreover, it is known that malignant tissues express more NK-1 receptors than benign tissues, that tumor cells expressing the most malignant phenotypes show an increased percentage of NK-1 receptor expression, and that the NK-1 receptor is involved in the viability of tumor cells (see Muñoz and Coveñas 2013a for review). Aprepitant is an excellent candidate for testing its antitumor, antimigratory and antiangiogenic action in human clinical trials since a large part of the required safety and characterization studies for aprepitant have already been carried out (aprepitant is already available in clinical practice for the treatment of emesis) (see Muñoz and Coveñas 2013a). In an in vivo study, it has also been demonstrated that fosaprepitant significantly reduces the tumor volume of MG-63 human osteosarcoma xenografts (Muñoz et al. 2014).

It seems that by increasing the number of days on which aprepitant is administered and using higher doses of aprepitant than those used in CINV this NK-1 receptor antagonist could be effective in cancer (see Muñoz and Coveñas 2013a). These issues should be investigated in depth. By increasing the dose of aprepitant, higher and hitherto unreported side effects may occur, although it has been described that in patients with depression a dose of 300 mg/day of aprepitant is well tolerated and no significant difference in the frequency of adverse events is observed as compared with placebo (see Muñoz and Coveñas 2013a). Carcinogenicity studies have been carried for aprepitant and fosaprepitant in mice and in rats (see Muñoz and Coveñas 2013a for review). By extrapolating the concentrations of aprepitant used as an antitumor agent in in vitro studies, the doses of aprepitant for the possible treatment of cancer would be very low in comparison with carcinogenetic doses (40–50 mg/kg/day for cancer treatment versus 125–1,000 mg/kg/day for carcinogenesis).

It has also been reported that vestipitant, although well tolerated, either alone or in combination with paroxetine, was not effective in ameliorating tinnitus (see Muñoz and Coveñas 2013b). However, according to the results of a recent clinical pilot study, it seems that aprepitant decreases severe pruritus induced by biological cancer treatments. No adverse events related to aprepitant were observed (Santini et al. 2012) (Table 3). Moreover, aprepitant decreased the average daily number of micturitions in subjects with urge incontinence (Green et al. 2006) (Table 3). In this pilot study, aprepitant was overall well tolerated and adverse events were generally mild.

Elevated levels of SP have been reported in major depression, anxiety disorders, people attempting suicide, chronic pain, schizophrenia and fibromyalgia (see Ebner et al. 2009 for review). It has been reported that the NK-1 receptor antagonists L-759,274 and CP-122,721 (Table 1) decrease the symptoms of depression and that the antidepressant action of CP-122,721 is similar to that found for fluoxetine or paroxetine, but the NK-1 receptor antagonist showed fewer adverse side effects (see Ebner et al. 2009).

Moreover, it is known that the NK-1 receptor antagonist GR-205,171 alleviates anxious symptoms in patients with social phobia (Furmark et al. 2005) (Table 1). Several NK-1 receptor antagonists (e.g., casopitant, orvepitant, vestipitant, vofopitant) have also been tested in human clinical trials for the treatment of depression, anxiety disorders, post-traumatic stress disorder, panic disorder and schizophrenia (see Ebner et al. 2009 for review). In some trials, these antagonists exerted an anxiolytic or an antidepressant action and showed a low side effect profile.

More clinical trials are required to fully determine the efficacy and safety of other NK-1 receptor antagonists, such as L-759,274, CP-122,721, GW-679,769, TAK-637, orvepitant, rolapitant and serlopitant in diseases in which the SP/NK-1 receptor system is upregulated (see Muñoz and Coveñas 2013b). In sum, to date, we think that pharmacological therapy has not fully exploited the many possible therapies offered by NK-1 receptor antagonists. For example, the use of NK-1 receptor antagonists as an additional therapy in the treatment of depression and anxiety should be investigated in-depth (Ebner et al. 2009).

Conclusion

SP peptide is ubiquitous throughout the body, being present in organic fluids and tissues. SP is the natural ligand of the NK-1 receptor; after binding to this receptor, it regulates many pathophysiological functions, such as emotional behavior, stress, depression, anxiety, emesis, vomiting, migraine, alcohol addiction, seizures and neurodegeneration. SP has been also implicated in pain, inflammation, hepatitis, hepatotoxicity, cholestasis, pruritus, myocarditis, bronchiolitis, abortus, bacteria and viral infection (e.g., HIV infection) and it plays an important role in cancer. Thus, a profound knowledge of this system will be the key for an in-depth understanding and consequently a better handling of many human diseases. We suggest therapeutic interventions using NK-1 receptor antagonists in human pathologies in which the SP/NK-1 receptor system is upregulated (depression, cancer, neural degeneration, inflammatory bowel disease, viral infection and pruritus). Accordingly, in the future, the use of NK-1 receptor antagonists should be tested clinically in these pathologies.

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Conflict of interest USPTO Application no. 20090012086 "Use of non-peptide NK-1 receptor antagonists for the production of apoptosis in tumor cells" (Miguel Muñoz).

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