Amino Acids (2006) 31: 251–272
DOI 10.1007/s00726-006-0335-9

Amino Acids
Printed in Austria

The role of substance P in stress and anxiety responses

Review Article

K. Ebner and **N. Singewald**

Department of Pharmacology and Toxicology, Institute of Pharmacy, Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Innsbruck, Austria

Received December 8, 2005
Accepted February 21, 2006
Published online July 6, 2006;

Summary. Substance P (SP) is one of the most abundant peptides in the central nervous system and has been implicated in a variety of physiological and pathophysiological processes including stress regulation, as well as affective and anxiety-related behaviour. Consistent with these functions, SP and its preferred neurokinin 1 (NK1) receptor has been found within brain areas known to be involved in the regulation of stress and anxiety responses. Aversive and stressful stimuli have been shown repeatedly to change SP brain tissue content, as well as NK1 receptor binding. More recently it has been demonstrated that emotional stressors increase SP efflux in specific limbic structures such as amygdala and septum and that the magnitude of this effect depends on the severity of the stressor. Depending on the brain area, an increase in intracerebral SP concentration (mimicked by SP microinjection) produces mainly anxiogenic-like responses in various behavioural tasks. Based on findings that SP transmission is stimulated under stressful or anxiety-provoking situations it was hypothesised that blockade of NK1 receptors may attenuate stress responses and exert anxiolytic-like effects. Preclinical and clinical studies have found evidence in favour of such an assumption. The status of this research is reviewed here.

Keywords: Substance P – Tachykinin – Neurokinin – In vivo release – Anxiety – Depression – Antidepressant – Anxiolytic – Stress – HPA axis – NK1 receptor – NK1 receptor antagonist – Emotional behaviour – Microdialysis – Stress-related disorders

1. Introduction

SP is the most abundant neurokinin peptide in mammals and is widely distributed in the central, peripheral, and enteric nervous systems of many species (for review see Pernow, 1983; Severini et al., 2002). Together with neurokinin A, neurokinin B and the recently described hemokinin 1 (Zhang et al., 2000; Duffy et al., 2003) it belongs to a family of bioactive peptides that are defined by their common carboxy-terminal amino acid sequence. SP and neurokinin A are encoded by the preprotachykinin A (PPTA or Tac1) gene (Krase et al., 1994), which produces four splice variants; α- and δ-PPTA yield SP alone, whereas β- and γ-PPTA produce both SP and NKA (Nawa et al., 1983, 1984; Harmar et al., 1990; Severini et al., 2002). Neurokinin B and hemokinin 1/endokinins are generated from the PPTB and PPTC genes, respectively (Kotani et al., 1986; Zhang et al., 2000; Page, 2004). So far, three types of neurokinin receptors have been identified in mammals, NK1, NK2 and NK3 receptors (Regoli et al., 1994; Maggi, 1995). The NK1 and NK3 binding sites are widely distributed in the brain (Nakaya et al., 1994; Ding et al., 1996), while NK2 receptors are observed only in a few particular areas such as prefrontal cortex, hippocampus, septum and thalamus (Saffroy et al., 2003). Although all endogenous neurokinins can interact with all three receptor types, SP exhibits high affinity to the NK1 receptor, whereas NKA and NKB preferentially bind to the NK2 and NK3 receptors, respectively (Regoli et al., 1994; Maggi, 1995). However, it is important to keep in mind that endogenous neurokinins possess limited selectivity and that there is a notable cross talk among the three receptor types (Maggi and Schwartz, 1997). A further complication is the species-dependent variation of the amino acid sequence of the NK1 receptor protein (Fong et al., 1992; Sachais et al., 1993) as well as species differences in the receptor pharmacology (Beresford et al., 1991). Although these variations do not

affect the affinity of endogenous SP, however, they determine the species-related differences in the potency of non-peptide antagonists (Fong et al., 1992; Sachais et al., 1993), probably caused by different binding epitopes on the NK1 receptor for SP and antagonists (Gether et al., 1993).

SP has been proposed to function in the central and peripheral nervous systems as a neurotransmitter, co-transmitter, or neuromodulator (Otsuka and Yoshioka, 1993). In the central nervous system, SP has been implicated not only in the regulation of autonomic and physiological functions such as nociception and pain transmission, respiration, thermoregulation and cardiovascular control, but also in the modulation of many behavioural and cognitive functions including emotional and anxiety-related behaviour (for review see Otsuka and Yoshioka, 1993; Severini et al., 2002). The physiological and behavioural effects of SP are mediated by binding to the G-protein-coupled neurokinin receptors (Nakanishi, 1991; Maggi, 1995; Khawaja and Rogers, 1996) that activate several second messenger systems including the phospholipase C-inositol triphosphate-calcium signal transduction cascade, arachidonic acid mobilization via phospholipase A2 and cyclic adenosine monophosphate accumulation via adenylyl cyclase (Mitsuhashi et al., 1992; Nakajima et al., 1992; Takeda et al., 1992; Nakanishi et al., 1993; Seabrook and Fong, 1993; Garcia et al., 1994; Mochizuki-Oda et al., 1994; Quartara and Maggi, 1997). Moreover it has been shown in several cell types that SP activates p38 mitogen-activated protein kinase, NF-kappa B and other signal transduction pathways implicated in neurogenic inflammation (Fiebich et al., 2000; Lieb et al., 1997). The localisation of SP/NK1 receptor system in brain regions known to be involved in the regulation of stress and affective behaviour, as well as functional preclinical and clinical findings suggest this system as a new target in stress-related disorders including depression and anxiety disorders. Since neurokinins similar to other neuropeptides are thought to play a predominant role in pathological conditions, when these systems are strongly activated or upregulated (Hökfelt et al., 2000), the hypothesis is that neurokinin ligands (in particular antagonists) will cause less side effects as existing drugs used in the treatment of these disorders. Indeed, in studies revealing clinical efficacy of NK1 receptor antagonists in the treatment of depression and anxiety (Kramer et al., 1998; Kramer et al., 2004) less side effects of these drugs have been found compared to classical antidepressants such as paroxetine used as comparator drugs in these studies.

2. Distribution of SP and NK1 receptors in the mammalian brain

2.1 SP distribution

The first descriptions of SP distribution in the mammalian brain by detecting SP immunoreactivity are dating back to the 70 s (Hökfelt et al., 1975; Ljungdahl et al., 1978). These initial reports utilised antibodies raised against the carboxy-terminal sequence of the peptide, shared by all neurokinins, and thus would be expected to cross-react with the other neurokinins. Subsequent studies using antibodies that recognised only SP provided a comprehensive description of the localisation of SP-immunoreactive cell bodies, fibres and terminals in the mammalian central nervous system (Shults et al., 1984; Ribeiro-da-Silva and Hökfelt, 2000). High levels of SP immunoreactivity have been identified in areas known to be implicated in the modulation of stress and anxiety reactions such as the cingulate cortex, caudate putamen, nucleus accumbens, septum, hippocampus, amygdala, various hypothalamic areas as well as periaqueductal gray, dorsal raphe nucleus, locus coeruleus, parabrachial nuclei and in the nucleus of the tractus solitarius. In these regions, SP frequently coexists in the same neuron with other neurokinins and with 'classical' neurotransmitters such as dopamine, acetylcholine, serotonin, noradrenaline, GABA and glutamate or neuropeptides such as thyrotropin releasing hormone and enkephalin (Pickel et al., 1979; Hökfelt et al., 1982; Kachidian et al., 1991; Nicholas et al., 1992; Dean et al., 1993).

2.2 NK1 receptor distribution

The distribution of the SP-preferring NK1 receptors in the mammalian central nervous system has been investigated by autoradiography (Shults et al., 1982; Mantyh et al., 1984, 1989; Saffroy et al., 1988, 2003), by in situ hybridisation studying the expression of messenger ribonucleic acid mRNA encoding for the receptor (Maeno et al., 1993; Caberlotto et al., 2003) and by immunohistochemistry (Nakaya et al., 1994) providing evidence for a wide, but distinct distribution of NK1 receptors in the mammalian brain. Accordingly, NK1 receptors have been identified in brain areas involved in the control of anxiety and stress responses, such as the prefrontal cortex, hippocampus, caudate putamen, lateral septum, nucleus accumbens, amygdala and various hypothalamic nuclei, as well as periaqueductal gray, habenula, dorsal raphe and locus coeruleus. Although in most of these areas there is good accordance between the distribution of SP-containing fibers and NK1

receptors, an interesting aspect is an apparent mismatch between SP and NK1 receptors in some areas (Shults et al., 1984; Nakaya et al., 1994). Most notably, in the substantia nigra the concentration of SP is extremely high, while the expression of NK1 receptors is very low. Reasons for such mismatches could be technical factors or the existence of yet undiscovered subtypes of neurokinin receptors (Herkenham, 1987). Another explanation for the anatomical discontinuity between the sites of release and potential targets might be the widespread release and neuromodulatory capacity of neuropeptides. Compared to classical transmitters, neuropeptides can be released non-synaptically from multiple sites of the neuronal membrane (including dendrites) and seem to persist in the extracellular fluid for relatively long periods of time. Thus, they might be able to diffuse considerable distances and act on relatively distant targets (for review see Landgraf and Neumann, 2004).

3. In vivo release of SP in the brain

Since neuropeptides such as SP only become biologically active after their release into the extracellular space, attempts to measure intracerebral release have to focus on approaches that are able to reflect concentrations and their fluctuations in the extracellular fluid. The investigation of in vivo efflux of neuropeptides including SP is hampered by the low concentrations in the extracellular fluid, which

Table 1. Characterisation of in vivo SP release in the brain

Stimulus	Method	Brain area	Response	Change (%)	References
High K^+ stimulation					
K^+ (50 mM; 2×)	via MD	PAG	↑ SP	+263, 339	Rosen et al. (1995)
K^+ (50 mM)	via MD	PAG	↑ SP	+200	Rosen et al. (2004)
	via PPS	SN	↑ SP		Michelot et al. (1979)
	via PPS	CN	↑ SP		Michelot et al. (1979)
	via PPS	SN	↑ SP	+50	Nagashima et al. (1987)
K^+ (100 mM; 2×)	via MD	PAG	↑ SP	+300	Rosen et al. (2004)
K^+ (100 mM)	via PPS	MeA	↑ SP	+240	Ebner et al. (2004a)
	via PPS	CeA	↑ SP	+50	Ebner et al. (2004a)
	via MD	SN	↑ SP	+500	Lindefors et al. (1989a)
	via MD	Striatum	↑ SP	+100	Lindefors et al. (1989b)
	via MD	SN	↑ SP	+450	Lindefors et al. (1989b)
	via MD	CN	↑ SP	+400	Brodin et al. (1983)
	via MD	PAG	↑ SP	+135	Stiller et al. (1995)
K^+ (150 mM)	via MD	PAG	↑ SP	+400	Rosen et al. (2004)
	via MD	NTS	↑ SP	+475	Potts and Fuchs (2001)
	via MD	NTS	↑ SP	+475	Potts et al. (1999)
	via MD	NTS	↑ SP	+330	Morilak et al. (1988)
	via MD	Striatum	↑ SP	+260	Lindefors et al. (1985)
K^+ (200 mM)	via MD	CN	↑ SP	+1600	Brodin et al. (1983)
Modulation of K^+ stimulated SP release					
Haloperidol	MD	SN	↓ SP		Lindefors et al. (1989b)
6-OHDA-lesion	MD	SN	↓ SP		Lindefors et al. (1989b)
Ca^{2+}-free medium					
Ca^{2+}-free + Mg (12 mM)	via MD	PAG	↓ SP		Rosen et al. (2004)
	via MD	PAG	↓ SP	−53	Stiller et al. (1995)
Pharmacological					
NMDA (50 μM)	via MD	MeA	↑ SP	+220	Ebner and Singewald (2005)
Capsaicin (33 μM)	via PPS	SN	↑ SP		Nagashima et al. (1987)
Apomorphine	via MD	NTS	↓ SP		Srinivasan et al. (1991)
Apomorphine (systemic)	MD	SN	↑ SP	+60–100	Orosz and Bennett (1990)
6-OHDA-lesion	MD	SN	↑ SP	+60	Orosz and Bennett (1990)
Estradiol treatment	PPS	POAH	↑ SP		Jarry et al. (1988)

CeA central amygdala; *CN* caudate nucleus; *MD* microdialysis; *MeA* medial amygdala; *NTS* nucleus tractus solitarius; *PAG* periaqueductal gray; *PBN* parabrachial nucleus; *POAH* preoptic anterior hypothalamus; *PPS* push-pull superfusion; *SN* substantia nigra; ↑ increase; ↓ decrease

are in the pM range (e.g. SP: 20–30 pM; Saleh, 1997; Ebner et al., 2004a) depending on the studied brain area. Compared with monoamines (nM range) or amino acids (μM range) the determination of neurokinins for example in microdialysates or push-pull superfusates is particularly challenging and needs extremely sensitive analytical procedures (Brodin et al., 1983; Lindefors et al., 1987). This is one reason why the tissue content of neurokinins or indirect methods such as receptor internalization has been widely used to estimate the release of neurokinins after challenges including aversive and stressful life experiences (for review see Herpfer and Lieb, 2005). However, these methods are not suitable to reliably gain information on SP transmission, since measurements of the tissue content reflects rather intracellular than extracellular concentrations and depends on various variables such as synthesis capacities, transport, storage and degradation of the neuropeptide. Therefore, for example reduced tissue measurements of SP after stimulation have been interpreted to represent either enhanced (Takayama et al., 1986) or reduced release (Siegel et al., 1987). As well, receptor internalization can only be seen as a crude, indirect measure of release prone to deliver false positive findings given that not only SP but also N-terminal SP fragments induce NK1 receptor internalization (Michael-Titus et al., 1999), despite very low affinity to NK1 receptors (Hanley et al., 1981; Igwe et al., 1990). Although these methods have provided important preliminary evidence in which brain areas SP transmission may be critical in particular situations they do not provide information on temporal dynamics of release which can be detected by using in vivo perfusion techniques. Push-pull superfusion and microdialysis have been successfully used in conjunction with highly sensitive radioimmunoassays for the determination of in vivo efflux of SP predominantly in the hindbrain/spinal cord, but more recently also in forebrain areas (Tables 1 and 2). For the use of microdialysis approaches, the recovery of neuropeptides is an important issue, which can vary in dependence on various factors such as flow rate or length and material of the dialysis membrane used (Kendrick, 1990; Ebner and Singewald, unpublished data). In our experiments cuprophan and hemophan membranes have been proved to be suitable for the determination of extracellular SP efflux in distinct forbrain areas. In our laboratory the SP concentration in microdialysates is assessed by radioimmunoassay using specific and highly sensitive antisera which provided a detection limit of approximately 0.3 fmol/sample (Ebner et al., 2004a). As mentioned before the sensitivity of the antibody is the critical component of the radioimmunolog-

Table 2. Changes in the central release of SP in response to various stimuli

Stimulus	Method	Brain area	Response	Change (%)	References
Stressors					
Immobilization	PPS	MeA	↑ SP	+150	Ebner et al. (2004a)
	PPS	CeA	≈ SP		Ebner et al. (2004a)
Elevated platform	MD	MeA	↑ SP	+40	Ebner et al. (2004a)
Swim stress	MD	LS	↑ SP	+90	Ebner et al. (2004b)
	MD	MeA	↑ SP	+200	Ebner and Singewald (2005)
Cold stress	MD	PAG	↑ SP	+202	Xin et al. (1997)
	MD	POAH	≈ SP		Xin et al. (1997)
Hypoxia	MD	NTS	↑ SP	+60	Srinivasan et al. (1991)
	MD	NTS	↑ SP	+150	Lindefors et al. (1986)
Modulation of stress-induced SP release					
PL017 (μ-agonist)	via MD	PAG	↓ SP		Xin et al. (1997)
Dynorphin (κ-agonist)	via MD	PAG	↓ SP		Xin et al. (1997)
Other stimuli					
Barosensitive afferent activation	MD	NTS	↑ SP	+16–39	Potts and Fuchs (2001)
Skeletal muscle contraction	MD	NTS	↑ SP	+150	Potts et al. (1999)
Aortic balloon inflation	MD	NTS	↑ SP	+37	Potts et al. (1999)
Aortic depressor nerve stimulation	MD	NTS	↑ SP	+175	Morilak et al. (1988)
Spinal cord stimulation	MD	PAG	≈ SP		Stiller et al. (1995)
Vagotomy	MD	NTS	↑ SP	+70	Lindefors et al. (1986)
Vagal stimulation	PPS	PBN	↑ SP	+100	Saleh (1997)

CeA central amygdala; *LS* lateral septum; *MD* microdialysis; *MeA* medial amygdala; *NTS* nucleus tractus solitarius; *PAG* periaqueductal gray; *PBN* parabrachial nucleus; *POAH* preoptic anterior hypothalamus; *PPS* push-pull superfusion; ↑ increase; ↓ decrease; ≈ no change

ical measurement. We found that the antibodies RD2 (donated by S. Leeman, Boston University School of Medicine, USA) and SP-2 (Euro-Diagnostica, Arnhem, The Netherlands) have sufficient sensitivity to detect even basal extracellular levels of SP in several forbrain and brainstem areas including amygdala, septum and locus coeruleus (Ebner et al., 2004a, b; Ebner and Singewald, unpublished data) and do not cross-react with other structurally related mammalian tachykinins. Using such approaches, changes in extracellular SP levels in various brain areas in response to different manipulations and stimuli were dectected (Tables 1 and 2). For example, it has been shown that the SP efflux in several brain areas is enhanced in response to neuronal depolarization induced by high potassium (Table 1). This evoked release can be modulated, e.g. by application of haloperidol, which reduces potassium-stimulated SP release in the substantia nigra (Lindefors et al., 1989b). Conversely, microdialysis with Ca^{2+} free perfusion medium reduced basal and potassium-evoked SP release (Stiller et al., 1995; Rosen et al., 2004) suggesting that the SP measured is of neuronal origin (Table 1). This idea has been further supported by in vitro studies showing that the depolarization-induced SP release was blocked by tetrodotoxin a voltage-dependent sodium channel inhibitor (Jessell, 1978; Torrens et al., 1981). Finally, it has been demonstrated that the in vivo efflux of SP is sensitive to pharmacological intervention including dopaminergic and glutamatergic pathways (Table 1) and is released after stimulation by different challenges including barosensitive nerve stimulation (Table 1) or exposure to aversive and stressful situations (see below and Table 2).

4. The role of the SP/NK1 receptor system in the modulation of stress

There is evidence that SP modulates physiological and behavioural stress responses in the brain. In conscious rats, SP administered centrally induces a pattern of cardiovascular and behavioural responses which closely resemble the responses to stressful stimuli (Unger et al., 1988; Culman and Unger, 1995). Further evidence corroborating the role of SP in stress mechanisms comes from studies investigating the neuronal activation in brain areas known to be implicated in the modulation of stress reactions in response to various aversive stimuli. Pharmacological blockade or genetic deletion of NK1 receptors has been found to attenuate stress-induced Fos expression (as marker for neuronal activation) in brain areas, such as prefrontal cortex, locus coeruleus, periaqueductal gray as well as some hypothalamic nuclei including the paraventricular nucleus (PVN) (Hahn and Bannon, 1999; Baulmann et al., 2000; Santarelli et al., 2002; Muigg et al., 2005; Ebner et al., unpublished data). However, if endogenous SP indeed is involved in the modulation of stress reactions including anxiety (see Section 6), it should be released centrally in response to different stressors. Until recently, information on stress-induced effects on intracerebral SP levels was mainly obtained from brain tissue measurements. Exposure to a variety of emotional, physical and painful stressors caused altered SP tissue levels or SP immunoreactivity in various brain regions. For example, increased SP concentrations were found in the septum, dentate gyrus after foot shock (Siegel et al., 1984) and in the periaqueductal gray after restraint and isolation stress (Rosen et al., 1992; Brodin et al., 1994a). Other reported increased SP concentrations in distinct hypothalamic areas after chronic adjuvant-induced arthritis (Chowdrey et al., 1995) and in the nucleus accumbens and amygdala after whole body vibration (Nakamura et al., 1990). However, also decreases in SP tissue content were observed (Lisoprawski et al., 1981; Takayama et al., 1986; Siegel et al., 1987; Nakamura et al., 1990), questioning the reliability of SP tissue content measurements to evaluate the sensitivity of SP neurotransmission to stressors (see also Section 3). In addition, internalization of NK1 receptors reflecting previous SP binding and receptor endocytosis has been observed in the amygdala following maternal separation in guinea-pig pups (Kramer et al., 1998; Boyce et al., 2001; Steinberg et al., 2002) and after immobilisation stress in gerbils (Smith et al., 1999). In this context, it seems that the degree of NK1 receptor internalization is proportional to the intensity of a noxious or stressful/aversive stimulus, which was demonstrated for thermal stimuli (Allen et al., 1997). However, although increased stress-induced receptor internalization is interpreted as increased local SP release this kind of measurement does not reflect the temporal dynamics of SP neurotransmission. Thus, these stress-induced changes both from tissue measurements and receptor internalization should be interpreted with caution (see Section 3). Using in vivo sampling methods it could be demonstrated that various stressors increase in vivo SP efflux (which is a direct and dynamic marker of SP neurotransmission) in discrete brain regions including areas known to be implicated in the generation of stress reactions (Table 2). By using specific small sized microdialysis probes and micro-push-pull cannulae constructed in our laboratory which allowed us to perfuse even subregions of the amygdala complex we found stress-induced increase of SP release in

the medial but not in the central amygdala (Ebner et al., 2004a), as well as in the lateral septum (Ebner et al., 2004b). Our finding of high extracellular SP levels in the medial amygdala is consistent with immunohistochemical studies demonstrating a dense plexus of SP containing cell bodies and terminals in this brain area (Roberts et al., 1982; Ribeiro-da-Silva and Hökfelt, 2000). Interestingly, the enhanced SP release in the medial amygdala seems considerably more pronounced and prolonged after a severe emotional stressor (immobilization) than in response to a rather mild stressor (elevated platform exposure), which has been shown to elicit only a moderate neuroendocrine stress response (Neumann et al., 2000). In a very recent study we found that basal and stress-induced release of SP in the medial amygdala is regulated differently by NK1 receptors (Ebner and Singewald, 2005). Under basal conditions endogenous SP can serve as a signal that tonically inhibits its own release via a NK1 receptor mediated negative feedback action, while under stress conditions SP release is further potentiated by activation of NK1 receptors, leading to high levels of SP which are then likely to activate further receptors in addition to NK1 (e.g. NK2 and NK3 receptors). Further studies should clarify whether and how other neurokinin receptors are involved in this mechanism.

It is further proposed that a specific genetic variability of the NK1 receptor has been selectively produced over the years in rats bred for extremes in high anxiety-related behaviour (HAB), an established psychopathological animal model of trait anxiety/depression (Landgraf and Wigger, 2002) suggesting that changes in NK1 receptors may contribute to their enhanced depression and anxiety-related behaviour. Indeed, preliminary molecular genetic approaches identified strict line polymorphisms on the NK1 receptor gene located on chromosome 4 (Landgraf and Wigger, 2003). In a very recent study we found differences in stress-related SP neurotransmission in HAB animals. Compared to their low anxiety (LAB) counterparts, HAB rats show a higher increase in the stress-induced SP release within the medial amygdala, whereas basal release of the neuropeptide as well as PPTA mRNA expression within this area did not differ between the two lines (Sartori et al., 2005). Thus, these data suggest a hyperactive SP neurotransmission in these high anxiety rats after exposure to an aversive stimulus which would be in line with clinical evidence of disturbed SP neurotransmission in patients with stress-related diseases such as depression and anxiety disorders (for review see Herpfer and Lieb, 2005). Beside genetic predisposition also adverse early life experiences have been shown to be linked to increased stress vulnerability. However, preliminary studies using maternal separation of neonate rats have revealed neither differences in PPTA mRNA levels (Stout et al., 2001) nor extracellular SP levels in the medial amygdala (Ebner et al., unpublished data), indicating that maternal separation may not be sufficient to induce changes in SP synthesis/release in adult animals.

In addition to changes in SP release, it has been shown that immobilization stress also leads to a decrease in the number of NK1 receptors in the amygdala (Takayama et al., 1986), which may represent endocytosis as the NK1 receptor has been shown to undergo internalization following receptor stimulation (Mantyh et al., 1995). Although most studies have found a decrease in receptor binding or receptor expression after stress exposure in brain areas implicated in stress processing such as in the amygdala and hippocampus (Takayama et al., 1986; Duric and McCarson, 2005) there is also evidence of opposite long term effects of stress exposure. Hwang et al. (2005) have shown that a 2 h restraint stress increases SP receptor binding in the central amygdala, 24 h and 48 h following stress exposure. Thus, it it likely that the duration of stress exposure and/or the severity of the stressor is an important factor for the observed effects on the SP transmission, at least in the amygdala. However, within the hippocampus both acute and chronic immobilization stress resulted in a down-regulation of the expression of the NK1 receptor gene (Duric and McCarson, 2005). Further studies should clarify whether repeated stress exposure causes enhanced SP transmission, contributing to deleterious effects of chronic stress. Supporting such a suggestion, it was observed that chronic mild stress causes elevated SP mRNA levels in the medial amygdala and parts of the hypothalamus (lateral, dorso- and ventromedial) (Sergeyev et al., 2005), and that NK1 receptor antagonist treatment can attenuate the chronic psychosocial stress-induced decrease in cell proliferation and hippocampal volume in subordinate tree shrews (van der Hart et al., 2002; Czeh et al., 2005a, b).

5. The role of the SP/NK1 receptor system in the regulation of the HPA axis

Hypersecretion of the stress hormones adrenocorticotrophin (ACTH) and cortisol is a common endocrine abnormality in stress-related disorders such as depression (Holsboer and Barden, 1996). There is growing evidence that SP regulates cortisol secretion through actions in the hypothalamus and the adrenal glands, and its effects are dependent on the site of action. Many hypothalamic nuclei

including the PVN are heavily innervated with SP-containing nerve fibres (Bittencourt et al., 1991; Larsen, 1992; Kawano and Masuko, 1995; Kang et al., 1999) and also NK1 receptors are present there (Mantyh et al., 1989; Quartara and Maggi, 1998). Thus, there is neuroanatomical potential and functional evidence for a central regulatory action of SP on the hypothalamo-pituitary-adrenal (HPA) axis activity. So far, most studies investigating the role of SP in HPA axis regulation were performed under basal conditions, while the modulatory capacity of endogenous SP under stress conditions is less well studied. Under basal conditions, intracerebroventricular injection of SP in rats has been reported to decrease plasma levels of ACTH (Jones et al., 1978; Chowdrey et al., 1990). This effect appears to be mediated via inhibition of the release of corticotrophin-releasing factor (CRF) from the hypothalamus, since SP inhibited the release of CRF from rat hypothalamic, but not from isolated median eminence tissue in vitro (Faria et al., 1991). Furthermore, administration of a NK1 receptor antagonist has been found to increase both plasma ACTH and corticosterone concentrations as well as CRF mRNA transcription in the parvocellular subdivision of the PVN in conscious, unstressed rats (Larsen et al., 1993; Jessop et al., 2000). In contrast, central injection of the NK1 receptor antagonist RP-67580 did not increase the magnitude of the ACTH and corticosterone response to restraint stress, but did maintain stress-elevated hormone levels (Jessop et al., 2000). Although these reports are consistent with the interpretation that SP exerts a central inhibitory influence on ACTH and glucocorticoid secretion at least under basal conditions, they have some limitations, such as the use of antagonists which were either rather unspecific for the NK1 receptor or are known to exert additional unspecific pharmacological effects such as the blockade of calcium channels (Rupniak et al., 1993). Along these lines, results obtained in genetically modified mice lacking NK1 receptors (NK1R$-/-$) do not appear to be consistent with the proposed inhibitory role of endogenous SP on HPA axis stress responses. Basal plasma levels of corticosterone in NK1R$-/-$ mice did not differ from those in wildtype mice, and were lower in NK1R$-/-$ mice subjected to the psychological stressor of elevated plus-maze exposure (Santarelli et al., 2001). Reduced stress hormone secretion is consistent with the less anxious phenotype of NK1R$-/-$ mice (Rupniak et al., 2001; Santarelli et al., 2001). Furthermore, as described before the stress-induced Fos-expression in the PVN was lower in NK1R$-/-$ mice than in wildtypes (Santarelli et al., 2002), as well as in rats after pharmacological blockade of NK1 receptors (Muigg et al., 2005; Ebner et al. unpublished data). Although further research is needed to study the role of the SP/NK1 receptor system on HPA axis activity especially under stress conditions, most of these data point rather to a facilitatory than inhibitory role of endogenous SP on stress-induced HPA axis activity. In the future it would be interesting to clarify in which brain areas and via which pathways the SP/NK1 NK1 receptor system modulates HPA axis responses to stress.

6. Behavioural effects of SP and NK1 receptor related ligands in animal models of anxiety and depression

6.1 SP and related NK1 receptor agonists

Intracerebral injection of SP or NK1 receptor agonists has been reported to elicit anxiogenic-like effects in different behavioural tests (Table 3). These include the elevated plus-maze (Teixeira et al., 1996, 2004; Aguiar and Brandao, 1996; Gavioli et al., 1999; De Araujo et al., 1999; Baretta et al., 2001; Duarte et al., 2004; Ebner et al., 2004a), conditioned place aversion (Elliott, 1988; Aguiar and Brandao, 1994), potentiation of the acoustic startle response (Krase et al., 1994), passive avoidance (Lenard and Kertes, 2002) and distress vocalisations (Kramer et al., 1998; Rupniak et al., 2000). However, the effects of SP on anxiety and fear-related behaviours in rodents seem to depend on both brain region and dose administered (Table 3). For instance, SP injections into the dorsal periaqueductal gray, lateral septal nucleus and medial amygdala, was reported to elicit anxiogenic action (Aguiar and Brandao, 1996; Gavioli et al., 1999; De Araujo et al., 1999; Ebner et al., 2004a), whereas injections into the nucleus basalis magnocellularis of rats has been shown to exert anxiolytic-like effects in the elevated plus-maze test (Hasenöhrl et al., 1998; Nikolaus et al., 1999a). Moreover, studies have also indicated that distinct amino (N)- and carboxy (C)-terminal SP-fragments have different effects on anxiety-related behaviours depending on the brain site injected. For example, the C-terminal fragment SP 7–11 injected into the dorsal periaqueductal gray produced an anxiogenic effect (De Araujo et al., 1999, 2001a, b), while the N-terminal fragment SP 1-7, which has little affinity for the NK1 receptor, elicits opposite effects (De Araujo et al., 2001b). On the other hand, both C- and N-terminal fragments had anxiolytic effects when infused into the ventral pallidal region (Nikolaus et al., 2000). Table 3 summarises effects of administration of

Table 3. Behavioural effects of intracerebral injected SP and related NK1 receptor agonists in animal models of anxiety and depression

Substance	Brain area	Species	Models	Effects	References
Substance P					
SP	PAG	Rats	Place conditioning	Aversive	Aguiar and Brandao (1994)
	PAG	Rats	Elevated plus-maze	Anxiogenic	Aguiar and Brandao (1996), De Araujo et al. (1999)
	MeA	Rats	Elevated plus-maze	Anxiogenic	Ebner et al. (2004a)
	BLA	Rats	Passive avoidance	Aversive	Lenard and Kertes (2002)
	LS	Rats	Elevated plus-maze	Anxiogenic	Gavioli et al. (1999)
	PnC	Rats	Acoustic startle response	Anxiogenic	Krase et al. (1994)
	NbasVP	Rats	Elevated plus-maze	Anxiolytic	Hasenöhrl et al. (1998), Nikolaus et al. (1999a, 2000)
	NbasVP	Rats	Social interaction	Anxiolytic	Hasenöhrl et al. (1998)
	NbasVP	Rats	Place conditioning	Reinforcing place preference	Nikolaus et al. (1999b)
	ICV	Rats	Elevated plus-maze	Anxiogenic	Duarte et al. (2004), Gavioli et al. (1999, 2002)
	ICV	Mice	Elevated plus-maze	Anxiogenic	Baretta et al. (2001), Ribeiro and De Lima (2002), Teixeira et al. (1996), Teixeira and De Lima (2003), Teixeira et al. (2004)
	ICV	Gerbils	Hind foot tapping	Anxiogenic	Duffy et al. (2003)
SP-analogues					
GR73632	MH	Cats	Defensive rage	Aversive	Bhatt et al. (2003)
	PAG	Cats	Defensive rage	Aversive	Gregg and Siegel (2003)
	ICV	Gerbils	Hind foot tapping	Anxiogenic	Ballard et al. (2001), Cheeta et al. (2001), Duffy et al. (2002, 2003), Rupniak and Williams (1994), Rupniak et al. (2001, 2003a, b)
	ICV	Guinea pigs	Neonatal vocalisation	Aversive	Kramer et al. (1998), Rupniak et al. (2000)
DiMethyl-C7	ICV	Rats	Place conditioning	Aversive	Elliott (1988)
	NbasVP	Rats	Place conditioning	Reinforcing place preference	Nikolaus et al. (1999b)
SP methyl ester	ICV	Mice	Elevated plus-maze	Anxiogenic	Teixeira et al. (1996)
SP free acid	ICV	Rats	Elevated plus-maze	Anxiogenic	Duarte et al. (2004)
SP-fragments					
SP-C-fragment (7–11)	PAG	Rats	Elevated plus-maze	Anxiogenic	De Araujo et al. (1999)
SP-C-fragment (7–11)	PAG	Rats	Place conditioning	Aversive	De Araujo et al. (2001a)
SP-C-fragment (7–11)	NbasVP	Rats	Elevated plus-maze	Anxiolytic	Nikolaus et al. (2000)
SP-C-fragment (6–11)	PAG	Rats	Elevated plus-maze	Anxiogenic	De Araujo et al. (2001b)
SP-C-fragment (6–11)	ICV	Rats	Elevated plus-maze	Anxiogenic	Duarte et al. (2004)
SP-N-fragment (1–7)	PAG	Rats	Elevated plus-maze	Anxiolytic	De Araujo et al. (2001b)
SP-N-fragment (1–7)	NbasVP	Rats	Elevated plus-maze	Anxiolytic	Nikolaus et al. (2000)

BLA basolateral amygdala; *ICV* intracerebroventricular; *LS* lateral septum; *MeA* medial amygdala; *MH* medial hypothalamus; *NbasVP* nucleus basalis magnocellularis of ventral pallidum; *PAG* periaqueductal gray; *PnC* pontine reticular nucleus

SP or SP-like compounds given either intracerobroventricularly or locally into distinct brain areas.

6.2 NK1 receptor antagonists

Since the discovery of the first nonpeptide NK1 receptor antagonist, CP-96,345 (Snider et al., 1991), several groups have produced structurally diverse, highly selective antagonists. This created the opportunity to investigate whether selective blockade of central NK1 receptors is capable of modifying stress and anxiety/fear-related behaviour in preclinical studies. Table 4 gives an overview of the behavioural effects of systemic and intracerebral administered NK1 receptor antagonists in animal models of anxiety and depression. For example, the NK1 receptor antagonists, FK-888 and NKP-608 were shown to have anxiolytic-like effects in the mouse and rat elevated plus-maze test, respectively (Teixeira et al.,

Table 4. Behavioural effects of systemic or intracerebral injected NK1 receptor antagonists in animal models of anxiety and depression

Drug	Species	Site	Models	Effects	References
MK-869	Gerbils	i.p.	Hind foot tapping	Inhibited footshock-induced foot tapping	Ballard et al. (2001)
	Gerbils	p.o.	Hind foot tapping	Reduced NK1-agonist induced foot tapping	Duffy et al. (2002)
	Gerbils	p.o.	Tail suspension	Antidepressant	Varty et al. (2003)
	Gerbils	p.o.	Elevated plus-maze	Anxiolytic	Varty et al. (2002)
	Guinea pigs	p.o.	Maternal separation	Anxiolytic	Kramer et al. (1998)
CP-96,345	Rats	ICV	Noxious stress	Reduced behavioural stress responses	Culman et al. (1997)
	Rats	i.p.	Forced swim	Antidepressant	Dableh et al. (2005)
	Rats	PnC	Acoustic startle response	Anxiolytic	Krase et al. (1994)
	Mice	i.p.	Light/dark box	Anxiolytic (but also sedation effect)	Zernig et al. (1992)
CP-99,994	Rats	PnC	Acoustic startle response	Anxiolytic	Krase et al. (1994)
	Gerbils	i.p.	Hind foot tapping	Inhibited footshock-induced foot tapping	Ballard et al. (2001)
	Gerbils	p.o.	Hind foot tapping	Reduced NK1-agonist induced foot tapping	Duffy et al. (2002)
	Gerbils	i.v.	Hind foot tapping	Inhibited NK1-agonist induced foot tapping	Rupniak and Williams (1994)
	Gerbils	p.o.	Elevated plus-maze	weak anxiolytic effect	Varty et al. (2002)
	Gerbils	p.o.	Tail suspension	Antidepressant	Varty et al. (2003)
	Guinea pigs	s.c.	Neonatal vocalisation	Blocked NK1-agonist induced vocalisation	Rupniak et al. (2000)
CP-122,721	Gerbils	p.o.	Tail suspension	Antidepressant	Varty et al. (2003)
	Gerbils	p.o.	Elevated plus-maze	Anxiolytic	Varty et al. (2002)
	Gerbils	p.o.	Hind foot tapping	Reduced NK1-agonist induced foot tapping	Duffy et al. (2002)
CGP-49,823	Rats	p.o.	Social interaction	Anxiolytic	File (1997)
	Guinea pigs	s.c.	Maternal separation	No effect	Rupniak et al. (2000)
FK-888	Rats	ICV	Elevated plus-maze	No effect (but inhibited SP induced anxiogenic effect)	Duarte et al. (2004)
	Rats	LS	Elevated plus-maze	No effect (but blocked NK1-agonist induced anxiogenic effects)	Gavioli et al. (2002)
	Mice	ICV	Elevated plus-maze	Anxiolytic	Teixeira et al. (1996), Teixeira and De Lima (2003)
	Mice	ICV	Elevated plus-maze	No effect (but blocked PTZ-induced anxiogenic effects)	Ribeiro and De Lima (2002)
GR-82334	Cats	MH	Defensive rage	No effect (but blocked NK1 agonist-induced facilitation effect on the defensive rage)	Bhatt et al. (2003)
	Cats	PAG	Defensive rage	No effect (but reduced the NK1 agonist induced facilitation effect on the defensive rage)	Gregg and Siegel (2003)
GR-205,171	Rats	s.c.	Operant conflict paradigm	No effect	Loiseau et al. (2003)
	Rats	s.c.	T-maze	Antidepressant	Loiseau et al. (2005)
	Rats	s.c.	Elevated plus-maze	No effect	Rupniak et al. (2001)
	Rat	s.c.	Maternal separation	Anxiolytic	Rupniak et al. (2003b)
	Mice	s.c.	Matenal separation	Anxiolytic	Rupniak et al. (2000)
	Mice	s.c.	Forced swim	Antidepressant	Rupniak et al. (2001)
	Mice	s.c.	Tail suspension	No effect	Rupniak et al. (2001)
	Mice	s.c.	Elevated plus-maze	No effect	Rupniak et al. (2001)
	Mice	i.p.	Forced swim	Antidepressant	Zocchi et al. (2003)
	Gerbils	p.o.	Hind foot tapping	Reduced NK1-agonist induced foot tapping	Duffy et al. (2002)
	Guinea pigs	s.c.	Matenal separation	Anxiolytic	Rupniak et al. (2000)
L-733,060	Gerbils	p.o.	Hind foot tapping	Reduced NK1-agonist induced foot tapping	Duffy et al. (2002)
	Gerbils	p.o.	Elevated plus-maze	Anxiolytic	Varty et al. (2002)
	Gerbils	p.o.	Tail suspension	Antidepressant	Varty et al. (2003)

(*continued*)

Table 4 (*continued*)

Drug	Species	Site	Models	Effects	References
	Guinea pigs	s.c.	Maternal separation	Anxiolytic	Kramer et al. (1998), Rupniak et al. (2000)
	Guinea pigs	s.c.	Neonatal vocalisation	Blocked NK1-agonist induced vocalisation	Rupniak et al. (2000)
L-742,694	Gerbils	p.o.	Hind foot tapping	Reduced NK1-agonist induced foot tapping	Duffy et al. (2002)
	Gerbils	p.o.	Elevated plus-maze	Anxiolytic	Varty et al. (2002)
	Gerbils	p.o.	Tail suspension	Antidepressant	Varty et al. (2003)
L-743,310	Gerbils	i.v.	Hind foot tapping	No effect	Rupniak and Williams (1994)
L-760,735	Gerbils	i.p.	Social interaction	Anxiolytic	Cheeta et al. (2001)
	Gerbils	p.o.	Hind foot tapping	Reduced NK1-agonist induced foot tapping	Duffy et al. (2002)
	Gerbils	s.c.	Hind foot tapping	Inhibited NK1-agonist induced foot tapping	Rupniak et al. (2001)
	Gerbils	s.c.	Tail suspension	No effect	Rupniak et al. (2001)
	Gerbils	s.c.	Elevated plus-maze	No effect	Rupniak et al. (2001)
	Gerbils	i.p.	Hind foot tapping	Inhibited NK1-agonist induced foot tapping	Rupniak et al. (2003a)
	Gerbils	i.p.	Fear conditioning	Anxiolytic	Rupniak et al. (2003a)
	Guinea pigs	AMY	Maternal separation	Anxiolytic	Boyce et al. (2001)
	Guinea pigs	s.c.	Maternal separation	Anxiolytic	Kramer et al. (1998)
	Guinea pigs	s.c.	Tail suspension	No effect	Rupniak et al. (2001)
	Guinea pigs	s.c.	Elevated plus-maze	No effect	Rupniak et al. (2001)
	Tree shrews	p.o.[a]	Chronic social stress	Antidepressant	Van der Hart et al. (2005)
L-822,429	Rats	MeA	Elevated plus-maze	Anxiolytic	Ebner et al. (2004a)
	Rats	LS	Forced swim	Antidepressant	Ebner et al. (2004b)
	Rats	s.c.	Operant conflict paradigm	No effect	Loiseau et al. (2003)
	Rats (HAB)	i.p.	Forced swim	Antidepressant	Sartori et al. (2005)
LY-303,870	Guinea pigs	s.c.	Maternal separation	No effect	Rupniak et al. (2000)
NKP-608	Rats	p.o.	Social interaction	Anxiolytic	File (2000)
	Rats	p.o.[a]	Chronic mild stress	Antidepressant	Papp et al. (2000)
	Rats	p.o.[a]	Social interaction	Anxiolytic	Vassout et al. (2000)
	Rats	p.o.[a]	Social exploration	Anxiolytic	Vassout et al. (2000)
	Rats	p.o.	Elevated plus-maze	Anxiolytic	Vendruscolo et al. (2003)
	Rats	p.o.	Open field	Anxiolytic	Vendruscolo et al. (2003)
	Mice	p.o.	Elevated plus-maze	weak anxiolytic	Rodgers et al. (2004)
	Mice	p.o.	Stress-induced hyperthermia	Anxiolytic	Spooren et al. (2002)
	Gerbils	p.o.	Social interaction	Anxiolytic	Gentsch et al. (2002)
	Gerbils	p.o.[a]	Hind foot tapping	Inhibited NK1-agonist induced foot tapping	Vassout et al. (2000)
RP-67580	Rats	ICV	Noxious stress	Reduced behavioural stress responses	Culman et al. (1997)
	Rats	i.p.	Stress-induced defecation	Anxiolytic	Ikeda et al. (1995)
	Rats	s.c.	Operant conflict paradigm	No effect	Loiseau et al. (2003)
	Mice	i.p.	Elevated plus-maze	Anxiolytic	Santarelli et al. (2001)
	Mice	i.p.	Maternal separation	Anxiolytic	Santarelli et al. (2001)
SLV-323	Tree shrews	p.o.[a]	Chronic social stress	Antidepressant (not sign.)	Czeh et al. (2005a)
SSR-240,600	Guinea pigs	i.p. or p.o.	Maternal separation	Anxiolytic	Steinberg et al. (2002)
WIN-51,708	Rats	i.p.	Place conditioning	No effect (but blocked NK1-agonist induced aversive effects)	De Araujo et al. (2001a)
	Rats	i.p.	Elevated plus-maze	No effect (but blocked NK1-agonist induced anxiogenic effects)	De Araujo et al. (2001b)
	Rats	i.p.	Place conditioning	No effect (but reversed SP induced place preference)	Nikolaus et al. (1999a)
	Rats	i.p.	Elevated plus-maze	No effect (but blocked SP induced anxiolytic effects)	Nikolaus et al. (1999b)

AMY amygdala; *ICV* intracerebroventricular; *LS* lateral septum; *MeA* medial amygdala; *MH* medial hypothalamus; *PAG* periaqueductal gray; *PnC* pontine reticular nucleus; a subchronic administration (7 days); *i.p.* intraperitoneal; *i.v.* intraveneous; *p.o.* per os; *s.c.* subcutaneous; [a] chronic or subchronic oral administration

1996; Vendruscolo et al., 2003; Rodgers et al., 2004). Moreover, some studies have described anxiolytic-like effects of NKP-608 and CGP-49,823 in the rat social interaction test (File, 1997, 2000; Vassout et al., 2000). Antidepressant-like effects of NKP-608 were reported in the rat chronic mild stress paradigm (Papp et al., 2000) and of CP-96,345 and GR-205,171 in the mouse and rat forced swim test (Zocchi et al., 2003; Dableh et al., 2005). Interestingly, acute systemic application of a selective NK1 receptor antagonist reduced immobility time in HAB rats in the forced swim test indicating an antidepressant-like effect in these high anxiety/depression rats (Sartori et al., 2005), which was previously also found after chronic paroxetine treatment (Keck et al., 2003). One of the major obstacles in behavioural testing of NK1 receptor antagonists was the species-difference with regard to the compound's affinity at the NK1 receptor (Saria, 1999). Therefore, analogous versions of animal models of anxiety and/or depression in species others than rats and mice were developed and validated. In guinea pigs, a pharmacologically validated behavioural model of acute separation stress has been described. Specifically, maternal separation of guinea pig pups produces intense audible vocalizations that are reduced by the NK1 receptor antagonists MK-869, L-733,060 and L-760,735 (Kramer et al., 1998; Rupniak et al., 2000; Boyce et al., 2001), with similar efficacy to standard anxiolytic and antidepressant drugs such as diazepam and fluoxetine (Molewijk et al., 1996). In gerbils anxiolytic or antidepressant-like effects of NK1 antagonists were described in the social interaction (Cheeta et al., 2001; Gentsch et al., 2002) elevated plus-maze (Varty et al., 2002) and tail suspension test (Varty et al., 2003) as well as in a fear-conditioning paradigm (Ballard et al., 2001). The aim of further investigations now is to get more information about the brain sites where NK1 receptor antagonists affect anxiety and stress-related behavioural reactions.

Previous studies have identified the amygdala as one of the potential sites where NK1 receptor antagonists may act to modulate such behavioural reactions (for review see Rupniak, 2002; McLean, 2005). Very recently we could show that SP microinjected into the medial amygdala causes an anxiogenic effect in the elevated plus-maze test that could be abolished by preinjection of the selective NK1 receptor antagonist L-822,429 (=Compound A) (Ebner et al., 2004a). However, no effect of the NK1 receptor antagonist per se was noted, which may be related to the fact that exposures to novel elevated areas increases SP efflux only transiently by approximately 40% (Ebner et al., 2004a). In contrast, in rats that were subjected to immobilization stress prior to test exposure and thus displayed greatly enhanced SP efflux as well as enhanced anxiety, the injection of the antagonist into the medial amygdala exerted an anxiolytic effect (Ebner et al., 2004a). In accordance with these findings, Rogers et al. (2000) reported an anxiolytic effect in the elevated plus maze-test after ablation of SP receptor expressing neurons in the basolateral amygdala of rats, by administration of a SP-toxin conjugate. This effect was found despite the fact that the NK1 receptor density in the basolateral amygdala is much lower than in the medial amygdala (Maeno et al., 1993; Smith et al., 1999). However, in a similar study in mice, conflicting results have been obtained (Gadd et al., 2003). Apart from species and methodological differences, this discrepancy may also be due to the size and extension of the toxin-induced ablation of SP receptor expressing neurons. While the ablation was restricted to the basolateral amygdala in the study of Rogers et al. (2000), in the mouse study a loss of NK1 receptors has been observed in various subregions of the amygdala complex.

In the lateral septum and periaqueductal gray, the blockade of NK1 receptors attenuates SP induced anxiogenic effects (De Araujo et al., 2001b; Gavioli et al., 2002), indicating these areas as further candidate areas where NK1 receptor antagonists may affect anxiety and stress-related behaviours. Moreover, preliminary experiments from our laboratory have shown that the infusion of the NK1 receptor antagonist L-822,429 into the lateral septum significantly reduced the immobility time during forced swimming indicating an antidepressant-like effect (Ebner et al., 2004b). Based on these data it may be speculated that different aspects of affective behaviours are regulated in different brain areas via SP/NK1 receptor pathways (see also Adell et al., 2005). For example, it is conceivable that the amygdala is important in mediating anxiolytic effects of NK1 receptor antagonists, while antidepressant effects of these drugs may be processed in some other limbic areas such as the lateral septum. Preliminary data of our laboratory would support this view because NK1 receptor blockade within the medial amygdala had no effect on the immobility time (a parameter related to depressant-like behaviour) during forced swimming (Ebner et al., unpublished data) but has been shown to modulate anxiety-related behaviour in the elevated plus-maze test (Ebner et al., 2004a). Investigations into further candidate areas should be extended to other parts of anxiety and depression circuitries such as hypothalamic and cortical areas where SP as well as NK1 receptors have also been identified (see Section 2).

7. Interactions of the SP/NK1 receptor system and the monoaminergic systems

7.1 Interactions with the serotonergic system

Although the precise mechanisms of the anxiolytic and antidepressant actions of NK1 receptor antagonists are still unclear, a key question to be addressed is whether these effects involve alterations in serotonin (5-HT) and/or noradrenaline neurotransmission, believed to underlie the therapeutic effects of most currently available antidepressant drugs. Support for such a functional interaction is provided e.g. by the substantial anatomical overlap of the SP/NK1 receptor system with monoaminergic systems: In the dorsal raphe nucleus, the major source of 5-HT projections to forebrain areas (Vertes, 1991), both SP immunoreactive cells/fibers (Warden and Young, 1988; Nakaya et al., 1994) as well as NK1 receptors have been described (Mantyh et al., 1984; Maeno et al., 1993; Nakaya et al., 1994; Saffroy et al., 2003). Interestingly, NK1 receptors are not present on serotonergic cells in the dorsal raphe (Froger et al., 2001; Santarelli et al., 2001), but are found on glutamatergic and GABAergic cells (Ma and Bleasdale, 2002; Commons and Valentino, 2002) suggesting an indirect influence of SP on 5-HT neurotransmission within this area. It was consistently found in slice preparations that SP has no direct excitatory effects on 5-HT cell firing (Liu et al., 2002; Conley et al., 2002), although it induces a marked, presumably AMPA/kainate receptor-mediated increase in excitatory postsynaptic currents in dorsal raphe 5-HT neurons (Liu et al., 2002). In contrast, conflicting results have been obtained in vivo, where a decrease in 5-HT cell firing has been shown after infusion of SP into the dorsal raphe (Valentino et al., 2003). Moreover, the application of NK1 receptor antagonists predominantly produced an increase rather than a decrease in 5-HT cell firing (Haddjeri and Blier, 2001; Santarelli et al., 2001; Conley et al., 2002), which was also found in NK1R−/− mice (Santarelli et al., 2001). Interestingly, in contrast to studies in NK1R−/− mice, where a desensitization of 5-HT1A autoreceptor in the dorsal raphe has been suggested (Froger et al., 2001; Santarelli et al., 2001), chronic treatment with an NK1 receptor antagonist did not produce any detectable desensitization of these receptors (Conley et al., 2002). However, although these conflicting results could also result from species and methodological differences this discrepancy may reflect developmental adaptation in the NK1R−/− mice rather than a true desensitization of the 5-HT1A autoreceptors (Conley et al., 2002). Moreover, in contrast to previous findings in mice the same authors found no direct effect on neuronal firing in dorsal raphe slices in vitro after SP or NK1 receptor agonist treatment in guinea pig, suggesting that NK1 receptors are not present on 5-HT neurons in the dorsal raphe of this species (Conley et al., 2002). Together with the fact that only sparse expression of NK1 receptors has been found in the dorsal raphe of guinea pigs and primates (Saffroy et al., 1994; Conley et al., 2002), it is rather unlikely that NK1 receptor antagonists have direct effects on dorsal raphe neurons. Therefore, changes of NK1 receptor antagonist-induced neuronal firing in the dorsal raphe are likely to result from blockade of NK1 receptors elsewhere in the brain (Conley et al., 2002). In preliminary microdialysis experiments performed in rats we found a pronounced increase in 5-HT release within the lateral septum after the blockade of intraseptal NK1 receptors both, under basal and stress conditions (Ebner et al., unpublished data). The lateral septum has been shown to receive a dense 5-HT innervation from the dorsal raphe (Kohler et al., 1982; Gall and Moore, 1984; Leger et al., 2001). Concerning the localisation of NK1 receptors modulating serotonergic tone in the lateral septum, mechanism involving other areas and additional neurotransmitter systems (Conley et al., 2002; Adell, 2004) including possibly presynaptically localized NK1 receptors on 5-HT terminals may be proposed. Presynaptic inhibitory mechanism of SP on a co-localized neurotransmitter has already been postulated for the release of glutamate (Malcangio and Bowery, 1999; Sekizawa et al., 2003). Exact determination of the localization of NK1 receptors in the septum will be required as a first step to clarify these mechanisms.

7.2 Interactions with the noradrenergic system

In addition to alterations in 5-HT neuronal function, there is evidence that SP interacts with the noradrenergic system. Locus coeruleus neurons, the primary source of noradrenergic fibers are innervated by SP-containing fibres forming axo-dendritic contacts with tyrosine hydroxylase positive cells (Ljungdahl et al., 1978; Pickel et al., 1979; Halliday et al., 1988). Moreover, NK1 receptors are highly expressed in the locus coeruleus (Shults et al., 1984; Saffroy et al., 1994), where they are localized mainly on noradrenergic cells (Chen et al., 2000; Ma and Bleasdale, 2002). It has been shown that SP excites the majority of locus coeruleus neurons (Guyenet and Aghajanian, 1977; Cheeseman et al., 1983), whereas pharmacological blockade of NK1 receptors had no effect on basal firing rates in most studies (Haddjeri and Blier, 2000; Bert et al., 2002; Steinberg et al., 2002), However,

Millan et al. (2001) reported a 50% increase in firing rates following administration of GR-205,171 in the rat. NK1 receptor antagonist administration attenuates the suppressant effect of the alpha2-adrenoceptor agonist clonidine on locus coeruleus neuronal activity, suggesting an activation of the noradrenergic system via an attenuation of the function of these autoreceptors (Haddjeri and Blier, 2000). In contrast to acute administration, chronic NK1 receptor antagonist treatment did not affect somatically located alpha2 receptors, although it induced an increase in burst firing similar to that observed after chronic imipramine treatment (Maubach et al., 2002). Gobbi and Blier (2005) also described an increase in burst activity of noradrenergic neurons in the locus coeruleus of NK1R−/− mice compared to wild type mice, while spontaneous firing of these neurons was not changed. An increase in burst activity is thought to be correlated with an enhancement of noradrenaline release in terminal areas (Florin-Lechner et al., 1996), which has been successfully shown in the cerebral cortex of NK1R−/− mice (Herpfer et al., 2005) and in the frontal cortex and hippocampus of rats after pharmacological blockade of NK1 receptors (Millan et al., 2001). It remains to be determined how acute or chronic NK1 receptor blockade influences stress-induced noradrenaline release in target areas and within the locus coeruleus (Singewald et al., 1999). In rats it has been shown that intracerebroventricular administration of two different NK1 receptor antagonists attenuated the stress-induced increase in c-fos expression in the locus coeruleus (Hahn and Bannon, 1999). In HAB rats systemic administration of a NK1 receptor antagonist also attenuated stress-induced Fos expression in the locus coeruleus (Muigg et al., 2005). Thus, it is conceivable that the SP/NK1 receptor system modulates noradrenergic neurotransmission differently under stress and basal conditions, respectively.

8. Effects of antidepressant, anxiolytic and psychotropic drugs on the SP/NK1 receptor system

8.1 Effects of antidepressants and anxiolytics

Results of numerous studies suggest that anxiolytic and antidepressant treatment can interact with the SP/NK1 receptor system at different levels. Although studies investigating effects of anxiolytic drugs on the SP/NK1 receptor system are scarce, some studies found region-specific changes of SP levels in rats. For example, a single dose of diazepam reduced SP tissue levels in the hippocampus and periaqueductal gray (Brodin et al., 1994a) but increased SP levels in the substantia nigra (Koshiya and Kato, 1983). Moreover, it has been shown that acute diazepam treatment resulted in a 30% decrease in PPTA mRNA in the nucleus accumbens while expression in the striatum was not affected (Lucas et al., 1997). Interestingly, subchronic diazepam treatment induced an increase of PPTA mRNA levels in the striatum (Lucas et al., 1997). Inconsitent findings have been reported with respect to the effect of antidepressant treatment on SP tissue concentrations. Acute administration of a 5-HT reuptake inhibitor such as alaproclate increases tissue concentrations of SP in the periaqueductal gray in rats, while no significant effects were found on in vivo release of SP (Rosen et al., 1995). Treatment with several different antidepressant agents for 40 days resulted in a reduction of SP concentration in brain areas such as the striatum, substantia nigra and amygdala (Shirayama et al., 1996). 14 days of treatment had no effect (Oblin et al., 1984; Hamon et al., 1987; Brodin et al., 1994b) with one exception, the frontal cortex where a reduced SP tissue concentration was found after imipramine treatment (Brodin et al., 1987). However, also evidence was presented that chronic antidepressant treatment may actually increase SP concentration in some areas. For example, two-week administration of the 5-HT reuptake inhibitors zimelidine or alaproclate increases SP concentrations in the periaqueductal gray, while imipramine has no effect (Brodin et al., 1987). In a preliminary microdialysis experiment a blunted stress-induced SP efflux in the medial amygdala of rats treated chronically with the 5-HT reuptake inhibitor paroxetine was observed (Ebner et al. unpublished data). PPTA mRNA expression has been reported to be transiently decreased in medullary raphe after subchronic zimelidine and chlorgyline (a monoamine oxidase inhibitor) treatment (Riley et al., 1991), while the opposite effect has been found in the striatum after same treatment for 5 but not 14 days (Walker et al., 1991). In contrast, no changes in PPTA gene expression have been observed in various forebrain areas, including the striatum following chronic treatment (26 days) with the antidepressants fluoxetine, reboxetine, venlafaxine, or tranycypromine (Stout et al., 1999).

Repeated electroconvulsive shock (ECS) in rats, used as a model for human electroconvulsive therapy for treatment of depression, resulted in a 43% decrease in PPTA mRNA in the caudate putamen (Zachrisson et al., 1997), although others have not seen any changes in this area following ECS (Stenfors et al., 1992). Conversely, in other areas including cerebral cortex, periaqueductal gray and

Edinger-Westphal nucleus increased SP levels or PPTA mRNA expression have been observed following ECS (Brodin et al., 1989; Lindefors et al., 1991). NK1 receptor binding was increased in the frontal, cingulate and occipital cortex following repeated ECS without changing receptor expression, while no effects were noted in other areas such as hippocampus or amygdala (Burnet et al., 2001). More recently it was demonstrated that chronic treatment with different classes of antidepressants does not cause significant changes in NK1 receptor expression or binding in brain areas including cortical areas with one exception: a moderate increase in NK1 receptor binding was observed in the locus coeruleus after treatment with the non-selective MAO inhibitor tranylcypromine (Sartori et al., 2004). Taken together the available data provide some evidence that existing antidepressant drugs may modulate SP/NK1 receptor transmission. However, since the influence of antidepressants on SP transmission seems inconsitent and only slight in magnitude, the significance of this interaction for the therapeutic effects of antidepressants remains questionable. Hence, these data do not seem to support the hypothesis that reduction in SP neurotransmission may underly both the action of NK1 receptor antagonists and established antidepressants.

8.2 Effects of mood-stabilizing drugs

There is also some evidence that mood-stabilizing drugs may mediate their therapeutic properties through an interaction with the SP/NK1 receptor system. For example, in rats increased SP tissue levels have been found in brain areas such as frontal cortex, nucleus accumbens and striatum after subchronic treatment with lithium (Hong et al., 1983; Sivam et al., 1989), as well as in the striatum and hypothalamus after long-term treatment (Mathé et al., 1990, 1994). Moreover, it was found that long-term lithium treatment changed basal tissue SP levels in Flinders Sensitive Line rats, an animal model of depression (Husum et al., 2001). Compared to control rats, these rats displayed lower SP tissue levels in the striatum and higher SP levels in the frontal cortex that were abolished by the lithium treatment. Carbamazepine, another agent with mood-stabilizing properties, increased SP tissue levels after chronic administration in forebrain areas including the cerebral cortex, striatum and substantia nigra as well as brainstem areas (Mitsushio et al., 1988; Kuang et al., 1991). Unfortunately, no information are available on effects of mood stabilizers on extracellular levels of SP. Interestingly, repeated carbamazepine treatment increased the responsiveness of neurones in the cingulate cortex of rats to the excitatory effects of iontophoretically applied SP (Jones et al., 1985). Very recently, Lieb et al. (2003) have shown that the mood-stabilizing agent valproic acid, but not lithium or carbamzepine, dose-dependently inhibited SP-induced interleukin-6 syntheses in primary rat astrocytes and human astrocytoma cells, which both express functional NK1 receptors. Notably, this pro-inflammatory cytokine has been shown to be elevated during the acute depressive state (Maes et al., 1995; Frommberger et al., 1997). Moreover, evidence was found that valproic acid inhibits the expression of NK1 receptors in human astrocytoma cells suggesting that therapeutic properties of these drugs are mediated by a downregulation of the binding sites for SP (Lieb et al., 2003). Thus, these data identify NK1 receptors as possible targets for the therapeutic effects of mood-stabilizing pschotropic agents.

9. NK1 receptor antagonists as new drugs for anxiety- and mood disorders

The existing pharmacological treatment of mood and anxiety disorders is dominated by drugs that directly target monoamine or GABA neurotransmitter systems. Monoamine reuptake inhibitors (predominantly noradrenaline and 5-HT) are first-line treatments for depression and are also, along with benzodiazepines, routinely prescribed for anxiety disorders (Argyropoulos et al., 2000; Nemeroff, 2003). Current antidepressant treatments have a series of disadvantages most notable a delayed onset of therapeutic action leaving a significant number of patients nonresponsive. Moreover, many patients discontinue treatment with monoamine reuptake inhibitors because of adverse side-effects, including nausea, sexual dysfunction, anorexia, sweating, asthenia (loss or lack of strength) and tremor. The tolerability of benzodiazepine anxiolytics is reduced by sedation, cognitive impairment and development of dependence. For these reasons there is a need for antidepressant/anxiolytic drugs with novel mechanisms of action. One promising avenue in the search for novel antidepressant/anxiolytic therapies is focused on the SP/NK 1 receptor system. The antidepressant and anxiolytic efficacy of the first NK1 receptor antagonist to be developed clinically, MK-869 (Aprepitant; Merck), was originally demonstrated in patients with major depression and high anxiety (Kramer et al., 1998), and has recently been replicated with a second compound, L-759,274 (Kramer et al., 2004). In both studies the NK1 antagonist was generally well-tolerated and side effects such as sexual dysfunction or gastrointestinal effects did

not occur or were very low. However, very recently conflicting results have been obtained from a clinical phase III study showing a lack of efficacy of aprepitant (Keller et al., 2005). Patient selection may be an important factor contributing to the outcome of such trials, since it is not clear at the moment whether and which defined subpopulation(s) of depressed patients may in particular benefit from NK1 receptor antagonist treatment. Thus, it is conceivable that depressed patients with high degree of comorbid anxiety and/or impaired stress perception system may preferentially respond to NK1 receptor antagonists. Indeed, NK1 receptor treatment has been found to alleviate symptoms of social phobia during a stressful public speaking task (Furmark et al., 2005). Interestingly, the NK1 receptor antagonist GW-597,599 has recently been reported to be active against CO_2-induced panic response (Glaxo Smith Kline). Despite these mixed results in particular in the depression trials, several other pharmaceutical companies are currently conducting clinical studies with their structurally different and possibly more potent NK1 receptor antagonists such as GW-597,599 (GlaxoSmith-Kline) and CP-122,721 (Pfizer) (for more detailed information on the clinical use of NK1 receptor antagonists in the treatment of depression and anxiety disorders see (Bosker et al., 2004; Herpfer and Lieb, 2005; McLean, 2005)). Moreover, the idea of combining serotonin reuptake inhibition with NK1 antagonism may result in a new class of antidepressants with an improved onset of action and better efficacy (Ryckmans et al., 2002a, b). Although several of these dual NK1 antagonist-serotonin reuptake inhibitor compounds have already been synthesized their clinical efficacy has to be proved.

10. Concluding remarks

In the past decade, a vast and growing body of evidence has demonstrated the involvement of the SP/NK1 receptor system in the regulation of stress and anxiety mechanisms. Until recently, SP was thought to be primarily a neurotransmitter/neuromodulator in pain transmission. We are now just beginning to understand how the widespread and divergent anatomical organization of the SP/NK1 receptor system in the brain might serve to modulate the complex physiological and behavioural responses to aversive and stressful stimuli. Considerable evidence suggests that stressful challenges lead to the activation of circuits utilizing SP signalling and feeding in brain regions that play a major role in the modulation of stress responses and the regulation of affective behaviour (such as the amygdala, septum, hypothalamus, hippocampus, striatum, nucleus accumbens, periaqueductal gray, raphe nucleus and locus coeruleus). Mimicking this activation by centrally administered SP or other NK1 receptor agonists induces a pattern of cardiovascular and behavioural responses that closely resemble the responses to various stressful stimuli. Along these lines, preclinical and clinical studies have shown that blocking SP transmission either by antagonists or genetic disruption attenuates the effects of stress including changes in behaviour (such as anxiety), neuronal activation and proliferation of hippocampal neurons. Given such widespread interaction with systems comprising (adaptive) responses to stress, it is likely that a dysregulation of the SP/NK1 receptor system might contribute to the pathophysiology of specific stress-related psychiatric disorders such as anxiety- and depressive disorders. Hence, the pharmacological interaction with the modulatory function of SP offers an attractive new target for drug development in psychiatric disorders including anxiety and depression. Indeed, first clinical trials have shown efficacy of NK1 receptor antagonists to alleviate symptoms of depression and anxiety, although negative findings were also reported in particular in depression trials. Since the mechanisms underlying these effects are still incompletely understood, it is important to further clarify the modulatory function of the SP/NK1 receptor system in physiological and behavioural adaptation to stressful life events, as well as in the occurrence of maladaptive consequences of chronic stressful challenges. This information is essential for further development of improved therapeutic strategies involving SP pathways.

Acknowledgements

The work of the authors in this review was funded by grants from the Fonds der Österreichische Nationalbank, Österreichischen Akademie der Wissenschaften and Fonds zur Förderung der Wissenschaftlichen Forschung.

References

Adell A (2004) Antidepressant properties of substance P antagonists: relationship to monoaminergic mechanisms? Curr Drug Targets CNS Neurol Disord 3: 113–121

Adell A, Castro E, Celada P, Bortolozzi A, Pazos A, Artigas F (2005) Strategies for producing faster acting antidepressants. Drug Discov Today 10: 578–585

Aguiar MS, Brandao ML (1994) Conditioned place aversion produced by microinjections of substance P into the periaqueductal gray of rats. Behav Pharmacol 5: 369–373

Aguiar MS, Brandao ML (1996) Effects of microinjections of the neuropeptide substance P in the dorsal periaqueductal gray on the behaviour of rats in the plus-maze test. Physiol Behav 60: 1183–1186

Allen BJ, Rogers SD, Ghilardi JR, Menning PM, Kuskowski MA, Basbaum AI, Simone DA, Mantyh PW (1997) Noxious cutaneous thermal stimuli induce a graded release of endogenous substance P in the spinal cord: imaging peptide action in vivo. J Neurosci 17: 5921–5927

Argyropoulos SV, Sandford JJ, Nutt DJ (2000) The psychobiology of anxiolytic drug. Part 2: Pharmacological treatments of anxiety. Pharmacol Ther 88: 213–227

Ballard TM, Sanger S, Higgins GA (2001) Inhibition of shock-induced foot tapping behaviour in the gerbil by a tachykinin NK1 receptor antagonist. Eur J Pharmacol 412: 255–264

Baretta IP, Assreuy J, De Lima TC (2001) Nitric oxide involvement in the anxiogenic-like effect of substance P. Behav Brain Res 121: 199–205

Baulmann J, Spitznagel H, Herdegen T, Unger T, Culman J (2000) Tachykinin receptor inhibition and c-Fos expression in the rat brain following formalin-induced pain. Neuroscience 95: 813–820

Beresford IJ, Birch PJ, Hagan RM, Ireland SJ (1991) Investigation into species variants in tachykinin NK1 receptors by use of the non-peptide antagonist, CP-96,345. Br J Pharmacol 104: 292–293

Bert L, Rodier D, Bougault I, Allouard N, Le-Fur G, Soubrie P, Steinberg R (2002) Permissive role of neurokinin NK(3) receptors in NK(1) receptor-mediated activation of the locus coeruleus revealed by SR 142801. Synapse 43: 62–69

Bhatt S, Gregg TR, Siegel A (2003) NK1 receptors in the medial hypothalamus potentiate defensive rage behavior elicited from the midbrain periaqueductal gray of the cat. Brain Res 966: 54–64

Bittencourt JC, Benoit R, Sawchenko PE (1991) Distribution and origins of substance P-immunoreactive projections to the paraventricular and supraoptic nuclei: partial overlap with ascending catecholaminergic projections. J Chem Neuroanat 4: 63–78

Bosker FJ, Westerink BH, Cremers TI, Gerrits M, van der Hart MG, Kuipers SD, van der Pompe G, ter Horst GJ, den Boer JA, Korf J (2004) Future antidepressants: what is in the pipeline and what is missing? CNS Drugs 18: 705–732

Boyce S, Smith D, Carlson E, Hewson L, Rigby M, O'Donnell R, Harrison T, Rupniak NM (2001) Intra-amygdala injection of the substance P [NK(1) receptor] antagonist L-760735 inhibits neonatal vocalisations in guinea-pigs. Neuropharmacology 41: 130–137

Brodin E, Lindefors N, Ungerstedt U (1983) Potassium evoked in vivo release of substance P in rat caudate nucleus measured using a new technique of brain dialysis and an improved substance P-radioimmunoassay. Acta Physiol Scand Suppl 515: 17–20

Brodin E, Ogren SO, Theodorsson-Norheim E (1987) Effects of subchronic treatment with imipramine, zimelidine and alaproclate on regional tissue levels of substance P- and neurokinin A/neurokinin B-like immunoreactivity in the brain and spinal cord of the rat. Neuropharmacology 26: 581–590

Brodin E, Rosen A, Schott E, Brodin K (1994a) Effects of sequential removal of rats from a group cage, and of individual housing of rats, on substance P, cholecystokinin and somatostatin levels in the periaqueductal grey and limbic regions. Neuropeptides 26: 253–260

Brodin K, Ogren SO, Brodin E (1994b) Clomipramine and clonazepam increase cholecystokinin levels in rat ventral tegmental area and limbic regions. Eur J Pharmacol 263: 175–180

Brodin K, Rosen A, Iwarsson K, Ogren SO, Brodin E (1989) Increased levels of substance P and cholecystokinin in rat cerebral cortex following repeated electroconvulsive shock and subchronic treatment with a serotonin uptake inhibitor. Acta Physiol Scand 136: 613–614

Burnet PW, Miller R, Lewis LJ, Pei Q, Sharp T, Harrison PJ (2001) Electroconvulsive shock increases tachykinin NK(1) receptors, but not the encoding mRNA, in rat cortex. Eur J Pharmacol 413: 213–219

Caberlotto L, Hurd YL, Murdock P, Wahlin JP, Melotto S, Corsi M, Carletti R (2003) Neurokinin 1 receptor and relative abundance of the short and long isoforms in the human brain. Eur J Neurosci 17: 1736–1746

Cheeseman HJ, Pinnock RD, Henderson G (1983) Substance P excitation of rat locus coeruleus neurones. Eur J Pharmacol 94: 93–99

Cheeta S, Tucci S, Sandhu J, Williams AR, Rupniak NM, File SE (2001) Anxiolytic actions of the substance P (NK1) receptor antagonist L-760735 and the 5-HT1A agonist 8-OH-DPAT in the social interaction test in gerbils. Brain Res 915: 170–175

Chen LW, Wei LC, Liu HL, Rao ZR (2000) Noradrenergic neurons expressing substance P receptor (NK1) in the locus coeruleus complex: a double immunofluorescence study in the rat. Brain Res 873: 155–159

Chowdrey HS, Jessop DS, Lightman SL (1990) Substance P stimulates arginine vasopressin and inhibits adrenocorticotropin release in vivo in the rat. Neuroendocrinology 52: 90–93

Chowdrey HS, Larsen PJ, Harbuz MS, Lightman SL, Jessop DS (1995) Endogenous substance P inhibits the expression of corticotropin-releasing hormone during a chronic inflammatory stress. Life Sci 57: 2021–2029

Commons KG, Valentino RJ (2002) Cellular basis for the effects of substance P in the periaqueductal gray and dorsal raphe nucleus. J Comp Neurol 447: 82–97

Conley RK, Cumberbatch MJ, Mason GS, Williamson DJ, Harrison T, Locker K, Swain C, Maubach K, O'Donnell R, Rigby M, Hewson L, Smith D, Rupniak NM (2002) Substance P (neurokinin 1) receptor antagonists enhance dorsal raphe neuronal activity. J Neurosci 22: 7730–7736

Culman J, Unger T (1995) Central tachykinins: mediators of defence reaction and stress reactions. Can J Physiol Pharmacol 73: 885–891

Culman J, Klee S, Ohlendorf C, Unger T (1997) Effect of tachykinin receptor inhibition in the brain on cardiovascular and behavioral responses to stress. J Pharmacol Exp Ther 280: 238–246

Czeh B, Pudovkina O, van der Hart MG, Simon M, Heilbronner U, Michaelis T, Watanabe T, Frahm J, Fuchs E (2005a) Examining SLV-323, a novel NK1 receptor antagonist, in a chronic psychosocial stress model for depression. Psychopharmacology 180: 548–557

Czeh B, Simon M, van der Hart MG, Schmelting B, Hesselink MB, Fuchs E (2005b) Chronic stress decreases the number of parvalbumin-immunoreactive interneurons in the hippocampus: prevention by treatment with a substance P receptor (NK1) antagonist. Neuropsychopharmacology 30: 67–79

Dableh LJ, Yashpal K, Rochford J, Henry JL (2005) Antidepressant-like effects of neurokinin receptor antagonists in the forced swim test in the rat. Eur J Pharmacol 507: 99–105

De Araujo JE, Silva RC, Huston JP, Brandao ML (1999) Anxiogenic effects of substance P and its 7–11 C terminal, but not the 1–7 N terminal, injected into the dorsal periaqueductal gray. Peptides 20: 1437–1443

De Araujo JE, Huston JP, Brandao ML (2001a) Place aversion induced by microinjections of C-fragment of substance P into the dorsal periaqueductal gray of rats is mediated by tachykinin NK1 receptors. Peptides 22: 1447–1452

De Araujo JE, Huston JP, Brandao ML (2001b) Opposite effects of substance P fragments C (anxiogenic) and N (anxiolytic) injected into dorsal periaqueductal gray. Eur J Pharmacol 432: 43–51

Dean C, Marson L, Kampine JP (1993) Distribution and co-localization of 5-hydroxytryptamine, thyrotropin-releasing hormone and substance P in the cat medulla. Neuroscience 57: 811–822

Ding YQ, Shigemoto R, Takada M, Ohishi H, Nakanishi S, Mizuno N (1996) Localization of the neuromedin K receptor (NK3) in the central nervous system of the rat. J Comp Neurol 364: 290–310

Duarte FS, Testolin R, De Lima TC (2004) Further evidence on the anxiogenic-like effect of substance P evaluated in the elevated plus-maze in rats. Behav Brain Res 154: 501–510

Duffy RA, Varty GB, Morgan CA, Lachowicz JE (2002) Correlation of neurokinin (NK) 1 receptor occupancy in gerbil striatum with behavioral effects of NK1 antagonists. J Pharmacol Exp Ther 301: 536–542

Duffy RA, Hedrick JA, Randolph G, Morgan CA, Cohen-Williams ME, Vassileva G, Lachowicz JE, Laverty M, Maguire M, Shan LS, Gustafson E, Varty GB (2003) Centrally administered hemokinin-1 (HK-1), a neurokinin NK1 receptor agonist, produces substance P-like behavioral effects in mice and gerbils. Neuropharmacology 45: 242–250

Duric V, McCarson KE (2005) Hippocampal neurokinin-1 receptor and brain-derived neurotrophic factor gene expression is decreased in rat models of pain and stress. Neuroscience 133: 999–1006

Ebner K, Rupniak NM, Saria A, Singewald N (2004a) Substance P in the medial amygdala: emotional stress-sensitive release and modulation of anxiety-related behavior in rats. Proc Natl Acad Sci USA 101: 4280–4285

Ebner K, Saria A, Singewald N (2004b) Forced swim-induced release of substance P within the lateral septum modulates stress-coping behavior in rats. FENS Forum A127.6: 299

Ebner K, Singewald N (2005) Effects of NK1 receptor antagonist administration on basal and stress-induced substance P release in the amygdala. J Neurochem 94 [Suppl 2]: 183

Elliott PJ (1988) Place aversion induced by the substance P analogue, dimethyl-C7, is not state dependent: implication of substance P in aversion. Exp Brain Res 73: 354–356

Faria M, Navarra P, Tsagarakis S, Besser GM, Grossman AB (1991) Inhibition of CRH-41 release by substance P, but not substance K, from the rat hypothalamus in vitro. Brain Res 538: 76–78

Fiebich BL, Schleicher S, Butcher RD, Craig A, Lieb K (2000) The neuropeptide substance P activates p38 mitogen-activated protein kinase resulting in IL-6 expression independently from NF-kappa B. J Immunol 165: 5606–5611

File SE (1997) Anxiolytic action of a neurokinin1 receptor antagonist in the social interaction test. Pharmacol Biochem Behav 58: 747–752

File SE (2000) NKP608, an NK1 receptor antagonist, has an anxiolytic action in the social interaction test in rats. Psychopharmacology 152: 105–109

Florin-Lechner SM, Druhan JP, Aston-Jones G, Valentino RJ (1996) Enhanced norepinephrine release in prefrontal cortex with burst stimulation of the locus coeruleus. Brain Res 742: 89–97

Fong TM, Yu H, Strader CD (1992) Molecular basis for the species selectivity of the neurokinin-1 receptor antagonists CP-96,345 and RP67580. J Biol Chem 267: 25668–25671

Froger N, Gardier AM, Moratalla R, Alberti I, Lena I, Boni C, De Felipe C, Rupniak NM, Hunt SP, Jacquot C, Hamon M, Lanfumey L (2001) 5-hydroxytryptamine (5-HT)1A autoreceptor adaptive changes in substance P (neurokinin 1) receptor knock-out mice mimic antidepressant-induced desensitization. J Neurosci 21: 8188–8197

Frommberger UH, Bauer J, Haselbauer P, Fraulin A, Riemann D, Berger M (1997) Interleukin-6-(IL-6) plasma levels in depression and schizophrenia: comparison between the acute state and after remission. Eur Arch Psychiatry Clin Neurosci 247: 228–233

Furmark T, Appel L, Michelgard A, Wahlstedt K, Ahs F, Zancan S, Jacobsson E, Flyckt K, Grohp M, Bergstrom M, Pich EM, Nilsson LG, Bani M, Langstrom B, Fredrikson M (2005) Cerebral blood flow changes after treatment of social phobia with the neurokinin-1 antagonist GR205171, citalopram, or placebo. Biol Psychiatry 58: 132–142

Gadd CA, Murtra P, De Felipe C, Hunt SP (2003) Neurokinin-1 receptor-expressing neurons in the amygdala modulate morphine reward and anxiety behaviors in the mouse. J Neurosci 23: 8271–8280

Gall C, Moore RY (1984) Distribution of enkephalin, substance P, tyrosine hydroxylase, and 5-hydroxytryptamine immunoreactivity in the septal region of the rat. J Comp Neurol 225: 212–227

Garcia M, Sakamoto K, Shigekawa M, Nakanishi S, Ito S (1994) Multiple mechanisms of arachidonic acid release in Chinese hamster ovary cells transfected with cDNA of substance P receptor. Biochem Pharmacol 48: 1735–1741

Gavioli EC, Canteras NS, De Lima TC (1999) Anxiogenic-like effect induced by substance P injected into the lateral septal nucleus. Neuroreport 10: 3399–3403

Gavioli EC, Canteras NS, De Lima TC (2002) The role of lateral septal NK1 receptors in mediating anxiogenic effects induced by intracerebroventricular injection of substance P. Behav Brain Res 134: 411–415

Gentsch C, Cutler M, Vassout A, Veenstra S, Brugger F (2002) Anxiolytic effect of NKP608, a NK1-receptor antagonist, in the social investigation test in gerbils. Behav Brain Res 133: 363–368

Gether U, Johansen TE, Snider RM, Lowe JA 3rd, Nakanishi S, Schwartz TW (1993) Different binding epitopes on the NK1 receptor for substance P and non-peptide antagonist. Nature 362: 345–348

Gobbi G, Blier P (2005) Effect of neurokinin-1 receptor antagonists on serotoninergic, noradrenergic and hippocampal neurons: comparison with antidepressant drugs. Peptides 26: 1383–1393

Gregg TR, Siegel A (2003) Differential effects of NK1 receptors in the midbrain periaqueductal gray upon defensive rage and predatory attack in the cat. Brain Res 994: 55–66

Guyenet PG, Aghajanian GK (1977) Excitation of neurons in the nucleus locus coeruleus by substance P and related peptides. Brain Res 136: 178–184

Haddjeri N, Blier P (2000) Effect of neurokinin-I receptor antagonists on the function of 5-HT and noradrenaline neurons. Neuroreport 11: 1323–1327

Haddjeri N, Blier P (2001) Sustained blockade of neurokinin-1 receptors enhances serotonin neurotransmission. Biol Psychiatry 50: 191–199

Hahn MK, Bannon MJ (1999) Stress-induced C-fos expression in the rat locus coeruleus is dependent on neurokinin 1 receptor activation. Neuroscience 94: 1183–1188

Halliday GM, Li YW, Joh TH, Cotton RG, Howe PR, Geffen LB, Blessing WW (1988) Distribution of substance P-like immunoreactive neurons in the human medulla oblongata: co-localization with monoamine-synthesizing neurons. Synapse 2: 353–370

Hamon M, Gozlan H, Bourgoin S, Benoliel JJ, Mauborgne A, Taquet H, Cesselin F, Mico JA (1987) Opioid receptors and neuropeptides in the CNS in rats treated chronically with amoxapine or amitriptyline. Neuropharmacology 26: 531–539

Hanley M, Lee C, Watson S, Sandberg B, Iversen L (1981) Biochemical and pharmacological studies of substance P receptors. Protides Biol Fluids 29: 477–482

Harmar AJ, Hyde V, Chapman K (1990) Identification and cDNA sequence of delta-preprotachykinin, a fourth splicing variant of the rat substance P precursor. FEBS Lett 275: 22–24

Hasenöhrl RU, Jentjens O, De Souza Silva MA, Tomaz C, Huston JP (1998) Anxiolytic-like action of neurokinin substance P administered systemically or into the nucleus basalis magnocellularis region. Eur J Pharmacol 354: 123–133

Herkenham M (1987) Mismatches between neurotransmitter and receptor localizations in brain: observations and implications. Neuroscience 23: 1–38

Herpfer I, Hunt SP, Stanford SC (2005) A comparison of neurokinin 1 receptor knock-out (NK1$-/-$) and wildtype mice: exploratory behaviour and extracellular noradrenaline concentration in the cerebral cortex of anaesthetised subjects. Neuropharmacology 48: 706–719

Herpfer I, Lieb K (2005) Substance P receptor antagonists in psychiatry: rationale for development and therapeutic potential. CNS Drugs 19: 275–293

Hökfelt T, Kellerth JO, Nilsson G, Pernow B (1975) Substance P: localization in the central nervous system and in some primary sensory neurons. Science 190: 889–890

Hökfelt T, Vincent S, Dalsgaard CJ, Skirboll L, Johansson O, Schultzberg M, Lundberg JM, Rosell S, Pernow B, Jancso G (1982) Distribution of substance P in brain and periphery and its possible role as a co-transmitter. Ciba Found Symp 91: 84–106

Hökfelt T, Broberger C, Xu ZQ, Sergeyev V, Ubink R, Diez M (2000) Neuropeptides – an overview. Neuropharmacology 39: 1337–1356

Holsboer F, Barden N (1996) Antidepressants and hypothalamic-pituitary-adrenocortical regulation. Endocr Rev 17: 187–205

Hong JS, Tilson HA, Yoshikawa K (1983) Effects of lithium and haloperidol administration on the rat brain levels of substance P. J Pharmacol Exp Ther 224: 590–593

Husum H, Vasquez PA, Mathé AA (2001) Changed concentrations of tachykinins and neuropeptide Y in brain of a rat model of depression: lithium treatment normalizes tachykinins. Neuropsychopharmacology 24: 183–191

Hwang BH, Katner J, Iyengar S (2005) Corticotropin-releasing factor mRNA and substance P receptor binding in the paraventricular hypothalamic nucleus, central nucleus of the amygdala, and locus coeruleus of Sprague-Dawley rats following restraint-induced stress. J Mol Neurosci 25: 239–250

Igwe OJ, Kim DC, Seybold VS, Larson AA (1990) Specific binding of substance P aminoterminal heptapeptide [SP(1–7)] to mouse brain and spinal cord membranes. J Neurosci 10: 3653–3663

Ikeda K, Miyata K, Orita A, Kubota H, Yamada T, Tomioka K (1995) RP67580, a neurokinin1 receptor antagonist, decreased restraint stress-induced defecation in rat. Neurosci Lett 198: 103–106

Jarry H, Perschl A, Meissner H, Wuttke W (1988) In vivo release rates of substance P in the preoptic/anterior hypothalamic area of ovariectomized and ovariectomized estrogen-primed rats: correlation with luteinizing hormone and prolactin levels. Neurosci Lett 88: 189–194

Jessell TM (1978) Substance P release from the rat substantia nigra. Brain Res 151: 469–478

Jessop DS, Renshaw D, Larsen PJ, Chowdrey HS, Harbuz MS (2000) Substance P is involved in terminating the hypothalamo-pituitary-adrenal axis response to acute stress through centrally located neurokinin-1 receptors. Stress 3: 209–220

Jones MT, Gillham B, Holmes MC, Hodges JR, Buckingham JC (1978) Influence of substance P on hypothalamo-pituitary-adrenocorticol activity in the rat. J Endocrinol 76: 183–184

Jones RS, Mondadori C, Olpe HR (1985) Neuronal sensitivity to substance P is increased after repeated treatment with tranylcypromine, carbamazepine or oxaprotaline, but decreased after repeated electroconvulsive shock. Neuropharmacology 24: 627–633

Kachidian P, Poulat P, Marlier L, Privat A (1991) Immunohistochemical evidence for the coexistence of substance P, thyrotropin-releasing hormone, GABA, methionine-enkephalin, and leucin-enkephalin in the serotonergic neurons of the caudal raphe nuclei: a dual labeling in the rat. J Neurosci Res 30: 521–530

Kang TC, Seo J, Jeon GS, Shin DH, Kim YW, Lim HS, Lee KH, Hwang DH, Won MH, Cho SS (1999) Are substance P neurons of the paraventricular nucleus related to the osmotic regulation in the Mongolian gerbil? Brain Res 820: 101–104

Kawano H, Masuko S (1995) Substance P innervation of neurons projecting to the paraventricular hypothalamic nucleus in the rat nucleus tractus solitarius. Brain Res 689: 136–140

Keck ME, Welt T, Muller MB, Uhr M, Ohl F, Wigger A, Toschi N, Holsboer F, Landgraf R (2003) Reduction of hypothalamic vasopressinergic hyperdrive contributes to clinically relevant behavioral and neuroendocrine effects of chronic paroxetine treatment in a psychopathological rat model. Neuropsychopharmacology 28: 235–243

Keller M, Montgomery S, Ball W, Morrison M, Snavely D, Liu G, Hargreaves R, Hietala J, Lines C, Beebe K, Reines S (2006) Lack of efficacy of the substance p (neurokinin(1) receptor) antagonist aprepitant in the treatment of major depressive disorder. Biol Psychiatry 59: 216–223

Kendrick KM (1990) Microdialysis measurement of in vivo neuropeptide release. J Neurosci Methods 34: 35–46

Khawaja AM, Rogers DF (1996) Tachykinins: receptor to effector. Int J Biochem Cell Biol 28: 721–738

Kohler C, Chan-Palay V, Steinbusch H (1982) The distribution and origin of serotonin-containing fibers in the septal area: a combined immunohistochemical and fluorescent retrograde tracing study in the rat. J Comp Neurol 209: 91–111

Koshiya K, Kato T (1983) Acute changes in nigral substance P content induced by drugs acting on dopamine, muscarine and GABA receptors. Naunyn Schmiedebergs Arch Pharmacol 324: 223–227

Kotani H, Hoshimaru M, Nawa H, Nakanishi S (1986) Structure and gene organization of bovine neuromedin K precursor. Proc Natl Acad Sci USA 83: 7074–7078

Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, Reines SA, Liu G, Snavely D, Wyatt-Knowles E, Hale JJ, Mills SG, MacCoss M, Swain CJ, Harrison T, Hill RG, Hefti F, Scolnick EM, Cascieri MA, Chicchi GG, Sadowski S, Williams AR, Hewson L, Smith D, Carlson EJ, Hargreaves RJ, Rupniak NM (1998) Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science 281: 1640–1645

Kramer MS, Winokur A, Kelsey J, Preskorn SH, Rothschild AJ, Snavely D, Ghosh K, Ball WA, Reines SA, Munjack D, Apter JT, Cunningham L, Kling M, Bari M, Getson A, Lee Y (2004) Demonstration of the efficacy and safety of a novel substance P (NK1) receptor antagonist in major depression. Neuropsychopharmacology 29: 385–392

Krase W, Koch M, Schnitzler HU (1994) Substance P is involved in the sensitization of the acoustic startle response by footshocks in rats. Behav Brain Res 63: 81–88

Kuang P, Lang S, Liu J, Zhang F, Wu W (1991) The investigation of antiepileptic action of qingyangshen (QYS) – effect of QYS on the concentrations of neuropeptides in rat brain. J Trad Chin Med 11: 40–46

Landgraf R, Neumann ID (2004) Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. Front Neuroendocrinol 25: 150–176

Landgraf R, Wigger A (2002) High vs low anxiety-related behavior rats: an animal model of extremes in trait anxiety. Behav Genet 32: 301–314

Landgraf R, Wigger A (2003) Born to be anxious: neuroendocrine and genetic correlates of trait anxiety in HAB rats. Stress 6: 111–119

Larsen PJ (1992) Distribution of substance P-immunoreactive elements in the preoptic area and the hypothalamus of the rat. J Comp Neurol 316: 287–313

Larsen PJ, Jessop D, Patel H, Lightman SL, Chowdrey HS (1993) Substance P inhibits the release of anterior pituitary adrenocorticotrophin via a central mechanism involving corticotrophin-releasing factor-containing neurons in the hypothalamic paraventricular nucleus. J Neuroendocrinol 5: 99–105

Leger L, Charnay Y, Hof PR, Bouras C, Cespuglio R (2001) Anatomical distribution of serotonin-containing neurons and axons in the central nervous system of the cat. J Comp Neurol 433: 157–182

Lenard L, Kertes E (2002) Influence of passive avoidance learning by substance P in the basolateral amygdala. Acta Biol Hung 53: 95–104

Lieb K, Fiebich BL, Berger M, Bauer J, Schulze-Osthoff K (1997) The neuropeptide substance P activates transcription factor NF-kappa B and kappa B-dependent gene expression in human astrocytoma cells. J Immunol 159: 4952–4958

Lieb K, Treffurth Y, Hamke M, Akundi RS, von Kleinsorgen M, Fiebich BL (2003) Valproic acid inhibits substance P-induced activation of protein kinase C epsilon and expression of the substance P receptor. J Neurochem 86: 69–76

Lindefors N, Brodin E, Theodorsson-Norheim E, Ungerstedt U (1985) Regional distribution and in vivo release of tachykinin-like immunoreactivities in rat brain: evidence for regional differences in relative proportions of tachykinins. Regul Pept 10: 217–230

Lindefors N, Yamamoto Y, Pantaleo T, Lagercrantz H, Brodin E, Ungerstedt U (1986) In vivo release of substance P in the nucleus tractus solitarii increases during hypoxia. Neurosci Lett 69: 94–97

Lindefors N, Brodin E, Ungerstedt U (1987) Microdialysis combined with a sensitive radioimmunoassay. A technique for studying in vivo release of neuropeptides. J Pharmacol Methods 17: 305–312

Lindefors N, Brodin E, Ungerstedt U (1989a) Subchronic haloperidol treatment decreases the in vivo release of tachykinins in rat substantia nigra. Eur J Pharmacol 161: 95–98

Lindefors N, Brodin E, Tossman U, Segovia J, Ungerstedt U (1989b) Tissue levels and in vivo release of tachykinins and GABA in striatum and substantia nigra of rat brain after unilateral striatal dopamine denervation. Exp Brain Res 74: 527–534

Lindefors N, Brodin K, Stiller CO, Persson H, Brodin E (1991) Repeated electroconvulsive shock increases tachykinin and cholecystokinin mRNA expression in ventral periaqueductal gray. Neuroscience 45: 73–80

Lisoprawski A, Blanc G, Glowinski J (1981) Activation by stress of the habenulo-interpeduncular substance P neurons in the rat. Neurosci Lett 25: 47–51

Liu R, Ding Y, Aghajanian GK (2002) Neurokinins activate local glutamatergic inputs to serotonergic neurons of the dorsal raphe nucleus. Neuropsychopharmacology 27: 329–340

Ljungdahl A, Hökfelt T, Nilsson G (1978) Distribution of substance P-like immunoreactivity in the central nervous system of the rat I. Cell bodies and nerve terminals. Neuroscience 3: 861–943

Loiseau F, Le Bihan C, Hamon M, Thiebot MH (2003) Distinct effects of diazepam and NK1 receptor antagonists in two conflict procedures in rats. Behav Pharmacol 14: 447–455

Loiseau F, Le Bihan C, Hamon M, Thiebot MH (2005) Antidepressant-like effects of agomelatine, melatonin and the NK1 receptor antagonist GR205171 in impulsive-related behaviour in rats. Psychopharmacology 182: 24–32

Lucas LR, Pompei P, McEwen BS (1997) Effects of deoxycorticosterone acetate and diazepam on neuropeptidergic neurons in rat striatum. Neuroreport 8: 811–816

Ma QP, Bleasdale C (2002) Modulation of brain stem monoamines and gamma-aminobutyric acid by NK1 receptors in rats. Neuroreport 13: 1809–1812

Maeno H, Kiyama H, Tohyama M (1993) Distribution of the substance P receptor (NK-1 receptor) in the central nervous system. Brain Res Mol Brain Res 18: 43–58

Maes M, Meltzer HY, Bosmans E, Bergmans R, Vandoolaeghe E, Ranjan R, Desnyder R (1995) Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. J Affect Disord 34: 301–309

Maggi CA (1995) The mammalian tachykinin receptors. Gen Pharmacol 26: 911–944

Maggi CA, Schwartz TW (1997) The dual nature of the tachykinin NK1 receptor. Trends Pharmacol Sci 18: 351–355

Malcangio M, Bowery NG (1999) Peptide autoreceptors: does an autoreceptor for substance P exist? Trends Pharmacol Sci 20: 405–407

Mantyh PW, Hunt SP, Maggio JE (1984) Substance P receptors: localization by light microscopic autoradiography in rat brain using SP as the radioligand. Brain Res 307: 147–165

Mantyh PW, Gates T, Mantyh CR, Maggio JE (1989) Autoradiographic localization and characterization of tachykinin receptor binding sites in the rat brain and peripheral tissues. J Neurosci 9: 258–279

Mantyh PW, Allen CJ, Ghilardi JR, Rogers SD, Mantyh CR, Liu H, Basbaum AI, Vigna SR, Maggio JE (1995) Rapid endocytosis of a G protein-coupled receptor: substance P evoked internalization of its receptor in the rat striatum in vivo. Proc Natl Acad Sci USA 92: 2622–2626

Mathé AA, Jousisto-Hanson J, Stenfors C, Theodorsson E (1990) Effect of lithium on tachykinins, calcitonin gene-related peptide, and neuropeptide Y in rat brain. J Neurosci Res 26: 233–237

Mathé AA, Wikner BN, Stenfors C, Theodorsson E (1994) Effects of lithium on neuropeptide Y, neurokinin A and substance P in brain and peripheral tissues of rat. Lithium 5: 241–247

Maubach KA, Martin K, Chicchi G, Harrison T, Wheeldon A, Swain CJ, Cumberbatch MJ, Rupniak NM, Seabrook GR (2002) Chronic substance P (NK1) receptor antagonist and conventional antidepressant treatment increases burst firing of monoamine neurones in the locus coeruleus. Neuroscience 109: 609–617

McLean S (2005) Do substance P and the NK1 receptor have a role in depression and anxiety? Curr Pharm Des 11: 1529–1547

Michael-Titus AT, Blackburn D, Connolly Y, Priestley JV, Whelpton R (1999) N- and C-terminal substance P fragments: differential effects on striatal substance P binding and NK1 receptor internalization. Neuroreport 10: 2209–2213

Michelot R, Leviel V, Torrens Y, Glowinski J, Cheramy A (1979) In vivo release of substance P in the cat substantia nigra. Neurosci Lett 15: 141–146

Millan MJ, Lejeune F, De Nanteuil G, Gobert A (2001) Selective blockade of neurokinin (NK)(1) receptors facilitates the activity of adrenergic pathways projecting to frontal cortex and dorsal hippocampus in rats. J Neurochem 76: 1949–1954

Mitsuhashi M, Ohashi Y, Shichijo S, Christian C, Sudduth-Klinger J, Harrowe G, Payan DG (1992) Multiple intracellular signaling pathways of the neuropeptide substance P receptor. J Neurosci Res 32: 437–443

Mitsushio H, Takashima M, Mataga N, Toru M (1988) Effects of chronic treatment with trihexyphenidyl and carbamazepine alone or in combination with haloperidol on substance P content in rat brain: a possible implication of substance P in affective disorders. J Pharmacol Exp Ther 245: 982–989

Mochizuki-Oda N, Nakajima Y, Nakanishi S, Ito S (1994) Characterization of the substance P receptor-mediated calcium influx in cDNA transfected Chinese hamster ovary cells. A possible role of inositol 1,4,5-trisphosphate in calcium influx. J Biol Chem 269: 9651–9658

Molewijk HE, Hartog K, van der Poel AM, Mos J, Olivier B (1996) Reduction of guinea pig pup isolation calls by anxiolytic and antidepressant drugs. Psychopharmacology 128: 31–38

Morilak DA, Morris M, Chalmers J (1988) Release of substance P in the nucleus tractus solitarius measured by in vivo microdialysis: response to stimulation of the aortic depressor nerves in rabbit. Neurosci Lett 94: 131–137

Muigg P, Sartori SB, Sparber T, Landgraf R, Singewald N (2005) Reduced depression-like behaviour in HAB rats after NK1-receptor antagonist treatment is associated with an altered neuronal activation pattern in specific brain areas. Abstract at the Meeting of the Austrian Neuroscience Association

Nagashima A, Takano Y, Masui H, Kamiya H (1987) Evidence that neurokinin A (substance K) neurons project from the striatum to the substantia nigra in rats. Neurosci Lett 77: 103–108

Nakajima Y, Tsuchida K, Negishi M, Ito S, Nakanishi S (1992) Direct linkage of three tachykinin receptors to stimulation of both phosphatidylinositol hydrolysis and cyclic AMP cascades in transfected Chinese hamster ovary cells. J Biol Chem 267: 2437–2442

Nakamura H, Moroji T, Nohara S, Nakamura H, Okada A (1990) Effects of whole-body vibration stress on substance P- and neurotensin-like immunoreactivity in the rat brain. Environ Res 52: 155–163

Nakanishi S (1991) Mammalian tachykinin receptors. Annu Rev Neurosci 14: 123–136

Nakanishi S, Nakajima Y, Yokota Y (1993) Signal transduction and ligand-binding domains of the tachykinin receptors. Regul Pept 46: 37–42

Nakaya Y, Kaneko T, Shigemoto R, Nakanishi S, Mizuno N (1994) Immunohistochemical localization of substance P receptor in the central nervous system of the adult rat. J Comp Neurol 347: 249–274

Nawa H, Hirose T, Takashima H, Inayama S, Nakanishi S (1983) Nucleotide sequences of cloned cDNAs for two types of bovine brain substance P precursor. Nature 306: 32–36

Nawa H, Kotani H, Nakanishi S (1984) Tissue-specific generation of two preprotachykinin mRNAs from one gene by alternative RNA splicing. Nature 312: 729–734

Nemeroff CB (2003) Anxiolytics: past, present, and future agents. J Clin Psychiatry 64 [Suppl 3]: 3–6

Neumann ID, Kromer SA, Toschi N, Ebner K (2000) Brain oxytocin inhibits the (re)activity of the hypothalamo-pituitary-adrenal axis in male rats: involvement of hypothalamic and limbic brain regions. Regul Pept 96: 31–38

Nicholas AP, Pieribone VA, Arvidsson U, Hökfelt T (1992) Serotonin-, substance P- and glutamate/aspartate-like immunoreactivities in medullo-spinal pathways of rat and primate. Neuroscience 48: 545–559

Nikolaus S, Huston JP, Hasenöhrl RU (1999a) The neurokinin-1 receptor antagonist WIN51,708 attenuates the anxiolytic-like effects of ventralpallidal substance P injection. Neuroreport 10: 2293–2296

Nikolaus S, Huston JP, Hasenöhrl RU (1999b) Reinforcing effects of neurokinin substance P in the ventral pallidum: mediation by the tachykinin NK1 receptor. Eur J Pharmacol 370: 93–99

Nikolaus S, Huston JP, Hasenöhrl RU (2000) Anxiolytic-like effects in rats produced by ventral pallidal injection of both N- and C-terminal fragments of substance P. Neurosci Lett 283: 37–40

Oblin A, Zivkovic B, Bartholini G (1984) Involvement of the D-2 dopamine receptor in the neuroleptic-induced decrease in nigral substance P. Eur J Pharmacol 105: 175–177

Orosz D, Bennett JP Jr (1990) Baseline and apomorphine-induced extracellular levels of nigral substance P are increased in an animal model of Parkinson's disease. Eur J Pharmacol 182: 509–514

Otsuka M, Yoshioka K (1993) Neurotransmitter functions of mammalian tachykinins. Physiol Rev 73: 229–308

Page NM (2004) Hemokinins and endokinins. Cell Mol Life Sci 61: 1652–1663

Papp M, Vassout A, Gentsch C (2000) The NK1-receptor antagonist NKP608 has an antidepressant-like effect in the chronic mild stress model of depression in rats. Behav Brain Res 115: 19–23

Pernow B (1983) Substance P. Pharmacol Rev 35: 85–141

Pickel VM, Joh TH, Reis DJ, Leeman SE, Miller RJ (1979) Electron microscopic localization of substance P and enkephalin in axon terminals related to dendrites of catecholaminergic neurons. Brain Res 160: 387–400

Potts JT, Fuchs IE, Li J, Leshnower B, Mitchell JH (1999) Skeletal muscle afferent fibres release substance P in the nucleus tractus solitarii of anaesthetized cats. J Physiol 514: 829–841

Potts JT, Fuchs IE (2001) Naturalistic activation of barosensitive afferents release substance P in the nucleus tractus solitarius of the cat. Brain Res 893: 155–164

Quartara L, Maggi CA (1997) The tachykinin NK1 receptor. Part I: ligands and mechanisms of cellular activation. Neuropeptides 31: 537–563

Quartara L, Maggi CA (1998) The tachykinin NK1 receptor. Part II: Distribution and pathophysiological roles. Neuropeptides 32: 1–49

Regoli D, Boudon A, Fauchere JL (1994) Receptors and antagonists for substance P and related peptides. Pharmacol Rev 46: 551–599

Ribeiro-da-Silva A, Hökfelt T (2000) Neuroanatomical localisation of substance P in the CNS and sensory neurons. Neuropeptides 34: 256–271

Ribeiro RL, De Lima TC (2002) Participation of GABAA receptors in the modulation of experimental anxiety by tachykinin agonists and antagonists in mice. Prog Neuropsychopharmacol Biol Psychiatry 26: 861–869

Riley LA, Hart RP, Jonakait GM (1991) Both zimelidine and clorgyline decrease preprotachykinin mRNA in adult medullary raphe nuclei. Mol Cell Neurosci 2: 139–144

Roberts GW, Woodhams PL, Polak JM, Crow TJ (1982) Distribution of neuropeptides in the limbic system of the rat: the amygdaloid complex. Neuroscience 7: 99–131

Rodgers RJ, Gentsch C, Hoyer D, Bryant E, Green AJ, Kolokotroni KZ, Martin JL (2004) The NK1 receptor antagonist NKP608 lacks anxiolytic-like activity in Swiss-Webster mice exposed to the elevated plus-maze. Behav Brain Res 154: 183–192

Rogers SD, Salak-Johnson JL, Schwei MJ, Pomonis JD, Mantyh PW (2000) Reduced anxiety related behavior following ablation of amygdala neurons expressing substance P receptor. Soc Neurosci Abstr

Rosen A, Brodin K, Eneroth P, Brodin E (1992) Short-term restraint stress and s.c. saline injection alter the tissue levels of substance P and cholecystokinin in the periaqueductal grey and limbic regions of rat brain. Acta Physiol Scand 146: 341–348

Rosen A, Franck J, Brodin E (1995) Effects of acute systemic treatment with the 5 HT-uptake blocker alaproclate on tissue levels and release of substance P in rat periaqueductal grey. Neuropeptides 28: 317–324

Rosen A, Zhang YX, Lund I, Lundeberg T, Yu LC (2004) Substance P microinjected into the periaqueductal gray matter induces antinociception and is released following morphine administration. Brain Res 1001: 87–94

Rupniak NM (2002) New insights into the antidepressant actions of substance P (NK1 receptor) antagonists. Can J Physiol Pharmacol 80: 489–494

Rupniak NM, Boyce S, Williams AR, Cook G, Longmore J, Seabrook GR, Caeser M, Iversen SD, Hill RG (1993) Antinociceptive activity of NK1 receptor antagonists: non-specific effects of racemic RP67580. Br J Pharmacol 110: 1607–1613

Rupniak NM, Williams AR (1994) Differential inhibition of foot tapping and chromodacryorrhoea in gerbils by central nervous system penetrant and non-penetrant tachykinin NK1 receptor antagonists. Eur J Pharmacol 265: 179–183

Rupniak NM, Carlson EC, Harrison T, Oates B, Seward E, Owen S, de Felipe C, Hunt S, Wheeldon A (2000) Pharmacological blockade or genetic deletion of substance P (NK1) receptors attenuates neonatal vocalisation in guinea-pigs and mice. Neuropharmacology 39: 1413–1421

Rupniak NM, Carlson EJ, Webb JK, Harrison T, Porsolt RD, Roux S, de Felipe C, Hunt SP, Oates B, Wheeldon A (2001) Comparison of the phenotype of NK1R−/− mice with pharmacological blockade of the substance P (NK1) receptor in assays for antidepressant and anxiolytic drugs. Behav Pharmacol 12: 497–508

Rupniak NM, Webb JK, Fisher A, Smith D, Boyce S (2003a) The substance P (NK1) receptor antagonist L-760735 inhibits fear conditioning in gerbils. Neuropharmacology 44: 516–523

Rupniak NM, Carlson EJ, Shepheard S, Bentley G, Williams AR, Hill A, Swain C, Mills SG, Di Salvo J, Kilburn R, Cascieri MA, Kurtz MM, Tsao KL, Gould SL, Chicchi GG (2003b) Comparison of the functional blockade of rat substance P (NK1) receptors by GR205171, RP67580, SR140333 and NKP-608. Neuropharmacology 45: 231–241

Ryckmans T, Balancon L, Berton O, Genicot C, Lamberty Y, Lallemand B, Pasau P, Pirlot N, Quere L, Talaga P (2002a) First dual NK(1) antagonists-serotonin reuptake inhibitors: synthesis and SAR of a new class of potential antidepressants. Bioorg Med Chem Lett 12: 261–264

Ryckmans T, Berton O, Grimee R, Kogej T, Lamberty Y, Pasau P, Talaga P, Genicot C (2002b) Dual NK(1) antagonists – serotonin reuptake inhibitors as potential antidepressants. Part 2: SAR and activity of benzyloxyphenethyl piperazine derivatives. Bioorg Med Chem Lett 12: 3195–3198

Sachais BS, Snider RM, Lowe JA 3rd, Krause JE (1993) Molecular basis for the species selectivity of the substance P antagonist CP-96,345. J Biol Chem 268: 2319–2323

Saffroy M, Beaujouan JC, Torrens Y, Besseyre J, Bergstrom L, Glowinski J (1988) Localization of tachykinin binding sites (NK1, NK2, NK3 ligands) in the rat brain. Peptides 9: 227–241

Saffroy M, Beaujouan JC, Petitet F, Torrens Y, Glowinski J (1994) Differential localization of 3H-[Pro9]SP binding sites in the guinea pig and rat brain. Brain Res 633: 317–325

Saffroy M, Torrens Y, Glowinski J, Beaujouan JC (2003) Autoradiographic distribution of tachykinin NK2 binding sites in the rat brain: comparison with NK1 and NK3 binding sites. Neuroscience 116: 761–773

Saleh TM (1997) Visceral afferent stimulation-evoked changes in the release of peptides into the parabrachial nucleus in vivo. Brain Res 778: 56–63

Santarelli L, Gobbi G, Debs PC, Sibille ET, Blier P, Hen R, Heath MJ (2001) Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. Proc Natl Acad Sci USA 98: 1912–1917

Santarelli L, Gobbi G, Blier P, Hen R (2002) Behavioral and physiologic effects of genetic or pharmacologic inactivation of the substance P receptor (NK1). J Clin Psychiatry 63 [Suppl 11]: 11–17

Saria A (1999) The tachykinin NK1 receptor in the brain: pharmacology and putative functions. Eur J Pharmacol 375: 51–60

Sartori SB, Burnet PW, Sharp T, Singewald N (2004) Evaluation of the effect of chronic antidepressant treatment on neurokinin-1 receptor expression in the rat brain. Neuropharmacology 46: 1177–1183

Sartori SB, Ebner K, Muigg P, Landgraf R, Singewald N (2005) Differences in substance P neurotransmission between rats with high vs. low trait anxiety/depression. J Neurochem 94 [Suppl 2]: 229

Seabrook GR, Fong TM (1993) Thapsigargin blocks the mobilisation of intracellular calcium caused by activation of human NK1 (long) receptors expressed in Chinese hamster ovary cells. Neurosci Lett 152: 9–12

Sekizawa S, Joad JP, Bonham AC (2003) Substance P presynaptically depresses the transmission of sensory input to bronchopulmonary neurons in the guinea pig nucleus tractus solitarii. J Physiol 552: 547–559

Sergeyev V, Fetissov S, Mathe AA, Jimenez PA, Bartfai T, Mortas P, Gaudet L, Moreau JL, Hökfelt T (2005) Neuropeptide expression in rats exposed to chronic mild stresses. Psychopharmacology 178: 115–124

Severini C, Improta G, Falconieri-Erspamer G, Salvadori S, Erspamer V (2002) The tachykinin peptide family. Pharmacol Rev 54: 285–322

Shirayama Y, Mitsushio H, Takashima M, Ichikawa H, Takahashi K (1996) Reduction of substance P after chronic antidepressants treatment in the striatum, substantia nigra and amygdala of the rat. Brain Res 739: 70–78

Shults CW, Quirion R, Jensen RT, Moody TW, O'Donohue TL, Chase TN (1982) Autoradiographic localization of substance P receptors using 125I substance P. Peptides 3: 1073–1075

Shults CW, Quirion R, Chronwall B, Chase TN, O'Donohue TL (1984) A comparison of the anatomical distribution of substance P and substance P receptors in the rat central nervous system. Peptides 5: 1097–1128

Siegel RA, Duker EM, Fuchs E, Pahnke U, Wuttke W (1984) Responsiveness of mesolimbic, mesocortical, septal and hippocampal cholecystokinin and substance P neuronal systems to stress, in the male rat. Neurochem Int 6: 783–789

Siegel RA, Duker EM, Pahnke U, Wuttke W (1987) Stress-induced changes in cholecystokinin and substance P concentrations in discrete regions of the rat hypothalamus. Neuroendocrinology 46: 75–81

Singewald N, Kaehler ST, Philippu A (1999) Noradrenaline release in the locus coeruleus of conscious rats is triggered by drugs, stress and blood pressure changes. Neuroreport 10: 1583–1587

Sivam SP, Krause JE, Takeuchi K, Li S, McGinty JF, Hong JS (1989) Lithium increases rat striatal beta- and gamma-preprotachykinin messenger RNAs. J Pharmacol Exp Ther 248: 1297–1301

Smith DW, Hewson L, Fuller P, Williams AR, Wheeldon A, Rupniak NM (1999) The substance P antagonist L-760,735 inhibits stress-induced NK(1) receptor internalisation in the basolateral amygdala. Brain Res 848: 90–95

Snider RM, Constantine JW, Lowe JA 3rd, Longo KP, Lebel WS, Woody HA, Drozda SE, Desai MC, Vinick FJ, Spencer RW, et al. (1991) A potent nonpeptide antagonist of the substance P (NK1) receptor. Science 251: 435–437

Spooren WP, Schoeffter P, Gasparini F, Kuhn R, Gentsch C (2002) Pharmacological and endocrinological characterisation of stress-induced hyperthermia in singly housed mice using classical and candidate anxiolytics (LY314582, MPEP and NKP608). Eur J Pharmacol 435: 161–170

Srinivasan M, Goiny M, Pantaleo T, Lagercrantz H, Brodin E, Runold M, Yamamoto Y (1991) Enhanced in vivo release of substance P in the nucleus tractus solitarii during hypoxia in the rabbit: role of peripheral input. Brain Res 546: 211–216

Steinberg R, Alonso R, Rouquier L, Desvignes C, Michaud JC, Cudennec A, Jung M, Simiand J, Griebel G, Emonds-Alt X, Le Fur G, Soubrie P (2002) SSR240600 (R)-2-(1-[2-[4-[2-[3,5-bis(trifluoromethyl)phenyl acetyl]-2-(3,4-dichloro phenyl)-2-morpholinyl]ethyl]-4-piperidinyl)-2-methylpropanamide], a centrally active nonpeptide antagonist of the tachykinin neurokinin 1 receptor: II. Neurochemical and behavioral characterization. J Pharmacol Exp Ther 303: 1180–1188

Stenfors C, Srinivasan GR, Theodorsson E, Mathe AA (1992) Electroconvulsive stimuli and brain peptides: effect of modification of seizure duration on neuropeptide Y, neurokinin A, substance P and neurotensin. Brain Res 596: 251–258

Stiller CO, Linderoth B, O'Connor WT, Franck J, Falkenberg T, Ungerstedt U, Brodin E (1995) Repeated spinal cord stimulation decreases the extracellular level of gamma-aminobutyric acid in the periaqueductal gray matter of freely moving rats. Brain Res 699: 231–241

Stout SC, Owens MJ, Nemeroff CB (1999) Preprotachykinin-A and neurokinin 1 receptor mRNA expression are unchanged in rat forebrain after chronic antidepressant administration. Soc Neurosci Abstr 25: 1066

Stout SC, Owens MJ, Nemeroff CB (2001) Neurokinin(1) receptor antagonists as potential antidepressants. Annu Rev Pharmacol Toxicol 41: 877–906

Takayama H, Ota Z, Ogawa N (1986) Effect of immobilization stress on neuropeptides and their receptors in rat central nervous system. Regul Pept 15: 239–248

Takeda Y, Blount P, Sachais BS, Hershey AD, Raddatz R, Krause JE (1992) Ligand binding kinetics of substance P and neurokinin A receptors stably expressed in Chinese hamster ovary cells and evidence for differential stimulation of inositol 1,4,5-trisphosphate and cyclic AMP second messenger responses. J Neurochem 59: 740–745

Teixeira RM, Santos AR, Ribeiro SJ, Calixto JB, Rae GA, De Lima TC (1996) Effects of central administration of tachykinin receptor agonists and antagonists on plus-maze behavior in mice. Eur J Pharmacol 311: 7–14

Teixeira RM, De Lima TC (2003) Involvement of tachykinin NK1 receptor in the behavioral and immunological responses to swimming stress in mice. Neuropeptides 37: 307–315

Teixeira RM, Duarte FS, De Lima TC (2004) Behavioral and immunological effects of substance P in female and male mice. Pharmacol Biochem Behav 79: 1–9

Torrens Y, Beaujouan JC, Besson MJ, Michelot R, Glowinski J (1981) Inhibitory effects of GABA, L-glutamic acid and nicotine on the potassium-evoked release of substance P in substantia nigra slices of the rat. Eur J Pharmacol 71: 383–392

Unger T, Carolus S, Demmert G, Ganten D, Lang RE, Maser-Gluth C, Steinberg H, Veelken R (1988) Substance P induces a cardiovascular defense reaction in the rat: pharmacological characterization. Circ Res 63: 812–820

Valentino RJ, Bey V, Pernar L, Commons KG (2003) Substance P acts through local circuits within the rat dorsal raphe nucleus to alter serotonergic neuronal activity. J Neurosci 23: 7155–7159

Van der Hart MG, Czeh B, de Biurrun G, Michaelis T, Watanabe T, Natt O, Frahm J, Fuchs E (2002) Substance P receptor antagonist and clomipramine prevent stress-induced alterations in cerebral metabolites, cytogenesis in the dentate gyrus and hippocampal volume. Mol Psychiatry 7: 933–941

Van der Hart MG, de Biurrun G, Czeh B, Rupniak NM, den Boer JA, Fuchs E (2005) Chronic psychosocial stress in tree shrews: effect of the substance P (NK1 receptor) antagonist L-760735 and clomipramine on endocrine and behavioral parameters. Psychopharmacology 181: 207–216

Varty GB, Cohen-Williams ME, Morgan CA, Pylak U, Duffy RA, Lachowicz JE, Carey GJ, Coffin VL (2002) The gerbil elevated plus-maze II: anxiolytic-like effects of selective neurokinin NK1 receptor antagonists. Neuropsychopharmacology 27: 371–379

Varty GB, Cohen-Williams ME, Hunter JC (2003) The antidepressant-like effects of neurokinin NK1 receptor antagonists in a gerbil tail suspension test. Behav Pharmacol 14: 87–95

Vassout A, Veenstra S, Hauser K, Ofner S, Brugger F, Schilling W, Gentsch C (2000) NKP608: a selective NK-1 receptor antagonist with anxiolytic-like effects in the social interaction and social exploration test in rats. Regul Pept 96: 7–16

Vendruscolo LF, Takahashi RN, Bruske GR, Ramos A (2003) Evaluation of the anxiolytic-like effect of NKP608, a NK1-receptor antagonist, in two rat strains that differ in anxiety-related behaviors. Psychopharmacology 170: 287–293

Vertes RP (1991) A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. J Comp Neurol 313: 643–668

Walker PD, Riley LA, Hart RP, Jonakait GM (1991) Serotonin regulation of neostriatal tachykinins following neonatal 6-hydroxydopamine lesions. Brain Res 557: 31–36

Warden MK, Young WS (1988) Distribution of cells containing mRNAs encoding substance P and neurokinin B in the rat central nervous system. J Comp Neurol 272: 90–113

Xin L, Geller EB, Liu-Chen LY, Chen C, Adler MW (1997) Substance P release in the rat periaqueductal gray and preoptic anterior hypothalamus after noxious cold stimulation: effect of selective mu and kappa opioid agonists. J Pharmacol Exp Ther 282: 1055–1063

Zachrisson O, Mathe AA, Lindefors N (1997) Decreased levels of preprotachykinin-A and tachykinin NK1 receptor mRNA in specific region of the rat striatum after electroconvulsive stimuli. Eur J Pharmacol 319: 191–195

Zernig G, Dietrich H, Maggi CA, Saria A (1992) The substance P (NK1) receptor antagonist (±)-CP-96,345 causes sedation and motor impairment in Swiss albino mice in the black-and-white box behavioral paradigm. Neurosci Lett 143: 169–172

Zhang Y, Lu L, Furlonger C, Wu GE, Paige CJ (2000) Hemokinin is a hematopoietic-specific tachykinin that regulates B lymphopoiesis. Nat Immunol 1: 392–397

Zocchi A, Varnier G, Arban R, Griffante C, Zanetti L, Bettelini L, Marchi M, Gerrard PA, Corsi M (2003) Effects of antidepressant drugs and GR 205171, an neurokinin-1 (NK1) receptor antagonist, on the response in the forced swim test and on monoamine extracellular levels in the frontal cortex of the mouse. Neurosci Lett 345: 73–76

Authors' address: Dr. Karl Ebner, Department of Pharmacology and Toxicology, Institute of Pharmacy, Center for Molecular Biosciences Innsbruck (CMBI), Leopold-Franzens-University of Innsbruck, Peter Mayr-Straße 1, A-6020 Innsbruck, Austria,
Fax: +43-512-507-2760; E-mail: karl.ebner@uibk.ac.at