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# Selective Blockade of Neurokinin-2 Receptors Produces Antidepressant-Like Effects Associated with Reduced Corticotropin-Releasing Factor Function

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## ABSTRACT

The present study investigated the effects of the selective neurokinin-2 ($NK_2$) receptor antagonist SR48968 in behavioral, electrophysiological, and biochemical tests sensitive to the action of prototypical antidepressants (fluoxetine, imipramine) or to corticotropin-releasing factor (CRF) receptor antagonists, which have been proposed recently as potential antidepressants. Results showed that SR48968 (0.3–10 mg/kg i.p.) produced antidepressant-like activity because it reduced immobility in the forced swimming test in both mice and rats, and decreased the amount of maternal separation-induced vocalizations in guinea pig pups. This latter effect appears to involve a reduction of stress-induced substance P release because SR48968 reduced the separation-induced increase in the number of neurons displaying neurokinin-1 receptor internalization in the amygdala. Furthermore, SR48968 increased the expression of the cAMP response-element binding protein mRNA in the rat hippocampus after repeated (1 mg/kg i.p., 21 days), but not acute administration. Finally, neuronal firing of the locus coeruleus (LC) and noradrenergic (NE) release in the prefrontal cortex both elicited by an uncontrollable stressor or an intraventricular administration of CRF were reduced by SR48968 (0.3–1 mg/kg i.p.). The finding that SR48968 (1 mg/kg i.p.) blocked the cortical release of NE induced by an intra-LC infusion of the preferential $NK_2$ receptor agonist neurokinin A suggested the presence of $NK_2$ receptors in this latter region. Importantly, SR48965 (1–10 mg/kg i.p.), the optical antipode of SR48968, which is devoid of affinity for the $NK_2$ receptor, was inactive in all the models used. These data suggest that $NK_2$ receptor blockade may constitute a novel mechanism in the treatment of depression and CRF-related disorders.

Brain substance P (SP), via the activation of its preferred target, the neurokinin-1 ($NK_1$) receptor, has been suggested to be involved in the modulation of emotional processes. This idea was initially based on the finding that SP acts as an excitatory neurotransmitter within key neuronal circuits regulating emotional responses, and on the evidence that changes in the content or release of SP occur in discrete brain regions in response to stressful situations or after antidepressant drug challenge (Bannon et al., 1983, 1986; Brodin et al., 1994; Shirayama et al., 1996; Hahn and Bannon, 1999). The involvement of SP in the modulation of stress-related disorders has received considerable support from the findings that selective nonpeptide $NK_1$ receptor antagonists may be effective in animal models of anxiety and depression, and show therapeutic efficacy in the treatment of major depression (Kramer et al., 1998; Smith et al., 1999).

SP can coexist with neurokinin A (NKA), the endogenous ligand for neurokinin-2 ($NK_2$) receptors, within the same neuronal population, and may be coreleased with the latter peptide as is for example observed in the spinal cord (Duggan and Furmidge, 1994). Both peptides derive from the common precursor preprotachykinin-A, whose mRNA is encoded by the PPT-A gene (for review, see Otsuka and Yoshioka, 1993). Although during the last decade, studies aiming to detect the presence of central $NK_2$ receptors were largely inconclusive, experiments in rodents with reverse transcription-polymerase chain reaction and fluorescent ligands, together with in vivo microdialysis studies, argue in favor of the presence of functional $NK_2$ receptors in discrete brain regions, including the medial septum and the striatum (Steinberg et al., 1998; Preston et al., 2000). Together, these findings prompted speculation about the involvement of NKA/$NK_2$ receptor activation in the modulation of emotional processes.

By using the brain-penetrant $NK_2$- versus $NK_1$-selective receptor antagonist SR48968 (Saredutant) (Emonds-Alt et al., 1992), the aim of the first part of this study was to investigate whether acute $NK_2$ receptor blockade may affect the behavioral and biochemical responses to acute inescap-

**ABBREVIATIONS:** SP, substance P; $NK_2$, neurokinin-2 receptor; NKA, neurokinin A; $NK_1$, neurokinin-1 receptor; CREB, cAMP response element-binding protein; CRF, corticotropin-releasing factor; NE, norepinephrine; LC, locus coeruleus; ANOVA, analysis of variance; SSC, standard saline citrate; CE, capillary electrophoresis.

able stress elicited either by forced swimming (in rats and mice), maternal separation (in guinea pigs), or tail pinch (in rats). Moreover, we tested the ability of sustained blockade of $NK_2$ receptors (by daily injection of SR48968 for 3 weeks) to modulate the expression of the nuclear transcription factor cAMP response element-binding protein (CREB) in hippocampal areas, a brain region where CREB mRNA expression was shown to be increased by repeated administration of a variety of clinically effective antidepressants (Nibuya et al., 1996).

Corticotropin-releasing factor (CRF) is a neuropeptide that has been recognized to play a critical role in the ability of the body to cope with stress (Arborelius et al., 1999). Given the major involvement of brain noradrenergic (NE) systems in stress-related disorders, and the ability of CRF to activate these neuronal populations (Koob, 1999), the second part of this study explored the role of $NK_2$ receptor activation in mediating CRF-induced firing of the locus coeruleus (LC) and NE release in its projection field, the medial prefrontal cortex.

## Materials and Methods

### Animals

Male CD1 mice (24–27 g; Charles River, St. Aubin les Elbeuf France) were used in the forced swimming test. Male Sprague-Dawley rats (Charles River) were used in microdialysis and electrophysiological experiments (250–330 g) and for CREB mRNA expression studies (150–200 g at the beginning of treatment), whereas male Wistar rats (270–330 g) (Charles River) were used in the forced swimming test. Mice and rats were housed in groups varying from 3 to 10 animals/cage 1 week prior to testing. Female guinea pigs (Dunkin-Hartley) with four 5-day-old pups were obtained from Harlan (Horst, The Netherlands). Each mother was housed individually with her litter provided with sawdust. All animals were maintained under standard laboratory conditions (21 ± 1°C) with commercially available food and tap water freely available, and kept on a 12-h light/dark cycle with light onset at 7:00 AM. All procedures have been approved by the Comité d'Expérimentation Animale of Sanofi-Synthélabo Recherche and were carried out in accordance with the French legislation (decree 87-848, October 19, 1987; and order from April 19, 1988), which implemented the European directive 86/609/EEC.

### Drugs

SR48968, its *R*-enantiomer SR48965, which displays a 2000-fold lower affinity for the $NK_2$ receptor (Emonds-Alt et al., 1992), and the CRF receptor antagonist antalarmin were synthesized by Sanofi-Synthélabo (Montpellier, France). For systemic administration, the antagonists were suspended with 0.01% Tween 80 in distilled water. Rat-human CRF (Bachem, Voisins-le-bretonneux, France) and NKA (Novabiochem, Fontenay-sous-Bois, France) were dissolved in water and Ringer's solution, respectively. Imipramine hydrochloride and fluoxetine (Sigma/RBI, St. Quentin-Fallavier, France) were suspended with 0.01% Tween 80 in saline (0.9% NaCl). SR48968, SR48965, antalarmin, imipramine, and fluoxetine were administered by i.p. or oral (forced swimming test in rats) route in a volume of 20 ml/kg of body weight for mice and 5 ml/kg of body weight for rats and guinea pig pups.

### Experimental Procedures

**Forced Swimming Test in Mice and Rats.** The procedure was a modification of the technique described by Porsolt et al. (1977). Animals were placed in individual glass cylinders containing water. Two swimming sessions were conducted in rats (an initial 15-min pretest followed 24 h later by a 5-min test), whereas one session was performed in mice. The total duration of immobility was measured during the last 4 min of a single 6-min test in mice, and for the entire 5-min test in rats. The animal was judged to be immobile whenever it remained floating passively in the water. In mice, SR48968 (1–8 mg/kg), SR48965 (8 mg/kg), and imipramine (20 mg/kg) were administered 30 min prior to testing, whereas in rats, SR48968 (0.1–3 mg/kg), imipramine (10–40 mg/kg), and fluoxetine (3–30 mg/kg) were administered twice (15 min after the first session on day 1 and 60 min before session 2 on the second day). Data were analyzed by a one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t* test.

**Maternal Separation in Guinea Pig Pups.** *Vocalization.* The procedure was adapted from that described by Molewijk et al. (1996). Briefly, from day 9 of age, pups entered at two pretest sessions (with 2-day intervals) consisting of 5-min isolation in a sound-attenuated cage equipped with white noise and white illumination, and the duration of their vocalizations was recorded by the experimenter. Immediately after the 5-min isolation, the subjects were returned to their mothers and littermates. Pups emitting vocalization during at least 120 s entered subsequent drug experimentation. Each pup was tested with vehicle and two dose levels of a compound with a washout period of 3 days between each treatment. SR48968 (0.3–10 mg/kg), SR48965 (3–10 mg/kg), and fluoxetine (1–10 mg/kg) were administered 30 min before the experiment that lasted 5 min. A repeated measures ANOVA was used with an appropriate covariance structure for vocalization duration analysis. Dunnett's *t* test analysis was subsequently applied to determine which dose was significantly different from vehicle.

*$NK_1$ receptor internalization in amygdala.* Separation and drug treatments were performed as described above. After the 5-min period of maternal separation, guinea pig pups were deeply anesthetized with pentobarbital (80 mg/kg i.p.) and perfused transcardially with heparinized saline, followed by 4% (w/v) paraformaldehyde in 0.1 M phosphate-buffered saline (pH 7.4). Brains were removed and allowed to postfix overnight in 4% paraformaldehyde. Fifty-micrometer coronal slices were then serially sectioned from each brain at the level of the amygdala by using a vibratome. Immunohistochemical analysis of $NK_1$ receptor-positive neurons ($NK_1$R-IR) was performed on free-floating tissue sections according to Mantyh et al. (1995) by using a rabbit $NK_1$ receptor antibody and a Cy3-conjugated donkey anti-rabbit IgG (Chemicon, Temecula, CA). After washing in phosphate-buffered saline, sections were mounted, air-dried, dehydrated, and covered with Poly Mount (Polysciences, Warrington, PA). Serial sections were viewed at the lateral and the anterio-basolateral amygdala by using a Leica TCS four-dimensional confocal scanning system (Leica, Wetzlar, Germany) on a Leica DMIRB microscope. Sections were scanned using a 63× objective and 1.8 numeric zoom factor. For each slice, single optical sections of eight $NK_1$R-IR neurons were taken, and six to eight consecutive sections were analyzed for each animal. The images (512 × 512 pixels) were taken through the center of the cell body so that they included the nucleus. Dendrites were not analyzed in the present study. The presence or absence of $NK_1$ receptor endocytosis in each scanned cell was determined subsequently by two independent experimenters who were unaware of the treatment of the animals. For each neuron, $NK_1$ receptors were considered as internalized when the immunolabeling was predominantly intracytoplasmic in the form of bright immunofluorescent particles as opposed to neurons with noninternalized $NK_1$ receptors that exhibited a uniform labeling on the cell surface. All counts were expressed as the percentage of $NK_1$ immunoreactive neurons that contained internalized receptors. The mean ± S.E.M. percentage of neurons showing $NK_1$ receptor internalization was calculated for each treatment group and comparisons were performed using Student's *t* test.

**CREB mRNA in Situ Hybridization.** Rats were administered fluoxetine (5 mg/kg), SR48968 (1 mg/kg), or SR48965 (1 mg/kg) once daily for 21 days. Animals were killed 24 h after the last adminis-

tration. The *Eco*RI-*Hin*dIII cDNA fragment (231 base pairs) corresponding to nucleotide 2585 to 2716 of the rat CREB protein was subcloned into the transcription vector pGEM-4Z (Promega, Charbonniere, France), which contains two promoters for T7 and SP6 RNA polymerases.

Analysis of CREB mRNA expression was performed by in situ hybridization according to Hoefler et al. (1986). Coronal brain sections (12 $\mu$m) were pretreated successively in 4% paraformaldehyde (20 min), 0.3% Triton X-100 (15 min), 0.25% acetic anhydride in triethanolamine (0.1 M, pH 8) for 10 min, and prehybridized in preheated (37°C) 50% formamide/2× standard saline citrate (SSC) for 10 min. Section were then hybridized with $^{35}$S-labeled CREB riboprobes ($2.5 \times 10^6$ cpm/section) for 15 h at 55°C in buffer containing 50% formamide, 0.6 M NaCl, 10 mM Tris, 2 mM EDTA, 1× Denhardt's, 10% dextran sulfate, 250 $\mu$g/ml yeast tRNA, 50 $\mu$g/ml salmon sperm DNA. The sections were washed in 2× SSC at 25°C, treated with 20 $\mu$g/ml RNase A for 30 min in 0.5 M NaCl, 10 mM Tris and 1 mM EDTA, and then washed with 2× SSC 10 min at 25°C, 1× SSC 10 min at 25°C, 1× SSC 10 min at 55°C, 0.5× SSC 10 min at 55°C, 0.5× SSC 10 min at 25°C. Sections were dehydrated in 30, 70, 95, and 100% alcohol and exposed to Biomax MR film (Amersham Pharmacia Biotech, Orsay, France). $^{35}$S-Labeled sense CREB riboprobe did not yield any significant hybridization signal.

The levels of CREB mRNA in the dentate gyrus and CA1 regions of the hippocampus were determined by densitometric quantification of autoradiograms with an image analysis system (version 4.00; Samba Technology, Meylan, France). For analysis, six to eight measurements from adjacent sections were averaged, and the data from six different animals of each group were statistically analyzed by Student's $t$ test or by ANOVA followed by Dunnett's $t$ test.

**Microdialysis.** *Freely moving rats.* Two days before dialysis measurements, rats were anesthetized with equithezine (4% chloral hydrate, 6% pentobarbital; 4 ml/kg of body weight). Cannula guides were stereotaxically implanted in the medial prefrontal cortex. The coordinates were 2.5 mm anterior to bregma, 0.6 mm lateral to the midline, and 1.3 mm down from the dural surface for the prefrontal cortex (Paxinos and Watson, 1986). On the day of the experiment, animals were placed in a Plexiglas cage, and a microdialysis probe (CMA 12, length 3 mm; Carnegie Medicine AB, Stockholm, Sweden) was inserted in the prefrontal cortex. The probes were perfused with a gassed Ringer's solution containing 125 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 23 mM $NaHCO_3$, and 1.5 mM $KH_2PO_4$, pH 7.4, at a rate of 2 $\mu$l/min by using a microinjection pump (CMA 100; Carnegie Medicine AB). Microdialysis sampling started 150 min after probe placement. Serial samples were collected at 15-min intervals in microtubes containing 5 $\mu$l of 0.1 N $HClO_4$, 1 mM EDTA, and 4 mM metabisulphite and immediately frozen at −40°C before analysis. After five baseline samples were collected, the tail of the animal was pinched at a point 2 cm from the tip of the tail with a rubber-hosed clamp for 15 min.

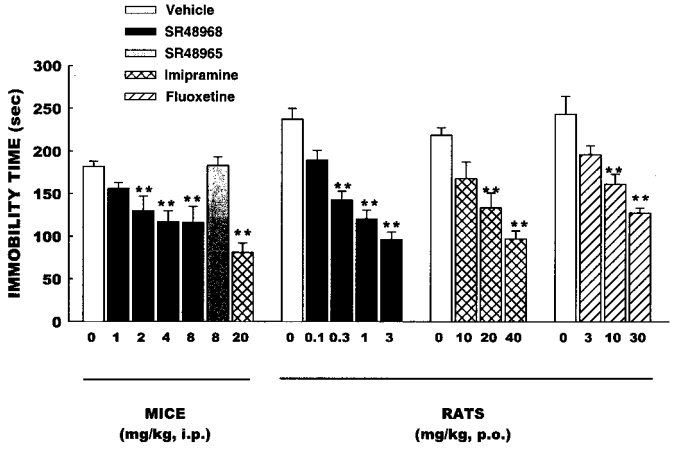


**Fig. 1.** Effect of SR48968, SR48965, imipramine, and/or fluoxetine on immobility time in the forced swimming test in mice and in rats. Data are expressed as mean ± S.E.M. [$n$ = 20–30 (mice) and $n$ = 7 (rats)] $^{**}p < 0.01$ compared with vehicle control group by ANOVA followed by Dunnett's $t$ test.

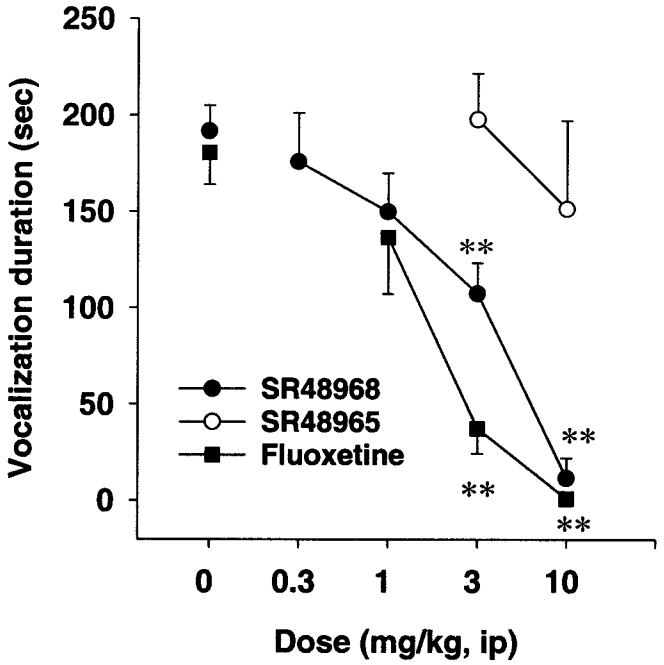


**Fig. 2.** Effects of SR48968, SR48965, and fluoxetine on the duration of isolation-induced calls of guinea pig pups. Data are expressed as mean ± S.E.M. of 7 to 55 animals/group. $^{**}p < 0.01$ compared with vehicle control group by ANOVA with repeated measures followed by Dunnett's $t$ test.

*Anesthetized rats.* Rats were anesthetized with urethane (1.4 g/kg i.p.) and then placed in a stereotaxic apparatus. Their body temperature was monitored by a rectal probe and adjusted (37 ± 1°C) by a homeothermic blanket. A 3-mm probe was implanted as described above in the prefrontal cortex 4.3 mm down from the dural surface and perfused with a gassed Ringer's solution adapted for capillary electrophoresis analysis (Bert et al., 1996). Microdialysis sampling started 90 min after the probe placement. For i.c.v. and intra-LC injections, samples were collected every 5 and 15 min, respectively. Dialysates were immediately frozen at −40°C before derivatization and analyzed by capillary electrophoresis with laser-induced fluorescence detection.

The pneumatic ejection procedure was performed as previously described (Marco et al., 1998). Briefly, for i.c.v. injection of CRF, ejection pipettes were implanted into the left lateral ventricle at the following coordinates: 0.8 mm posterior to bregma, 1.5 lateral to the midline, and 3.4 down from the dural surface. The ejection of CRF (3 $\mu$g/2 $\mu$l/90 s) was performed by applying air pressure with a 1-ml syringe connected to the nontapered side of the pipette by Tygon tubing.

Local injection of CRF or NKA in the LC was performed according to coordinates modified from the atlas of Paxinos and Watson (1986) with the tooth bar set 10 mm below the interaural plane (0.7 mm anterior to the interaural line, 1.1 mm lateral to the midline). The optimal depth of the pipette placement (around 5.5-mm depth below the cerebellar surface) was determined using a voltametric technique (Marco et al., 1998). The ejections (2 × 56 nl, 30 s) of CRF (37 ng) or NKA (0.15–15 ng) were performed as described above. Vehicle, SR48968 (0.1–1 mg/kg), SR48965 (0.3–1 mg/kg), and antalarmin (30 mg/kg) were given 30 min before tail pinch or CRF and NKA applications.

**Assay of Extracellular NE Levels.** For tail pinch experiments, NE levels were measured in 30-$\mu$l dialysates samples by a high-

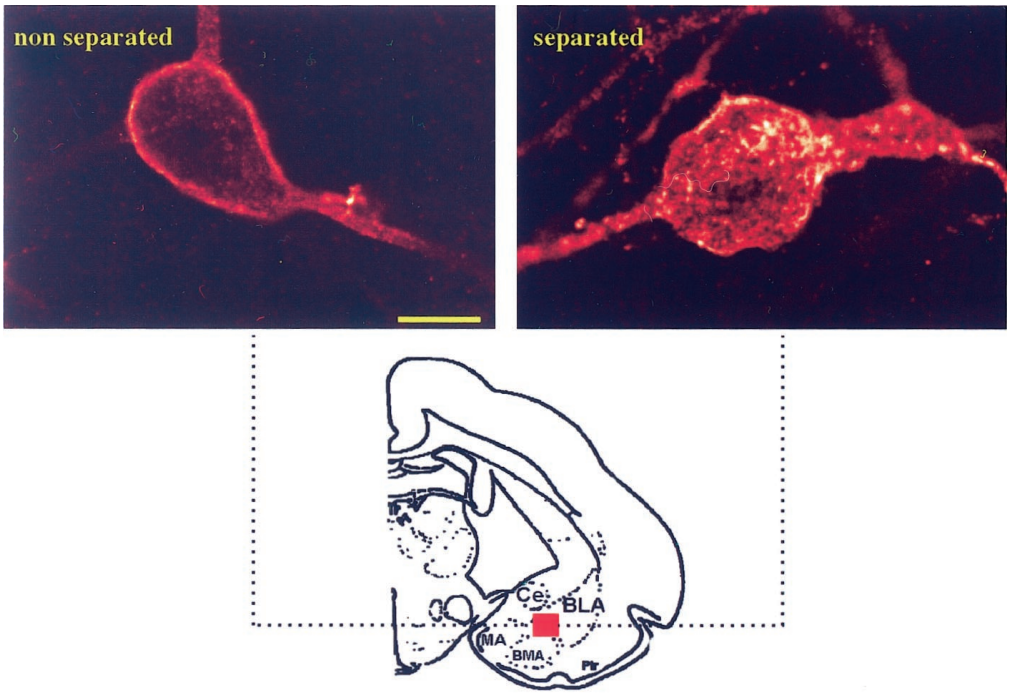


**Fig. 3.** Confocal microscopic imaging of $NK_1$ receptor immunolabeling in cells bodies of the amygdala. Representative images of neurons scanned in the basolateral amygdala (red square in the anatomical diagram) from nonseparated versus separated guinea pig pups. Note the intense immunolabeling within the cytoplasm in separated animals compared with the labeling restricted to the neuronal membrane in nonseparated pups. Images were taken using a 63× objective and 1.8 numeric zoom factor. BLA, basolateral; BMA, basomedian; MEA, median amygdala; CE, central amygdala. Scale bar, 5 $\mu$m.

TABLE 1
Effect of SR48968, SR48965, and fluoxetine on $NK_1$ receptor internalization induced by maternal separation in guinea pig pups

| | No. of Analyzed Cells | Percentage of Cells with $NK_1$ Receptor Internalization |
|---|---|---|
| Vehicle | 258 | 71.6 ± 0.8 |
| SR48968 3 mg/kg i.p. | 260 | 49.0 ± 1.1** |
| Vehicle | 260 | 71.2 ± 1.9 |
| SR48965 3 mg/kg i.p. | 249 | 75.5 ± 1.7 |
| Vehicle | 257 | 70.2 ± 2.1 |
| Fluoxetine 10 mg/kg i.p. | 262 | 44.9 ± 3.0** |

** $p < 0.01$ compared with vehicle-treated animals; $n = 4$ animals per group treatment.

performance liquid chromatography system with coulometric detection as previously described (Marco et al., 1998), except for the mobile phase containing 7% acetonitrile as organic agent. The analytical system consisted of an electrochemical detector ESA Coulochem II equipped with a model 5014 analytical cell (ESA, Chelmsford, MA).

For experiments with CRF and NKA injections and to optimize the temporal resolution of NE release, NE levels were evaluated in 10- or 30-$\mu$l dialysate samples by using capillary electrophoresis (CE) with laser-induced fluorescence detection according to Robert et al. (1995). Before analysis, the samples were derivatized using naphthalene-2,3-dicarboxaldehyde and sodium cyanide, as previously described (Bert et al., 1996). CE was performed on a PACE/MDQ (Beckman Coulter, Gagny, France) coupled to an external LIF-SA-2 detector (Picometrics, Toulouse, France). The excitation was performed by a Liconix helium-cadmium laser at a wavelength of 442 nm with a 15-mW excitation power. The emission intensity was measured at a wavelength of 490 nm. Separations were carried out with a fused silica capillary of 50 $\mu$m i.d. and 375 $\mu$m o.d. (Polymicro Technology, Phoenix, AZ) having a total length of 56 cm and an effective length of 46 cm with an applied voltage of 25 kV (i.e., 170-$\mu$A current). Phosphate buffer (110 mM), pH 7.05 ± 0.02, was used for CE running. A sample sacking procedure was used to separate NE from dopamine (Bert et al., 1996). The NE levels in fractional samples were converted to a percentage of the mean value of the 45-min baseline measurements before treatment. Time course effects of tail pinch, CRF, and NKA on NE levels were analyzed by an ANOVA with repeated measures. Dunnett's analysis was used for individual time comparisons. Drug antagonism of the effects of tail pinch was evaluated during the tail pinch sampling collection, whereas the antagonism of the effects of the peptides was evaluated by comparing the area under the curve during the 25 or 60 min after i.c.v. or local injection of the peptides, respectively. Statistical analysis was carried out by Student's $t$ test or by ANOVA followed by Dunnett's $t$ test.

**Electrophysiological Activity of LC Neurons.** Animals were anesthetized with chloral hydrate (400 mg/kg i.p.) and sustained with intravenous infusion (60 mg/kg/h). After appropriate surgery, 1-$\mu$m tipped glass micropipettes filled with 0.1% KCl (impedance 5–10 MΩ at 1000 Hz) were stereotaxically aimed to LC according to coordinates described above. LC neurons were identified using previously described criteria (Valentino et al., 1983). Quantification of discharge activity was made using an audio amplifier, and a transistologic pulse-generating window discriminator. On-line/off-line processing of transistologic pulses were made with an intelligent 1401 interface unit (Cambridge Electronic Design, Cambridge, UK) linked to a Compaq 5166PC running Spike2 software. On-line firing rate histograms were run continuously for at least 70 min. Infusions of CRF were made as described above for microdialysis experiments into the lateral ventricle (1.8 mm posterior to bregma, 1.1 mm lateral, 3.7-mm depth below the cortical surface).

The firing rate was averaged on consecutive 5-min periods throughout the 70-min recording time and converted to a percentage of the mean value of the 30-min baseline measurements before treatment. Time course of the effects of CRF were analyzed by ANOVA with repeated measures, and Dunnett's $t$ test was used for individual time comparisons. Drug antagonism of the effects of CRF was evaluated by comparing the area under the curve during the 30 min after peptide injection. Statistical analysis was carried out by Student's $t$ test or ANOVA followed by Dunnett's $t$ test.

## Results

### Blockade of $NK_2$ Receptors and Responses to Stress

**Forced Swimming Test in Mice and Rats.** In both species, SR48968 (mice: 1–8 mg/kg i.p.; rats: 0.3–3 mg/kg p.o.) dose dependently reduced immobility time (Fig. 1). Al-

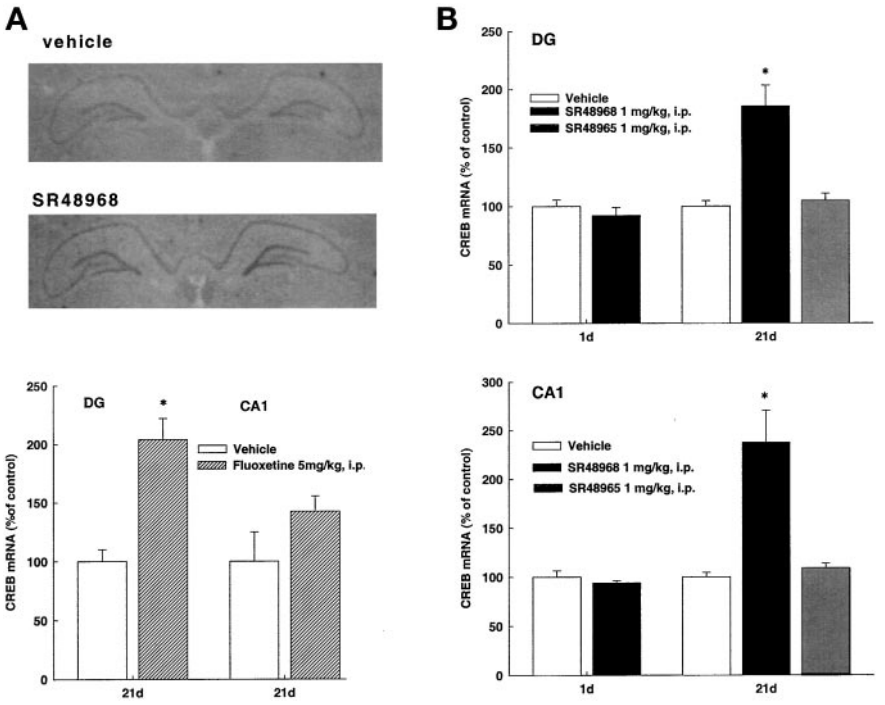


**Fig. 4.** Effect of SR48968, SR48965, and fluoxetine on CREB mRNA expression analyzed by in situ hybridization. Rats were treated with SR48968 for 1 or 21 days or with SR48965 and fluoxetine for 21 days. A, representative autoradiograms for vehicle and 21-day SR48968-treated animals. B, bar graphs: results are expressed as the mean ± S.E.M. of six to eight sections from six different animals per group. $p < 0.05$ compared with control group with Student's test, or ANOVA followed by Dunnett's $t$ test.

though the magnitude of this effect was somewhat less than that of imipramine in mice, it was quite comparable with that of imipramine and fluoxetine in rats. SR48965 (8 mg/kg i.p.), the *R*-enantiomer of SR48968, did not significantly modify immobility time.

### Maternal Separation in Guinea Pig Pups

**Vocalization.** In guinea pig pups, administration of SR48968 (0.3–10 mg/kg i.p.) 30 min before maternal separation caused a dose-dependent and complete inhibition of separation-induced vocalizations (Fig. 2). Significant reduction ($p < 0.01$) was observed at 3 and 10 mg/kg. In contrast, SR48965 (3 and 10 mg/kg i.p.) was inactive. Fluoxetine (3 and 10 mg/kg i.p.) also potently inhibited ($p < 0.01$) the duration of vocalizations.

**$NK_1$ Receptor Internalization in Amygdala.** As already reported, maternal separation of guinea pig pups was also found to produce endocytosis of $NK_1$ receptors in the amygdala, as assessed by immunocytochemical techniques (Kramer et al., 1998; Smith et al., 1999). $NK_1$ receptor immunoreactivity in cell bodies of the amygdala was mostly concentrated on the neuronal membrane in nonseparated guinea pig pups, whereas in separated animals, a majority of neurons exhibited bright immunofluorescent particles within the cytoplasm, indicating $NK_1$ receptor endocytosis (Fig. 3). Semiquantitative analysis indicates that in separated animals treated with vehicle, 72.0 ± 3.6% of neurons (mean ± S.E.M. of 278 neurons from 6 animals) have internalized $NK_1$ receptors compared with 22.7 ± 2.9% (mean ± S.E.M. of 260 neurons from 6 animals) in nonseparated animals treated with vehicle ($p < 0.01$). Administration of SR48968 (3 mg/kg i.p.), but not SR48965 (3 mg/kg i.p.), significantly ($p < 0.01$) reduced maternal separation-induced $NK_1$ receptor internalization (Table 1). A comparable reduction in the magnitude of $NK_1$ receptor internalization was observed in animals pretreated with fluoxetine (10 mg/kg i.p.; Table 1).

### Sustained Blockade of $NK_2$ Receptors Increased CREB mRNA Expression

In rats, repeated administration of SR48968 (once a day for 21 days; 1 mg/kg i.p.) increased the expression of CREB mRNA in dentate gyrus (85 ± 18% over controls, $p < 0.05$) and CA1 (137 ± 33% over controls, $p < 0.05$) cell layers of the hippocampus (Fig. 4). In comparison, chronic fluoxetine treatment (21 days; 5 mg/kg i.p.) induced an increase of 104 ± 18 ($p < 0.05$) and 43 ± 13% (N.S.) over control values in dentate gyrus and CA1, respectively. In contrast, no change in the expression of CREB mRNA was observed 24 h after an acute administration of SR48968 (1 mg/kg i.p.) or after repeated administration of SR48965 (once a day for 21 days; 1 mg/kg i.p.).

### Blockade of $NK_2$ Receptors Reduced Cortical NE Release Induced by Tail Pinch

In awake rats, a 15-min tail pinch induced a marked and transient increase in NE release in the prefrontal cortex (91 ± 16% stimulation over baseline level, $p < 0.01$, $n = 8$). NE release returned to basal level 60 min after cessation of the stimulation (Fig. 5A). SR48968 (0.3 mg/kg i.p.), but not SR48965 (0.3 mg/kg i.p.), administered 30 min before tail pinch, significantly ($p < 0.01$) reduced the evoked NE release (Fig. 5B). Similarly, the administration of the CRF receptor antagonist antalarmin (30 mg/kg i.p.) reduced ($p < 0.01$) the enhancing effect of tail pinch on cortical NE release. Under these conditions, basal extracellular NE levels in the prefrontal cortex were not affected by SR48968, SR48965, or antalarmin. Changes of extracellular NE levels were, respectively, −12 ± 3, +9 ± 13, and + 5 ± 14% ($n = 4$ animals/group) 45 min after administration.

### Blockade of $NK_2$ Receptors Suppressed CRF-Induced Activation of LC Neurons

In anesthetized rats, the i.c.v. injection of CRF (3 μg/2 μl) increased the discharge rate of LC-NE neurons by 220 ± 68%

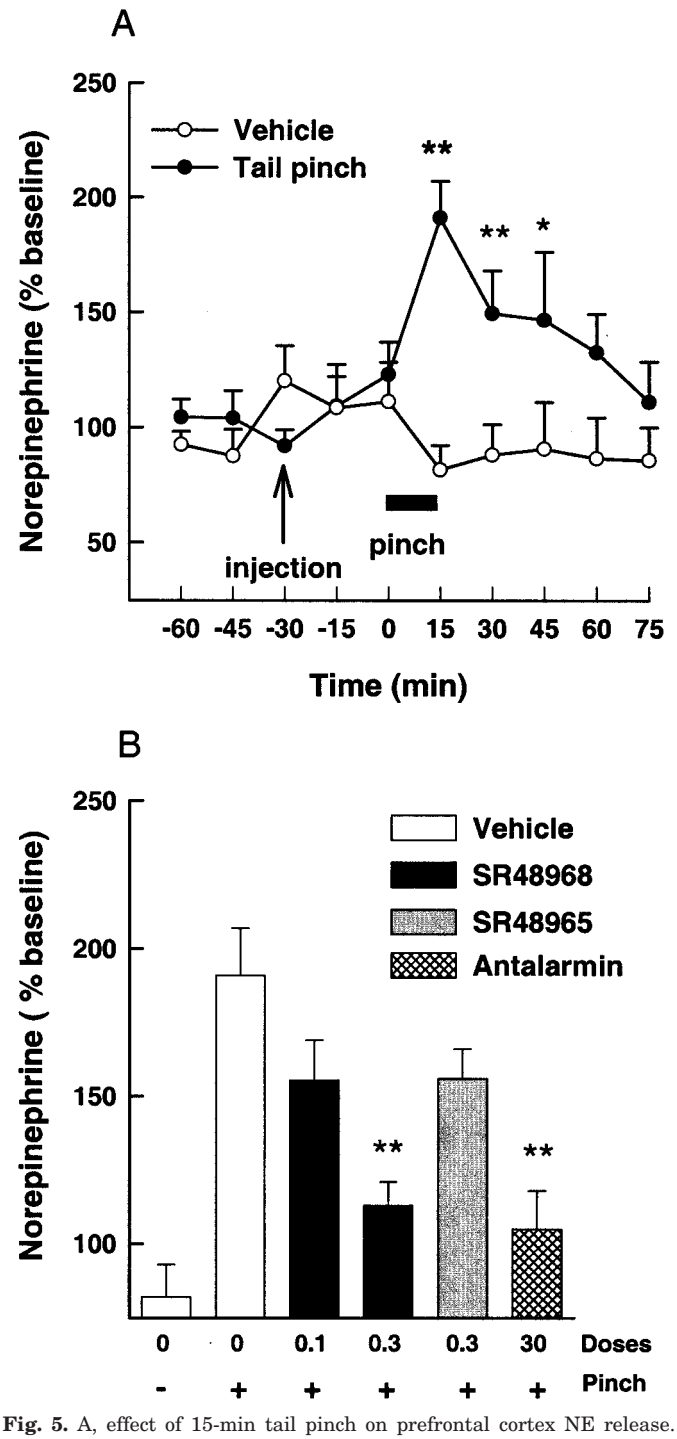


**Fig. 5.** A, effect of 15-min tail pinch on prefrontal cortex NE release. Changes in NE levels are expressed as a percentage of the mean value of the three basal samples before vehicle treatment. Each data point represents the mean value ± S.E.M. of six to eight animals $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control group by ANOVA with repeated measures followed by Dunnett's $t$ test. B, reversal of tail pinch-induced NE release by SR48968 versus SR48965 and antalarmin given i.p. 30 min before pinch. Each data point represents the mean value ± S.E.M. of NE release during tail pinch from four to eight animals. $^{**}p < 0.01$ compared with control group by ANOVA followed by Dunnett's $t$ test.

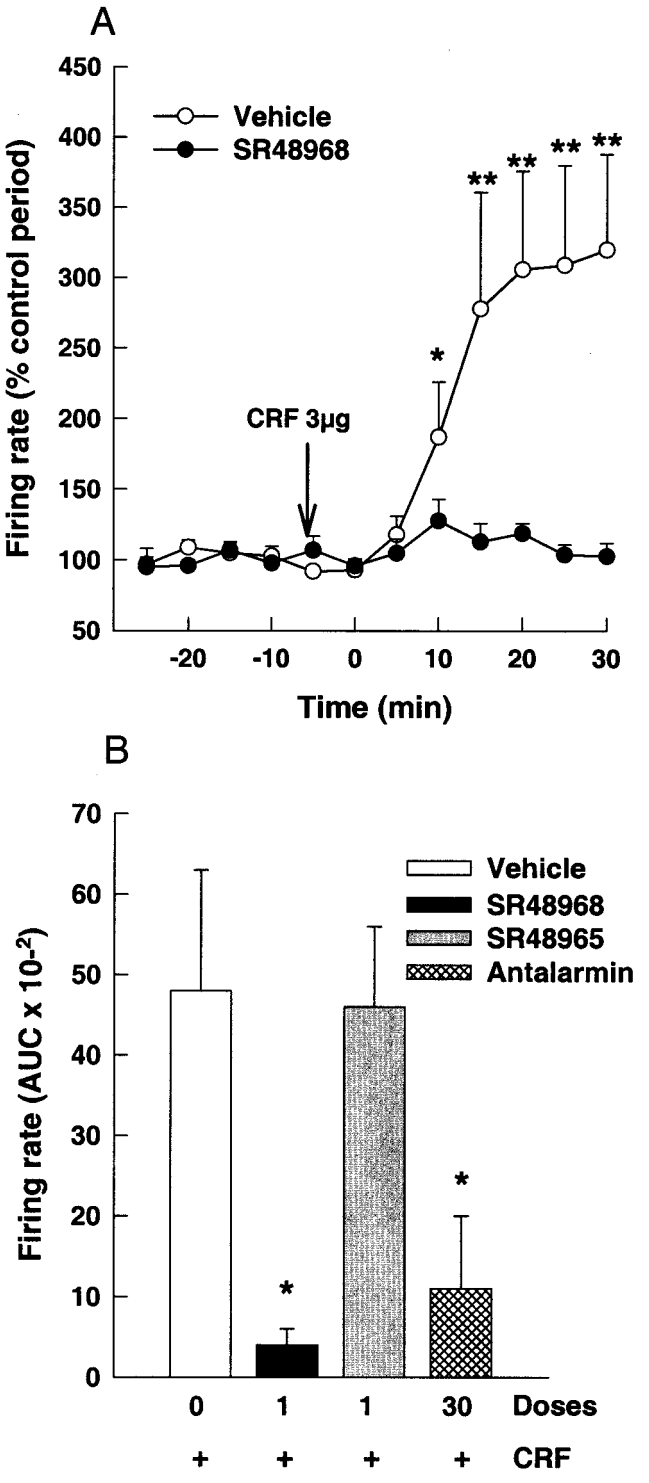


**Fig. 6.** A, effect of CRF (3 $\mu$g i.c.v.) on spontaneous discharge rate of LC neurons. The changes in firing rate are expressed as a percentage of the mean spontaneous discharge rate determined over 30 min before CRF injection. Each point is the mean of four animals. SR48968 was given i.p. 30 min before CRF. B, reversal of CRF-induced increase in firing rate in LC neurons by SR48968 (versus SR48965) and antalarmin given i.p. 30 min before CRF. The data are the mean area under the curve (AUC) ± S.E.M. of four animals per group for the 25 min after CRF injection. $^{*}p < 0.05$ compared with control group by Student's $t$ test.

at 30 min postinfusion (basal value = 1.6 ± 0.7 Hz, $n = 4$). SR48968 (1 mg/kg i.p), which did not modify the spontaneous discharge rate of LC-NE neurons (1.54 ± 0.14 Hz, $n = 4$), totally suppressed the enhancing effect of CRF during the entire recording period (Fig. 6A). As revealed by area under the curve examination (Fig. 6B), the inhibitory effect of SR48968 (−83.0 ± 4%, $n = 4$, $p < 0.05$) was comparable with that obtained with 30 mg/kg i.p. antalarmin (−78 ± 19%, $n =$

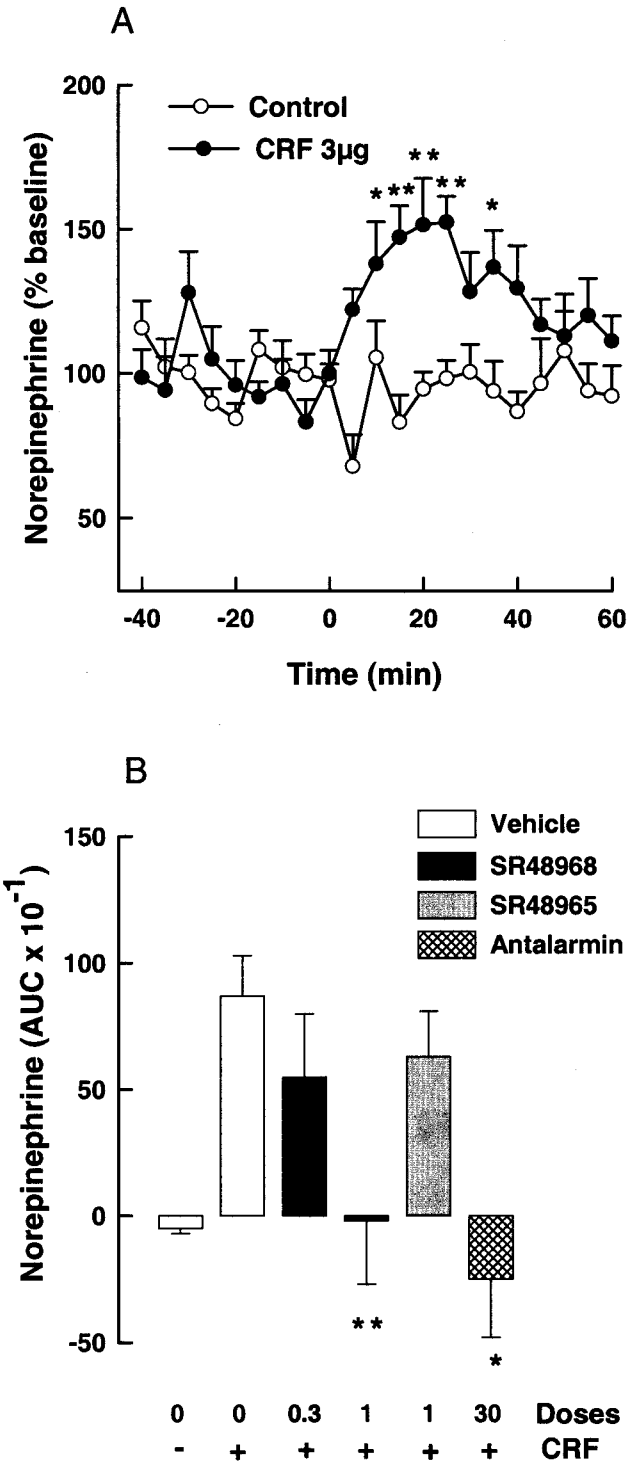


**Fig. 7.** A, effect of CRF (3 $\mu$g i.c.v.) on prefrontal cortex NE release. The changes in NE levels are expressed as a percentage of the mean value of the nine basal samples before treatment. Each data point represents the mean value ± S.E.M. of five to seven animals $^*p < 0.05$, $^{**}p < 0.01$ compared with control group by ANOVA with repeated measures and the Dunnett's $t$ test. B, reversal of CRF-induced NE release by SR48968 (SR48965) and antalarmin given i.p. 30 min before CRF. The data are the mean AUC ± S.E.M. of 3 to 10 animals for the 25 min after CRF injection. $^*p < 0.05$, $^{**}p < 0.01$ compared with control group by ANOVA followed by the Dunnett's $t$ test.

4, $p < 0.05$). In contrast, SR48965 (1 mg/kg i.p.) failed to modify the CRF-induced increase in LC firing (−0.5 ± 20%, $n = 4$).

Under similar conditions, CRF produced a rapid increase in extracellular NE measured in dialysates from the prefrontal cortex (Fig. 7A). A significant increase was obtained at 15 min ($p < 0.01$) after CRF injection, which persisted up to 35 min with a maximal effect at 25 min (+53 ± 9%, $n = 6$, $p < 0.01$). SR48968 (0.3–1 mg/kg i.p.) dose dependently and completely ($p < 0.01$) blocked the CRF-evoked cortical NE release as revealed by the area under the curve during the 25-min sampling period after CRF injection (Fig. 7B). Similarly, antalarmin (30 mg/kg i.p.) fully blocked ($p < 0.05$) the CRF response. In contrast, administration of SR48965 (1 mg/kg i.p.) was without effect.

### CRF and NKA Activation of Noradrenergic Neurons in LC

When CRF (37 ng/112 nl) was applied in the LC at a site densely packed with NE cell bodies, i.e., at the coordinates yielding the maximal 3,4-dihydroxyphenylacetic acid signal (Fig. 8A), a time-dependent increase in NE release in the prefrontal cortex was observed (Fig. 8B). In addition, NKA (0.15–15 ng/112 nl) injected in the LC also produced a significant and dose-dependent increase in cortical NE release (Fig. 8C). As shown in Fig. 8D, the enhancing effect of NKA (15 ng) on NE release was totally blocked by SR48968 (1 mg/kg i.p.).

## Discussion

The results of the present series of experiments, which combined behavioral, electrophysiological, and biochemical tests, with the selective $NK_2$ receptor antagonist SR48968, support further a critical role of $NK_2$ receptors and, by extension, endogenous NKA, in brain function. More specifically, we provide the first evidence that blockade of $NK_2$ receptors may yield antidepressant-like activity, and that $NK_2$ receptor activation is a key component in the action of the stress peptide CRF.

In the forced swimming test, a single injection of SR48968 significantly reduced immobility time in mice, whereas two administrations produced similar effects in rats. This profile is consistent with an antidepressant-like action of the drug and is comparable in terms of the magnitude of the effects with those of classical antidepressants, such as imipramine and fluoxetine (Sanchez and Meier, 1997). Although the forced swimming test is among the most frequent behavioral tests used to measure potential antidepressant activity, drug effects in this test alone have limited predictive value. We therefore sought to confirm this hypothesis in additional models. SR48968 was as effective as fluoxetine in inhibiting vocalization elicited by maternal separation in guinea pig pups. Although this model has been used to screen antidepressants, it has also proved to be useful to test anxiolytic agents (Molewijk et al., 1996). Thus, the activity of SR48968 in this paradigm may also be attributed to its anxiolytic-like properties. In line with this idea are several findings showing that SR48968 displayed anxiolytic-like activity in the murine light-dark choice task (Walsh et al., 1995), the rat and mouse elevated plus-maze tests, and in the mouse defense test battery (Teixeira et al., 1996; Griebel et al., 2001). Previous studies have demonstrated that stress produced by maternal separation induced the substance P in the amygdala of guinea pig pups, as revealed by an increase in the extent of $NK_1$ receptor endocytosis (Kramer et al., 1998; Smith et al.,

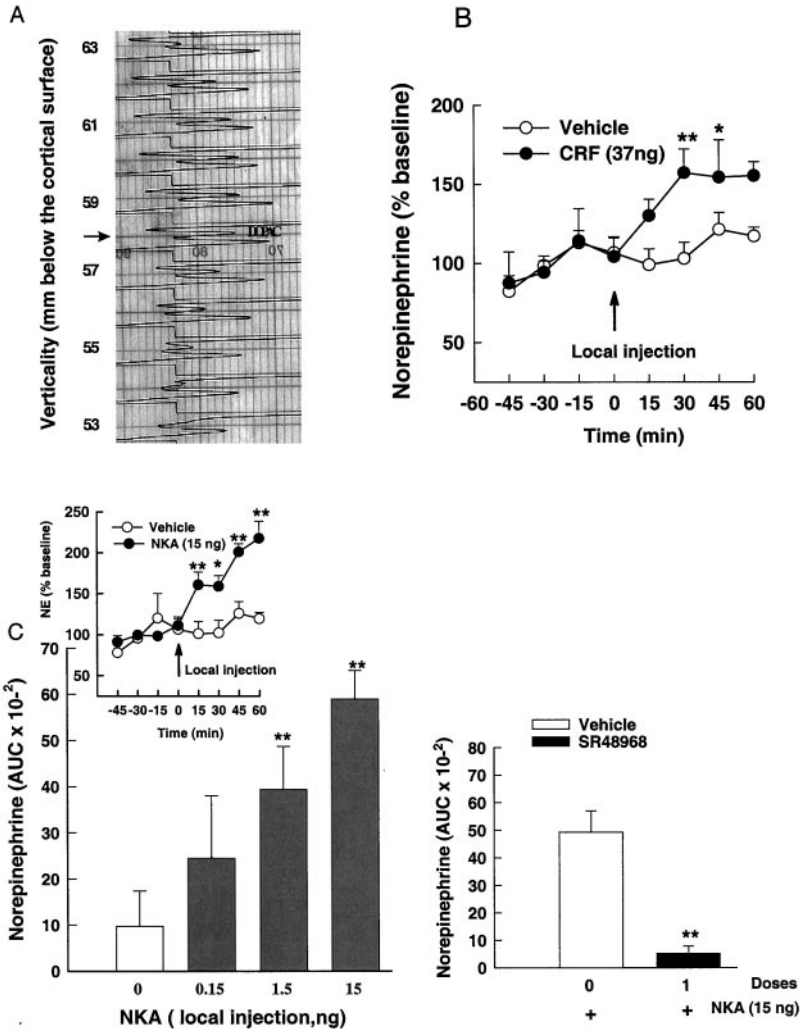


**Fig. 8.** Effect of CRF and NKA microinfused into the LC on prefrontal cortex NE release. A, variations in the voltamograms as a function of the depth of the recording electrode in the LC region. The arrow shows the voltamogram obtained in the LC at the coordinate use for local injection. Effects of pneumatic application of CRF (37 ng/112 nl) (B) or NKA (15 ng/112 nl) (C) in the LC on the prefrontal cortex NE release. The changes in NE levels are expressed as a percentage of the mean value of the four basal samples before treatment. Each data point represents the mean value ± S.E.M. of four to seven animals. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control group by ANOVA with repeated measures and the Dunnett's $t$ test. Inset, dose response for NKA (0.15, 1.5, and 15 ng/112 nl) on NE levels after area under the curve (AUC). Data are AUC ± S.E.M. of four to five animals for each group for the 60-min period after NKA ejection. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control group by ANOVA followed by the Dunnett's $t$ test. D, reversal of local LC NKA-induced NE release by SR48968 given i.p. 30 min before NKA. The data are AUC ± S.E.M. of five to six animals for the 60 min after NKA injection. $^{**}p < 0.01$ compared with control group by Student's $t$ test.

1999). In the present study, we found that SR48968 reduced separation-induced $NK_1$ receptor internalization in the amygdala to a similar extent as fluoxetine. This suggests that these compounds act through an inhibition of stress-induced substance P release. In all of these models, the inactivity of SR48965, the *R*-enantiomer of SR48968, which displays a 2000-fold lower affinity for $NK_2$ receptors (Emonds-Alt et al., 1992), suggests that blockade of $NK_2$ may be involved in the effects of SR48968.

Recent studies in rodents have implicated the postreceptor components of the cAMP second messenger cascade in the action of different classes of antidepressants, including 5-hydroxytryptamine- and NE-selective reuptake inhibitors, and monoamine oxidase inhibitors (Thome et al., 2000). The results of these studies demonstrated that chronic but not acute treatment with these drugs up-regulates the cAMP system at several levels, including expression of CREB in the cerebral cortex and hippocampus. Here, we show that daily injections of SR48968 for 21 days, but not acute administration of the compound (24 h prior analysis), increased the expression of CREB mRNA in the dentate gyrus granule and CA1 cell layer of the hippocampus. Similar effects were observed after repeated treatment with fluoxetine. In contrast, 21-day treatment with SR48965 did not alter CREB mRNA expression, thus suggesting the selective involvement of $NK_2$ receptors in the effects of SR48968.

It is well acknowledged that CRF plays a primary role in coordinating the overall response of the body to stressors. There is considerable evidence supporting an important role of the interaction between CRF and NE in the LC in mediating the behavioral response to stress (Curtis et al., 1997). Our findings further support this idea because we showed that tail pinch-induced increase in cortical NE release was prevented by prior administration of the CRF receptor antagonist antalarmin (Deak et al., 1999). Likewise, SR48968, which is devoid of affinity for CRF receptors, stereoselectively counteracted this stress response. This suggests that stress-induced activation of the prefrontal NE system may be under the control of both endogenously released CRF and NKA, thus opening the possibility of an interplay between the pathways containing these peptides. This hypothesis is substantiated by our data from the recently developed method of CE with laser-induced fluorescence, which achieves rapid analysis of NE in microdialysates. Intracerebroventricular administration of CRF induced an increase in extracellular concentrations of NE in the prefrontal cortex, which occurred concomitantly with an increase in the firing rate of NE neurons in the LC. Both these effects were prevented by systemic administration of antalarmin and SR48968 in a stereoselective manner, at doses that were similar to those blocking the tail pinch response. This raises the possibility that CRF provokes increases in LC discharge

rate and cortical NE release by stimulating NKA release. It is important to note that the blockade of $NK_2$ or CRF receptors with SR48968 or antalarmin, respectively, did not affect basal levels of NE in the prefrontal cortex, suggesting a lack of tonically active NKA or CRF control on NE release in anesthetized as well as awake rats.

Data from the further microdialysis experiments intended to localize the site of such CRF/NKA interaction. They showed that microinfusion of CRF or NKA performed under voltametric control in the LC increased extracellular NE levels in the prefrontal cortex. The effects of NKA were probably mediated by the activation of $NK_2$ receptors, because they were totally blocked by SR48968. Taken together, our data provide the first evidence that biochemical effects of endogenous or exogenous CRF might be mediated by NKA release, probably at the level of the LC area, and that NKA/$NK_2$ receptors may participate in the CRF-mediated regulation of NA systems in stress response. Further experiments are in progress to investigate whether this CRF/NKA cross talk is limited to NE systems or can be extended to the cholinergic system, because CRF and NKA have also been shown independently to release hippocampal acetylcholine (Day et al., 1998; Marco et al., 1998).

It must be noted that $NK_1$ receptors also have been implicated in various stress-related responses (Hahn and Bannon, 1999; Santarelli et al., 2001) and are expressed in relevant brain areas (Kramer et al., 1998; Chen et al., 2000). It is therefore legitimate to suggest that the effects of SR48968 result from a direct interaction with $NK_1$ receptors. This hypothesis seems unlikely because binding as well as functional in vitro experiments show that SR48968 behaved as a selective $NK_2$ receptor antagonist with no cross-reactivity for the other tachykinins receptors (Emonds-Alt et al., 1992, 1995). Moreover, within a similar dose range as in the present studies (1–3 mg/kg i.p.) SR48968 reversed the turning behavior in mice or striatal acetylcholine release in rat induced by the $NK_2$ receptor agonist NKA, but not that elicited by the $NK_1$ receptor agonist septide (Jung et al., 1994; Steinberg et al., 1995). Furthermore, that an $NK_2$ receptor with classical $NK_2$ pharmacology was implicated in our study is further strengthened by the stereoselective effects of SR48968 versus SR48965 in all models tested. On the other hand, we cannot exclude an indirect implication of $NK_1$ receptors in the effects of SR48968, which can be conceptualized through an $NK_2$/$NK_1$ heteroregulation, similarly to that previously described for neurokinin-3 and $NK_1$ receptors interaction in the spinal cord (Schmid et al., 1998).

Even though the present data as well as previous studies (Jung et al., 1994; Steinberg et al., 1995; Walsh et al., 1995; Teixeira et al., 1996; Preston et al., 2000; Griebel et al., 2001) provide firm support for a functional $NK_2$ receptors in the brain, their physical presence has been more difficult to demonstrate. Although $NK_2$ receptor mRNA has been detected by reverse transcription-polymerase chain reaction in the rat (Steinberg et al., 1998) and human brain (Bensaid et al., 2001), only a low level of rat brain $NK_2$ receptor expression was detected by autoradiography (Hagan et al., 1993; Watling et al., 1993). More sensitive techniques such as histofluorescence did provide direct evidence for the expression of $NK_2$ receptors in brain regions such as the septal area and the striatum where their functional role was clearly established (Steinberg et al., 1998; Preston et al., 2000). Our present observations should renew the interest for the study of $NK_2$ receptor localization in brain areas such as the locus coeruleus or the prefrontal cortex, with the use of highly sensitive techniques.

In conclusion, the present finding showed that blockade of $NK_2$ receptors produces antidepressant-like effects and prevents CRF-induced activation of NE neurons. This mechanism may represent an alternative approach in the pharmacotherapy of depressive disorders.

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**References**

Arborelius L, Owens MJ, Plotsky PM, and Nemeroff CB (1999) The role of corticotropin-releasing factor in depression and anxiety disorders. *J Endocrinol* **160:**1–12.

Bannon MJ, Deutch AY, Tam SY, Zamir N, Eskay RL, Lee JM, Maggio JE, and Roth RH (1986) Mild footshock stress dissociates substance P from substance K and dynorphin from Met- and Leu-enkephalin. *Brain Res Mol Brain Res* **381:**393–396.

Bannon MJ, Elliott PJ, Alpert JE, Goedert M, Iversen SD, and Iversen LL (1983) Role of endogenous substance P in stress-induced activation of mesocortical dopamine neurones. *Nature (Lond)* **306:**791–792.

Bensaid M, Faucheux BA, Hirsch E, Agid Y, Soubrié P, and Oury-Donat F (2001) Expression of tachykinin NK2 receptor mRNA in human brain. *Neurosci Lett* **303:**25–29.

Bert L, Robert F, Denoroy L, Stoppini L, and Renaud B (1996) Enhanced temporal resolution for the microdialysis monitoring of catecholamines and excitatory amino acids using capillary electrophoresis with laser-induced fluorescence detection. Analytical developments and in vitro validations. *J Chromatogr A* **755:**99–111.

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

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

Curtis AL, Lechner SM, Pavcovich LA, and Valentino RJ (1997) Activation of the locus coeruleus noradrenergic system by intracoerulear microinfusion of corticotropin-releasing factor: effects on discharge rate, cortical norepinephrine levels and cortical electroencephalographic activity. *J Pharmacol Exp Ther* **281:**163–172.

Day JC, Koehl M, Le Moal M, and Maccari S (1998) Corticotropin-releasing factor administered centrally, but not peripherally, stimulates hippocampal acetylcholine release. *J Neurochem* **71:**622–629.

Deak T, Nguyen KT, Ehrlich AL, Watkins LR, Spencer RL, Maier SF, Licinio J, Wong ML, Chrousos GP, Webster E, et al. (1999) The impact of the nonpeptide corticotropin-releasing hormone antagonist antalarmin on behavioral and endocrine responses to stress. *J Neurosci* **140:**79–86.

Duggan AW and Furmidge LJ (1994) Probing the brain and spinal cord with neuropeptides in pathways related to pain and other functions. *Front Neuroendocrinol* **15:**275–300.

Emonds-Alt X, Bichon D, Ducoux JP, Heaulme M, Miloux B, Poncelet M, Proietto V, Van Broeck D, Vilain P, Neliat G, et al. (1995) SR 142801, the first potent non-peptide antagonist of the tachykinin NK3 receptor. *Life Sci* **56:**PL27–PL32.

Emonds-Alt X, Vilain P, Goulaouic P, Proietto V, Van Broeck D, Advenier C, Naline E, Neliat G, Le Fur G, and Breliere JC (1992) A potent and selective non-peptide antagonist of the neurokinin A (NK2) receptor. *Life Sci* **50:**L101–L106.

Griebel G, Perrault G, and Soubrié P (2001) Effects of SR48968, a selective non-peptide $NK_2$ receptor antagonist on emotional processes in rodents. *Psychopharmacology*, in press.

Hagan RM, Beresford IJM, Stables J, Dupere J, Stubbs CM, Elliott PJ, Sheldrick RLG, Chollet A, Kawashima E, McElroy AB, et al. (1993) Characterisation, CNS distribution and function of $NK_2$ receptors studied using potent $NK_2$ receptor antagonists. *Regul Peptides* **46:**9–19.

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

Hoefler H, Childers H, Montminy MR, Lechan RM, Goodman RH, and Wolfe HJ (1986) In situ hybridization methods for the detection of somatostatin mRNA in tissue sections using antisense RNA probes. *Histochem J* **18:**597–604.

Jung M, Calassi R, Maruani J, Barnouin MC, Souilhac J, Poncelet M, Gueudet C, Emonds-Alt X, Soubrie P, Breliere JC, et al. (1994) Neuropharmacological characterization of SR 140333, a non peptide antagonist of NK1 receptors. *Neuropharmacology* **33:**167–179.

Koob GF (1999) Corticotropin-releasing factor, norepinephrine, and stress. *Biol Psychiatry* **46:**1167–1180.

Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, Reines SA, Liu G, Snavely D, Wyatt-Knowles E, et al. (1998) Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science (Wash DC)* **281:**1640–1645.

Mantyh PW, Allen CJ, Ghilardi JR, Rogers SD, Mantyh CR, Liu H, Basbaum AI, Vigna SR, and Maggio JE (1995) Rapid endocytosis of a G protein-coupled receptor:

substance P evoked internalization of its receptor in the rat striatum in vivo. *Proc Natl Acad Sci USA* **92:**2622–2626.
Marco N, Thirion A, Mons G, Bougault I, Le Fur G, Soubrie P, and Steinberg R (1998) Activation of dopaminergic and cholinergic neurotransmission by tachykinin NK3 receptor stimulation: an in vivo microdialysis approach in guinea pig. *Neuropeptides* **32:**481–488.
Molewijk HE, Hartog K, van der Poel AM, Mos J, and Olivier B (1996) Reduction of guinea pig pup isolation calls by anxiolytic and antidepressant drugs. *Psychopharmacology* **128:**31–38.
Nibuya M, Nestler EJ, and Duman RS (1996) Chronic administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *Neuroscience* **2:**363–372.
Otsuka M and Yoshioka K (1993) Neurotransmitter functions of mammalian tachykinins. *Physiol Rev* **73:**229–308.
Paxinos G and Watson C (1986) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, Sydney.
Porsolt RD, Bertin A, and Jalfre M (1977) Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* **229:**327–336.
Preston Z, Lee K, Widdowson L, Richardson PJ, and Pinnock RD (2000) Tachykinins increase [$^3$H] acetylcholine release in mouse striatum through multiple receptor subtypes. *Neuroscience* **95:**367–376.
Robert F, Bert L, Denoroy L, and Renaud B (1995) Capillary zone electrophoresis with laser-induced fluorescence detection for the determination of nanomolar concentrations of noradrenaline and dopamine: application to brain microdialysate analysis. *Anal Chem* **67:**1838–1844.
Sanchez C and Meier E (1997) Behavioral profiles of SSRIs in animal models of depression, anxiety and aggression. Are they all alike? *Psychopharmacology* **129:** 197–205.
Santarelli L, Gobbi G, Debs P, Sibille E, Blier P, Hen R, and Heath MJS (2001) Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. *Proc Natl Acad Sci USA* **98:**1912–1917.
Schmid G, Carita F, Bonanno G, and Raiteri M (1998) NK-3 receptors mediate enhancement of substance P release from capsaicin-sensitive spinal cord afferent terminals NK-3 receptors mediate enhancement of substance P release from capsaicin-sensitive spinal cord afferent terminals. *Br J Pharmacol* **125:**621–626.
Shirayama Y, Mitsushio H, Takashima M, Ichikawa H, and Takahashi K (1996) Reduction of substance P after chronic antidepressants treatment in the striatum, substantia nigra and amygdala of the rat. *Brain Res Mol Brain Res* **739:**70–78.
Smith DW, Hewson L, Fuller P, Williams AR, Wheeldon A, and Rupniak NMJ (1999) The substance P antagonist L-760,735 inhibits stress-induced NK1 receptor internalisation in the basolateral amygdala. *Brain Res Int* **848:**90–95.
Steinberg R, Marco N, Voutsinos B, Bensaid M, Rodier D, Souilhac J, Alonso R, Oury-Donat F, Le Fur G, and Soubrie P (1998) Expression and presence of septal neurokinin-2 receptors controlling hippocampal acetylcholine release during sensory stimulation in rat. *Eur J Neurosci* **10:**2337–2345.
Steinberg R, Rodier D, Souilhac J, Bougault I, Emonds-Alt X, Soubrie P, and Le Fur G (1995) Pharmacological characterization of tachykinin receptors controlling acetylcholine release from rat striatum: an in vivo microdialysis study. *J Neurochem* **65:**2543–2548.
Teixeira RM, Santos AR, Ribeiro SJ, Calixto JB, Rae GA, and De Lima TC (1996) Effects of central administration of tachykinin receptor agonists and antagonists on plus-maze behavior in mice. *Eur J Pharmacol* **311:**7–14.
Thome J, Sakai N, Shin KH, Steffen C, Zhang YJ, Impey S, Storm D, and Duman RS (2000) CAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. *Neuroscience* **20:**4030–4036.
Valentino RJ, Foote SL, and Aston-Jones G (1983) Corticotropin-releasing factor activates noradrenergic neurons of the locus coeruleus. *Brain Res Mol Brain Res* **270:**363–367.
Walsh DM, Stratton SC, Harvey FJ, Beresford IJ, and Hagan RM (1995) The anxiolytic-like activity of GR159897, a non-peptide NK2 receptor antagonist, in rodent and primate models of anxiety. *Psychopharmacology* **121:**186–191.
Watling KJ, Guard S, Krause JE, Takeda Y, Quirion R, Zarnegar R, Pain D, and Franks R (1993) On the presence of NK2 receptor subtypes in peripheral and central tissues. *Regul Peptides* **46:**311–313.

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