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ORIGINAL ARTICLE

Stress-induced release of substance P in the locus coeruleus modulates cortical noradrenaline release

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Abstract Several lines of evidence implicate the neuropeptide substance P (SP) in the modulation of emotional behavior. Interaction between SP and noradrenergic systems has been proposed to be important in the regulation of stress, depression, and anxiety mechanisms; however, most evidence so far is based on studies in unchallenged and/or anesthetized animals. Thus, by using a dual-probe microdialysis approach in freely moving animals, the aim of the present study was to investigate whether a relevant stressor can trigger the release of SP in the locus coeruleus (LC) and whether and how this response modulates noradrenaline (NA) transmission both in the LC and in the medial prefrontal cortex (mPFC), an important LC terminal region involved in emotional processing. While confirming previous reports that neurokinin 1 receptor (NK1R) antagonists activate cortical noradrenergic transmission under resting conditions, we present evidence that this interaction is opposite during stress challenge. Our results show that exposure to forced swimming considerably enhanced the release of SP and NA in the LC. Administration of a selective NK1R antagonist into the LC potentiated this NA response within the LC but abolished the stress-induced increase in NA release within the mPFC. These findings demonstrate stress-induced increase in endogenous extracellular SP levels within the LC exerting a facilitatory effect on the noradrenergic pathway to the mPFC. The attenuation of stress-induced hyperactivation of this pathway by NK1R antagonists, presumably via enhancing NA and autoinhibition in the LC, may contribute to the therapeutic efficacy of these drugs known to ameliorate symptoms of stress-related disorders.

Keywords Medial prefrontal cortex · Locus coeruleus · Substance P · Stress · NK1 receptor antagonist · Neuromodulation · Microdialysis · Rat

Abbreviations

LC	locus coeruleus
NA	noradrenaline
NK1R	neurokinin 1 receptor
SP	substance P
mPFC	medial prefrontal cortex

Introduction

In the search for new therapeutic targets for depression and other stress-related disorders, much attention has focused on the neuropeptide substance P (SP) opening up the possibility of a therapeutic effect for neurokinin 1 receptor (NK1R) antagonists in the treatment of these disorders (Rupniak 2002; Herpfer and Lieb 2005; Ebner and Singewald 2006). In two high-quality clinical trials, NK1R antagonists have been shown to relieve the symptoms of depression and anxiety (Kramer et al. 1998, 2004), although some conflicting results have been obtained more recently (Keller et al. 2006). While suggesting that NK1R antagonists may represent a putative new pharmacological

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class of antidepressant and anxiolytic drugs, these conflicting clinical data also call for additional studies to better understand the precise mechanisms mediating such effects. A key question to be addressed is whether NKR-mediated effects involve alterations in monoaminergic neurotransmission, believed to underlie the therapeutic effects of most currently available antidepressant drugs. Support for such a functional interaction is provided by the substantial anatomical overlap of the SP/NK1R system with monoaminergic systems (Adell 2004; Blier et al. 2004). For example, locus coeruleus (LC) neurons, the primary source of noradrenergic fibers in the brain, are targeted by SP-containing fibers forming axo-dendritic contacts with tyrosine hydroxylase-positive cells (Ljungdahl et al. 1978; Pickel et al. 1979). Moreover, NK1Rs are highly expressed in the LC (Shults et al. 1984; Saffroy et al. 1994), where they are localized mainly on noradrenergic cells (Hahn and Bannon 1999; Chen et al. 2000; Ma and Bleasdale 2002). Although in vitro studies have demonstrated that SP excites noradrenergic neurons in the LC (Guyenet and Aghajanian 1977; Cheeseman et al. 1983), an effect prevented by NK1R blockade (McLean et al. 1993), more recent in vivo studies have shown also an increase in the firing rate of LC noradrenergic neurons after NK1R antagonist administration (Millan et al. 2001; Blier et al. 2004; but see also Haddjeri and Blier 2000). Support for the latter finding comes from studies on NK1R knockout mice showing an increase in burst activity of noradrenergic neurons in the LC as compared to wild-type mice (Gobbi and Blier 2005); LC burst activity is thought to be correlated with an enhancement of noradrenaline (NA) release in terminal areas (Florin-Lechner et al. 1996). Indeed, in a very recent in vivo microdialysis study, it has been shown that basal NA efflux was higher in the cerebral cortex of knockout mice than in wild-type controls (Herpfer et al. 2005). However, none of these studies have investigated the effects of NK1R activation or blockade on noradrenergic transmission in conscious, freely moving animals during situations where SP pathways are thought to be highly activated, including exposure to aversive and stressful situations (Ebner et al. 2004). This issue seems particularly relevant as stress represents an important pathogenetic factor in many psychiatric disorders including depression and anxiety disorders (van Praag 2005), and NK1R antagonists have been shown to be particularly effective on "stressed" or pathophysiologically deranged systems (Hökfelt et al. 2000; Ebner et al. 2004). It is interesting to note that it has been shown in rats that intracerebroventricular administration of two different NK1R antagonists attenuated the stress-induced increase in c-Fos expression (used as marker for neuronal activation) in the LC (Hahn and Bannon 1999; but see also Hahn and Bannon 1998). This finding is consistent with our recent data from rats bred for extremes in high-anxiety-related behavior where we found also an attenuated stress-induced c-Fos expression in the LC after systemic administration of a NK1R antagonist (Muigg et al. 2006). Thus, it is conceivable that the SP/NK1R system modulates noradrenergic neurotransmission differently under basal and stress conditions, respectively. However, the effects of endogenous SP on central NA release under stress conditions have not been demonstrated so far.

On the basis of these observations, the intracerebral microdialysis experiments of this study were designed to investigate whether a stressful situation such as exposure to forced swimming triggers the release of SP in the LC, a key brain structure implicated in stress, anxiety, and depression (Stanford 1995; Bremner et al. 1996; Harro and Oreland 2001). Furthermore, we studied whether locally released SP within the LC is involved in the regulation of the local release of NA during basal and stress conditions. To further evaluate the putative role of endogenous SP on noradrenergic transmission, we also examined the effects of intracoerulear NK1R blockade on the NA release in the medial prefrontal cortex (mPFC), a main LC projection area (Morrison et al. 1979), which is thought to be involved in the regulation of behavioral, neuroendocrine, and autonomic responses during aversive and stressful situations (Heidbreder and Groenewegen 2003).

Materials and methods

Animals

Experiments were carried out on adult male Sprague–Dawley rats (250–350 g). Before use, the animals were housed in groups of four to six under controlled laboratory conditions (12:12 h light/dark cycle with lights on at 7:00 A.M., 21±1°C, 60% humidity, pelleted food and water ad libitum) for at least 1 week after delivery from the supplier. The experimental studies described here were designed to minimize animal suffering and number of animals used and were approved by the Bundesministerium für Wissenschaft und Verkehr, Kommission für Tierversuchsangelegenheiten, Austria.

Surgery

Rats were anesthetized by intraperitonal injections of sodium pentobarbital (40 mg/kg) and ketamine (50 mg/kg), and a microdialysis probe (hemophan membrane, 18 kDa molecular cutoff, 210 μm outer diameter) was stereotaxically implanted according to the stereotaxic atlas of Paxinos and Watson (1998). The probe with an active surface length of 1 mm was positioned with its U-shaped tip reaching the right LC

(Fig. 1b; implantation coordinates: 0.7 mm posterior to lambda, 1.0 mm lateral to the midline, 7.0 mm below the surface of the skull). In experiment 2, a second probe with an active surface length of 2 mm was implanted in the ipsilateral mPFC (Fig. 1a; implantation coordinates: 3.5 mm rostral to bregma, 0.6 mm lateral to the midline, 5.0 mm below the surface of the skull). The probes were fixed to the skull with two jeweler's screws and dental cement. The two endings of each probe were connected to approx. 5-cm-long pieces of PE-20 polyethylene tubing and fixed with dental cement.

Experimental protocol

After surgery, rats were housed individually in transparent Plexiglas cages until testing. They were handled for 3 min twice daily to familiarize them with the experimental procedure and to minimize nonspecific stress responses during the experiments. At least 24 h before the experiment, animals were kept in the experimental room and allowed to habituate. Experiments were performed between 9:00 A.M. and 4:00 P.M.

Experiment 1: effects of forced swimming on the release of SP within the LC

Two days after surgery, the microdialysis probe was connected to a syringe mounted onto a microinfusion pump and superfused with artificial cerebrospinal fluid (aCSF) of the following composition: 140 mM NaCl, 3.0 mM KCl, 1.25 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.2 mM Na_2HPO_4, 0.3 mM NaH_2PO_4, and 3.0 mM glucose (pH 7.2) at a rate of 6.5 μl/min. To minimize the binding of SP to the plastic surfaces and to reduce peptidase activity, bovine serum albumin (0.2%) and bacitracin (0.03%) were included in the perfusion medium. After an equilibration period of 120 min, 11 consecutive 30-min dialysates were collected directly into Eppendorf vials, which were stored at −80°C until assay. At the beginning of the fifth dialysis interval, animals (n=8) were transferred from their home cages to the swim tank for 10 min at 20°C with ongoing microdialysis; thereafter, they were gently dried using a towel and returned to their home cages. After stress exposure, animals were superfused for further 200 min. For the last dialysate, aCSF containing 100 mM KCl was used as a positive control to elicit local depolarization to confirm that the assay sensitivity was adequate to detect the release of SP.

Experiment 2: effects of intracoerulear NK1R blockade on basal and stress-induced NA release in the LC and mPFC

In the first series of experiments, three groups of animals were implanted with a microdialysis probe into the right LC. After the equilibration period and the collection of three basal samples, rats were dialyzed either with aCSF (controls, n=6) or aCSF containing the selective NK1R antagonist ([2-cyclopropoxy-5-(5-(trifluoromethyl)tetrazol-1-yl)benzyl]-(2-phenylpiperidin-3-yl)amine (L-822429; 10 μM; n=6; Ebner et al. 2004) or its low-affinity enantiomer (10 μM; n=7) at a rate of 3.3 μl/min similarly as described in experiment 1. To protect NA from decomposition, the tubes used to collect the microdialysates contained 3 μl of the following solution: 140 mM $HClO_4$, 1.6 mM HCl, 0.05 mM $Na_2S_2O_5$, 0.9 mM ethylenediamine tetraacetic acid, and 1.4 mM ethylene glycol tetraacetic acid. During dialysis period 7, rats were forced to swim for 10 min with ongoing microdialysis and collection of consecutive microdialysates. After the stress exposure, the

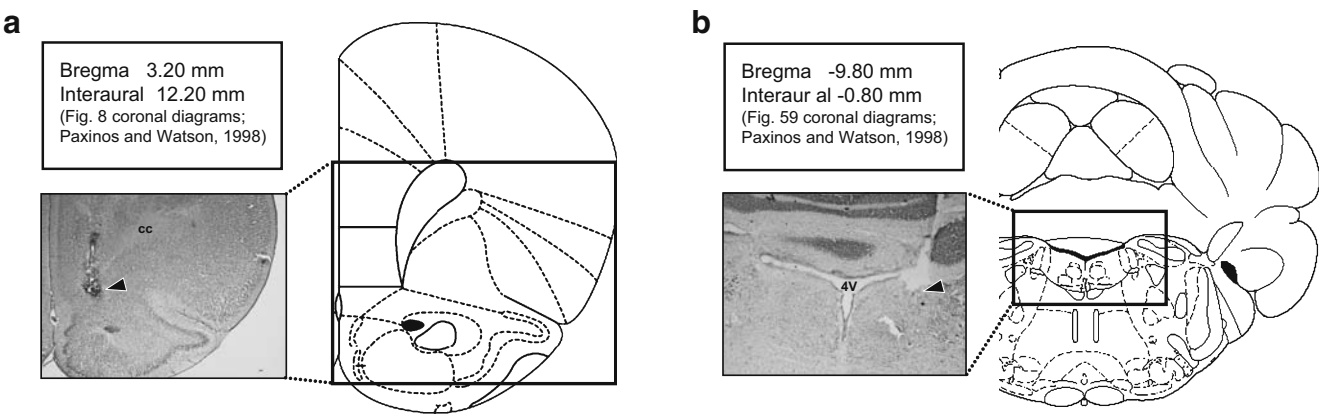

Fig. 1 A schematic drawing and a representative enlargement of a microphotograph of a cresyl-stained coronal section of the rat brain showing the localization of the tip of the membrane of a microdialysis probe (*arrowhead*) within the medial prefrontal cortex (**a**) and locus coeruleus (**b**). *cc* Corpus callosum; *4V* fourth ventricle

animals were returned to their home cages, and microdialysis was further continued for another 110 min.

In two additional groups of animals, a dual-probe microdialysis approach was applied for infusion of the NK1R antagonist into the LC and simultaneous detection of NA levels in the mPFC. After the equilibration period and the collection of three basal samples, rats were dialyzed within the LC either with aCSF (controls, n=6) or aCSF containing the selective NK1R antagonist (L-822429; 10 μM; n=6) before, during, and after forced swimming similar as described above. In the dual-probe microdialysis experiments, four channel swivel system were used to enable undisturbed movements of the animals.

Histology

At the end of the experiment, the animals were killed by an overdose of pentobarbital, and their brains were removed. For histological verification of the localization of the microdialysis probes, brains were sectioned, and 50-μm coronal sections were stained with cresyl violet (Fig. 1). The locations of LC and mPFC were determined on the basis of previous definitions of a brain atlas (Paxinos and Watson 1998). Judgment of successful implantation of probes into the LC and mPFC was made before analyzing data from release experiments.

Quantification of SP and NA in dialysates

The concentration of SP was measured in microdialysates by a highly sensitive and selective radioimmunoassay (detection limit: 0.3 fmol/sample; cross-reactivity of the antiserum RD2 with other related peptides, like neurokinin A and B, was less than 0.01 and 0, respectively) using synthetic SP (Sigma, St Louis, MO) as a standard and iodinated peptide (approximate specific activity 74 TBq/mmol=2,000 Ci/mmol; Amersham, UK) as a tracer (for a detailed description, see Ebner et al. 2004).

The NA content in microdialysates was determined by a radioenzymatic assay as described previously (Singewald and Philippu 1993). This assay involves COMT-catalyzed *O*-methylation using [^{3}H]*S*-adenosylmethionine as methyl donor and separation of the resulting [^{3}H]normetanephrine by thin-layer chromatography.

Statistics

Experimental subjects were included in the statistical analysis only if the microdialysis probes have been localized in the respective target brain area (Fig. 1). Statistical analysis was performed using a computer software package (GB-Stat 6.0, Dynamic Microsystems, Silver Springs, USA). Data were expressed either as raw data or as percentage of averaged baseline values and submitted to a one-way (SP, experiment 1) or two-way analysis of variance (ANOVA) with repeated measures (treatment×time, NA, experiment 2) followed by appropriate post-hoc analysis. Data are presented as means±SEM. Significance was accepted if P<0.05.

Results

Experiment 1: effects of forced swimming on the release of SP within the LC

The SP content of dialysates collected in the LC under basal conditions was more than one magnitude above the detection limit of the radioimmunoassay, reaching an average of 4.41±0.16 fmol/dialysate. As shown in Fig. 2, swim stress caused a significant increase in the SP content of microdialysates collected from the LC, which remained significantly elevated up to 60 min after onset of the stressor (one-way ANOVA $F_{7,\ 10}$=6.61, P<0.0001) and returned to prestress values within the next 30 min. Dialysis with aCSF containing 100 mM KCl caused an increase in SP release in the LC (by 250%).

Experiment 2: effects of intracoerulear NK1R blockade on basal and stress-induced NA release in the LC and mPFC

Basal NA concentrations in microdialysates collected in the LC were 0.79±0.01, 1.21±0.04, and 0.96±0.03 pg/dialysate for the aCSF, L-822429, and less active enantiomer group and in the mPFC, 0.48±0.01 and 0.47±0.01 pg/

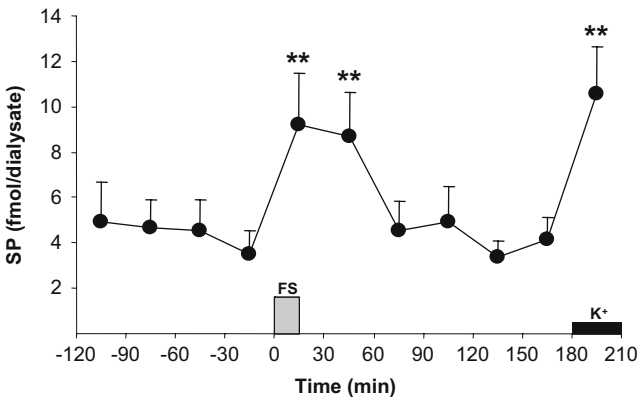

Fig. 2 Effects of forced swimming on the content of SP in 30-min microdialysates sampled consecutively in the locus coeruleus of freely moving male rats (means+SEM; n=8). Animals were exposed to forced swimming (*FS*, *gray-shaded bar*) for 10 min during collection of dialysis sample number 5. During sampling of the last microdialysate, the superfusion medium was changed to aCSF containing 100 mM KCl (*black bar*). *Double asterisk*, P<0.01 vs dialysates 1–4 (one-way ANOVA followed by Newman–Keuls post-hoc test)

dialysate for the aCSF and L-822429 group, respectively. As illustrated in Fig. 3a, forced swimming significantly increased NA efflux in the LC by 60% of baseline as reflected by a higher concentration of NA in microdialysates sampled during perfusion period 7 compared to dialysates sampled under basal conditions. Statistical analysis by two-way ANOVA with repeated measures indicated a significant effect of the factor group ($F_{2,\ 16}$= 3.51, P<0.05) and time ($F_{9,\ 144}$=13.43, P<0.0001) as well as a significant interaction between main factors ($F_{18,\ 144}$= 1.94, P=0.016). Post-hoc analysis was used to compare corresponding values of the different groups and revealed significant differences of NA concentrations during and after stress exposure between antagonist-treated animals and controls. Intracoerulear infusion of L-822429 significantly potentiated the swim stress-induced enhancement of the NA release in the LC, while the administration of the inactive enantiomer of the antagonist had no effect and did not differ to vehicle-treated controls. In contrast, basal NA release in the LC was not affected by the NK1R antagonist treatment.

Forced swimming also increased NA efflux in the mPFC by 50% of baseline. As shown in Fig. 3b, intracoerulear administration of the NK1R antagonist L-822429 caused changes of the NA release in the mPFC. Statistical analysis by two-way ANOVA with repeated measures revealed a significant effect of the factor time ($F_{9,\ 90}$=2.55, P=0.0116) and a significant interaction between main factors ($F_{9,\ 90}$= 5.59, P<0.0001). As illustrated in Fig. 3b, antagonist-treated animals show an increase in basal NA release and a suppression of the swim stress-induced enhancement of the NA release in the mPFC.

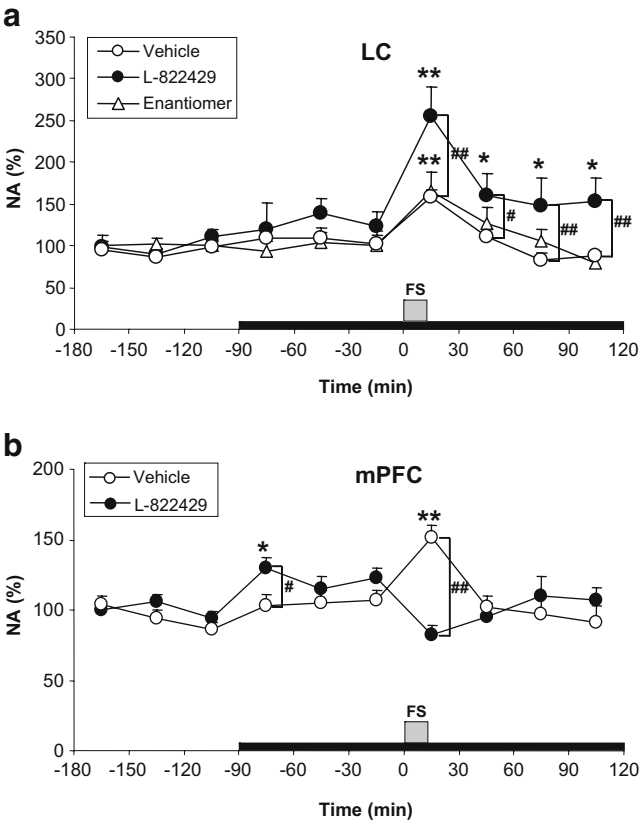

Fig. 3 Effects of intracoerulear NK1R antagonist administration on basal and swim stress-induced NA concentration in 30-min microdialysates collected in the locus coeruleus (**a**; *LC*) and medial prefrontal cortex (**b**; *mPFC*). The NK1R antagonist (L-822429, 10 μM, *black circles*) or its low-affinity enantiomer (10 μM, *open triangles*) was applied into the LC via retrodialysis for 210 min (*black bar*), starting 90 min before forced swimming (*FS*, *gray-shaded bars*). Animals were exposed to swim stress for 10 min during collection of dialysis sample number 7. Data (Mean+SEM) are expressed as the percentage of the averaged basal values. N=6–7 per group. *Asterisk*, P<0.05, *double asterisk*, P<0.01 vs basal (dialysates 1–3); *number sign*, P<0.05, *double number sign*, P<0.01 vs respective value in the vehicle (*aCSF*) treated controls (two-way ANOVA followed by Fisher's LSD post-hoc test)

Discussion

The results of the present study demonstrate for the first time that by using microdialysis in conjunction with a highly sensitive radioimmunoassay, SP release within the LC can be monitored in conscious rats both under stimulated and basal/resting conditions. SP release in the LC was considerably enhanced in response to a stressful situation by exposure of rats to forced swimming. Stress-induced SP release may suggest a possible functional role of SP within this brain area in the generation and modulation of appropriate stress responses. Numerous studies have implicated the LC in various physiological and pathological processes including stress, anxiety, and mood regulation (Stanford 1995; Bremner et al. 1996; Harro and Oreland 2001), and different neurotransmitter systems including neuropeptides are thought to be involved in the mediation of these LC responses (Singewald and Philippu 1998; Harro and Oreland, 2001). Although immunohistochemical studies demonstrated SP-positive fibers as well as binding sites within the LC (Shults et al. 1984; Nakaya et al. 1994), the present study provides the first direct in vivo evidence that SP is released under physiologically relevant conditions in the LC.

Because NK1Rs are highly expressed on noradrenergic LC neurons (Hahn and Bannon 1999; Chen et al. 2000; Ma and Bleasdale 2002), we examined whether endogenous SP has a functional role in the regulation of NA transmission during basal and stress conditions. After applying a selective NK1R antagonist into the LC of unstressed rats, slightly increased NA release in the mPFC was found indicating that the noradrenergic LC pathway activity is to some degree under a tonic inhibitory control of SP. This finding is in agreement with a previous microdialysis study in freely moving rats demonstrating increased NA release in

the mPFC after systemic NK1R antagonist administration (Millan et al. 2001). It is interesting to note that in other regions of the frontal cortex such as the motor cortex, no changes of NA release have been found either after intracoerulear (Ebner et al., unpublished) or systemic (Zocchi et al. 2003) NK1R antagonist administration. Microdialysis studies in anesthetized guinea pigs have observed an increase in NA release in the mPFC after intracoerulear NK1R activation, while NK1R blockade had no effect (Bert et al. 2002; Steinberg et al. 2002). One limitation of these microdialysis studies is the use of anesthetized animals. It is well known that anesthesia profoundly affects extracellular NA release in different brain areas including the mPFC (Pan and Lai 1995; Shimokawa et al. 1998; Kubota et al. 1999).

Increased release of NA in terminal regions of LC neurons such as mPFC is thought to result from increases in neuronal LC activity (Florin-Lechner et al. 1996; Devoto et al. 2005). Moreover, it has been shown that a linear relationship exists between the LC activity and NA dialysate concentrations in the mPFC (Berridge and Abercrombie 1999). Thus, the increase in NA release within the mPFC after NK1R blockade found in our study likely reflects activation of impulse flow in noradrenergic LC neurons. This finding is consistent with electrophysical studies demonstrating increased spiking activity and burst-firing activity of noradrenergic neurons in the LC of mice and rats after acute systemic NK1R antagonist administration (Millan et al. 2001; Blier et al. 2004). Notably, an increase in burst activity is thought to be correlated with an enhancement of NA release in terminal areas (Florin-Lechner et al. 1996). However, it should be mentioned that in several other studies, NK1R antagonists had no effect on basal firing rate of LC neurons (McLean et al. 1993; Haddjeri and Blier 2000; Maubach et al. 2002). Possible explanations for such inconsistent and discrepant findings might be species and methodological differences including the use of anesthetized vs awake animals or the use of drugs with different selectivity. Various antagonists used in these studies are known to exert additional unspecific pharmacological effects such as the blockade of Ca channels (McLean 1996; Saria 1999).

The mechanisms through which endogenous SP mediates this inhibitory tone on NA release in the mPFC of unstressed rats are still unclear. A direct action on noradrenergic neurons in the LC appears unlikely because SP administration into the LC has been shown to excite noradrenergic cells (Guyenet and Aghajanian 1977; Cheeseman et al. 1983). Thus, an indirect mechanism via activation of NK1Rs localized on non-noradrenergic LC neurons seems conceivable and has been suggested previously (Millan et al. 2001). Although the location of NK1Rs inhibitory to noradrenergic neurons remains to be determined, inhibitory neurotransmitter systems such as gamma-aminobutyric acid (GABA) are potential candidates. Indeed, there is some evidence for the presence of GABAergic interneurons within the LC (Iijima and Ohtomo 1988; van Bockstaele 1998). Furthermore, it has been shown that local administration of a $GABA_A$ agonist into the LC decreases while the administration of an antagonist increases NA release in the mPFC indicating that LC noradrenergic neurons terminating in the mPFC are under an inhibitory GABAergic tone (Kawahara et al. 1999). However, further studies are necessary to clarify whether NK1Rs are localized on GABAergic cells and whether endogenous SP indeed activates this GABAergic pathway within the LC.

Although modulation of noradrenergic neurons by SP/NK1R system is well documented (see above; Adell 2004; Blier et al. 2004), most studies have examined the effects of NK1R ligands on noradrenergic transmission under basal conditions only. The effects of this interaction during situations where SP pathways are thought to be highly activated, including exposure to aversive and stressful situations (Ebner et al. 2004), are so far largely unknown. Moreover, it is important to note that regulation of basal and evoked neurotransmitter release in a particular brain region may differ quantitatively and/or qualitatively, and effects on basal release may not generalize to effects on an activated system. Therefore, it was proposed that drug effects on resting neurotransmitter systems may be of little relevance for understanding the mechanisms responsible for their potential therapeutic effects (Sarter et al. 2007). To the best of our knowledge, the present study examined for the first time the role of endogenous SP on NA release during stress conditions. Our data show that exposure to swim stress enhanced not only SP release in the LC but also NA efflux in the LC as well as in a terminal area of LC neurons within the mPFC. This NA response is in line with previous findings demonstrating increased NA efflux in the LC and mPFC after various stressors such as immobilization, noise, and air jet stress (Nakane et al. 1994; Singewald et al. 1999; Morilak et al. 2005; Renoldi and Invernizzi 2006). In addition, we found that intracoerulear administration of a NK1R antagonist potentiated the stress-induced rise in NA release in the LC, which was associated with suppression of the NA response in the mPFC. This effect is mediated selectively by NK1Rs because the administration of the less active enantiomer of the antagonist had no effect on intracoerulear NA release. Thus, our data suggest that SP changes the activity of NA neurons in the LC differently under basal and stress conditions, respectively. Although the exact mechanisms for this varying modulation are unknown, it might be speculated that SP afferents arising from different sources may be active (or predominate) during basal and challenge conditions, respectively. Unfortunately, no systematic study

has investigated the origin of LC SP-containing afferents. Based on indirect evidence, it was proposed that SP-containing projections to the LC arise from different sources including the amygdala, the bed nucleus of stria terminalis, dorsomedial, paraventricular, lateral hypothalamic areas, and the central gray (Tamiya et al. 1994). Alternatively, differential modulation during unchallenged and challenged conditions may result from separate interneuronal signaling (wired vs volume transmission) within the LC area. It is well known that neuropeptides are released nonsynaptically from multiple sites of the neuronal membrane and act as neuromodulators on relatively distant targets (Landgraf and Neumann 2004). Thus, it is conceivable that under basal conditions, nonsynaptically released SP tonically activates inhibitory (e.g., GABAergic) interneurons that lead to an inhibition of LC neurons. Under stress conditions, focal synaptic SP release might add to the rather diffuse spread, thus combining high speed, spatial precision, and a theoretically unlimited variability in signaling. Consequently, SP is then likely to stimulate noradrenergic neurons in the LC directly. Indeed, the finding that intracoerulear $GABA_A$ receptors inhibit NA neurons under basal but not under stress conditions (see above and Kawahara et al. 2000) provides further support for differential modes of interneuronal communication that vary in dependence upon experimental conditions. Future studies on the role of SP in this respect will have to test this hypothesis.

Our finding that the NK1R antagonist potentiated the NA release in the LC during stress conditions but inhibited it in the mPFC raises the question whether the same population of neurons can induce opposite effects on NA release in the somatodendritic vs terminal regions. Indeed, somatodendritic release of neurotransmitters/neuromodulators can be regulated independently from impulse flow and terminal release (e.g., Ludwig and Pittman 2003). However, to which extent the intracoerulear NA release is a result of changes in electrical impulse flow of LC neurons is a matter of debate (Singewald et al. 1994, for review see Singewald and Philippu 1998), and data from dual-probe microdialysis experiments have shown that certain receptor interactions are able to modify the release of NA in the LC without affecting the electrical activity of these neurons (Pudovkina and Westerink 2005).

Similar opposing release patterns of NA in the LC and mPFC have been shown after intracoerulear infusion of the antidepressant desipramine under basal conditions (Mateo et al. 1998; Fernandez-Pastor et al. 2005). It is well known that an increase in NA levels in the LC leads (via activation of somatodendritic α2-adrenoceptors) to an inhibition of the firing of noradrenergic neurons and subsequent decrease in NA release in the terminal area (Aghajanian et al. 1977; Egan et al. 1983; Jorm and Stamford 1993). Possible sources of extracellular NA in the LC include somatodendritic sites, nerve terminals of noradrenergic afferents to LC neurons, as well as terminals of recurrent collaterals (Singewald and Philippu 1998; Pudovkina and Westerink 2005). Especially under stress conditions, it is thought that intracoerulear-released NA probably limits further excessive LC activation by increased autoinhibition (Singewald et al. 1999; Weiss et al. 1994). Thus, our data indicate that under stress conditions, NK1R blockade may potentiate this autoinhibition by increasing the NA response within the LC. The results of the present study are in agreement with previous studies in rats where the stress-induced increase in c-Fos expression, as a marker for neuronal activation, was attenuated in the LC after intracerebroventricular administration of two different NK1R antagonists (Hahn and Bannon 1999; but see also Hahn and Bannon 1998). This latter finding is consistent with our recent data from rats bred for extremes in high-anxiety-related behavior where we found also attenuated stress-induced c-Fos expression in the LC after systemic administration of a NK1R antagonist (Muigg et al. 2006). It is interesting to note that similar attenuating effects on stress-induced NA release within the mPFC have been shown after acute systemic administration of another NK1R antagonist (Renoldi and Invernizzi 2006) and a range of other anxiolytic and/or antidepressant drugs (Sacchetti et al. 1993; Nakane et al. 1994; Finlay et al. 1995; Dazzi et al. 2002; but see also Dalley et al. 1996; Page and Lucki 2002). Given that excessive noradrenergic transmission in cortical brain areas is related to stress-induced anxiety (Redmond and Huang 1979; Charney and Redmond 1983), it might be speculated that the suppressant effect of NK1R antagonist on stress-induced NA release in the mPFC may contribute to the anxiolytic properties of these drugs (Rupniak 2002; Herpfer and Lieb 2005; Ebner and Singewald 2006).

Taken together, the results of the present study demonstrate that extracellular SP in the LC is responsive to a relevant stressor (forced swim stress) and that this enhanced SP release modulates noradrenergic transmission during stress. By using a selective NK1R antagonist, it was shown that during stress exposure, endogenous SP exerts an inhibitory effect on intracoerulear NA release, which was associated with an activation of the noradrenergic pathway to the mPFC resulting in increased NA stress response in this terminal region of LC neurons. Conversely, the observed suppressant effect of the NK1R antagonist on stress-induced NA release within this terminal area may contribute to its anxiolytic and antidepressant-like efficacy given that excessive noradrenergic transmission in cortical brain areas is associated with stress-related disorders including anxiety and depression.

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