

The role of substance P in stress and anxiety responses

Review Article

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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 70s (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	Lindfors et al. (1989a)
	via MD	Striatum	↑ SP	+100	Lindfors et al. (1989b)
	via MD	SN	↑ SP	+450	Lindfors 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	Lindfors 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		Lindfors et al. (1989b)
6-OHDA-lesion	MD	SN	↓ SP		Lindfors 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 (μ M 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 cuprophane and hemophane membranes have been proved to be suitable for the determination of extracellular SP efflux in distinct forebrain 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 forebrain 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 detected (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 (Lindfors 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 McCarron, 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 is 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 McCarron, 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 wild-type 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	Hasenöhl et al. (1998), Nikolaus et al. (1999a, 2000)
	NbasVP	Rats	Social interaction	Anxiolytic	Hasenöhl 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)
DiMethyl-C7	ICV	Guinea pigs	Neonatal vocalisation	Aversive	Kramer et al. (1998), Rupniak et al. (2000)
	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 intracerebroventricularly 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.	Maternal 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)
L-733,060	Guinea pigs	s.c.	Maternal separation	Anxiolytic	Rupniak et al. (2000)
	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
L-742,694	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)
	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)
	Gerbils	i.v.	Hind foot tapping	No effect	Rupniak and Williams (1994)
	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)
L-743,310 L-760,735	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)
	Rats	MeA	Elevated plus-maze	Anxiolytic	Ebner et al. (2004a)
	Rats	LS	Forced swim	Antidepressant	Ebner et al. (2004b)
L-822,429	Rats	s.c.	Operant conflict paradigm	No effect	Loiseau et al. (2003)
	Rats (HAB)	i.p.	Forced swim	Antidepressant	Sartori et al. (2005)
	Guinea pigs	s.c.	Maternal separation	No effect	Rupniak et al. (2000)
	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)
LY-303,870 NKP-608	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)
	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)
	Tree shrews	p.o. ^a	Chronic social stress	Antidepressant (not sign.)	Czeh et al. (2005a)
	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. intravenous; p.o. per os; s.c. subcutaneous; ^achronic 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-HT_{1A} 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-HT_{1A} 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 α 2-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 α 2 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 hippo-

campus 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). Inconsistent 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 tranylcypromine (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 inconsistent 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 carbamazepine, 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 psychotropic 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/NK1 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 CO₂-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.

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