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Additional evidence for anxiolytic- and antidepressant-like activities of saredutant (SR48968), an antagonist at the neurokinin-2 receptor in various rodent-models

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Abstract

Central tachykinins have been shown to play a role in the modulation of stress-related behaviours. Saredutant, a tachykinin NK2 receptor antagonist, displayed mixed anxiolytic- and antidepressant-like activities in rodents. The present study aimed at further characterizing its psychotropic properties. Saredutant was tested in the rat social interaction test to further confirm its anxiolytic-like activity, and in a variety of behavioural models sensitive to antidepressant drugs. In the rat social interaction test, saredutant (20 mg/kg, i.p.) significantly increased the time spent in interaction, as did the prototypical anxiolytic agents, diazepam (1 mg/kg, i.p.) and buspirone (1 mg/kg, s.c.), but not the antidepressant, fluoxetine. In a differential reinforcement of low rate-72s task, saredutant (3 mg/kg, i.p.) displayed an antidepressant-like activity by increasing reinforced response rate and percentage of responses emitted in the inter-response time bin [49–96 s]. In bulbectomized rats, saredutant (20 mg/kg, i.p.) restored the deficit of acquisition of passive avoidance. In rat pups separated from their mother, saredutant (3–10 mg/kg, s.c.) reduced ultrasonic distress calls. Finally, in the chronic mild stress paradigm in mice, a 29-day treatment regimen with saredutant (10 mg/kg, i.p.) attenuated stress-induced physical degradation. Importantly, in the depression models, the effects of saredutant were comparable to those obtained under similar experimental conditions by reference antidepressants such as fluoxetine or imipramine. Together, these results suggest further that the NK2 receptor may represent an attractive target for the treatment of both depressive and anxiety disorders.

Keywords: Anxiety; Depression; Neurokinin-2; Saredutant; Rodents; Animal models

1. Introduction

The tachykinins (substance P (SP), neurokinin A and neurokinin B) are widely distributed within the mammalian peripheral and central nervous system (for review, see Severini et al., 2002). Biological activities of tachykinins are mediated by receptors, denoted NK1, NK2, and NK3, belonging to the superfamily of G-protein-coupled seven α-helical transmembrane spanning receptors (Buell et al., 1992; Gerard et al., 1990, 1991). Substance P is the natural endogenous ligand of tachykinin NK1 receptors, whereas neurokinin A and neurokinin B are the preferential ligands of tachykinin NK2 and NK3 receptors, respectively (Regoli et al., 1994).

In the periphery, the NK2 receptor is mainly found in the smooth muscle of the gastrointestinal, respiratory and urinary tracts (Maggi, 1995). In the central nervous system, the presence of NK2 binding sites in the rat brain has been demonstrated by [^{125}I]NKA labeling found on membranes from the hippocampus, the thalamus and the septum (Saffroy et al., 2001). In addition, autoradiographic experiments delineated [^{125}I]NKA binding in CA1 and CA2 areas of hippocampus and the prefrontal cortex (Saffroy et al., 2003). Such a presence of NK2 binding sites in several limbic structures suggests that the NKA/NK2 system could be involved in the modulation of emotional processes (Otsuka and Yoshioka, 1993). Moreover, some experimental data suggest an interaction between tachykininergic neurotransmission and the corticotropin-releasing factor (CRF), that is a neuropeptide recognized to play a critical role in the ability of the body to cope with stress, through its involvement in the regulation of

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hypothalamic–pituitary–adrenal axis functioning (Steinberg et al., 2001). Indeed, intraventricular administration of CRF increases the firing of the noradrenergic neuronal populations of the locus coeruleus. Such an activation is blocked by a peripheral administration of saredutant (Steinberg et al., 2001), suggesting that NK2 receptor blockade may constitute a novel mechanism in the treatment of CRF-related disorders, such as anxiety and depression (Risbrough and Stein, 2006).

The therapeutic potential of several selective NK2 receptor antagonists has been investigated in animal models of anxiety and depression. GR159897 and saredutant (SR48968) (Emonds-Alt et al., 1992; Maggi et al., 1993), were effective in exploration-based procedures sensitive to anxiolytics, such as the elevated plus-maze and the light/dark tests in rodents (Griebel et al., 2001a, b; Steinberg et al., 2001; Stratton et al., 1993b; Teixeira et al., 1996; Walsh et al., 1995). Anxiolytic-like effects of saredutant have also been reported in the mouse defense battery test (Griebel et al., 2001a), the marmoset human intruder test (Walsh et al., 1995) and the social interaction test in gerbils (Salomé et al., 2006). In addition to its anxiolytic-like activity, saredutant displayed antidepressant-like effects in the forced-swim test in rats (Dableh et al., 2005; Steinberg et al., 2001) and in the tonic immobility test in gerbils (Salomé et al., 2006).

Here, we summarize additional evidence for the potential anxiolytic- and antidepressant-like potential of saredutant in several behavioural paradigms sensitive to acute treatment with anxiolytics and antidepressants. Thereby, different aspects of behaviour have been addressed such as frustration (in the DRL-72s procedure in rats), ability to learn (passive avoidance testing in olfactory bulbectomized rats) and 'distress' (separation-induced ultrasonic vocalization test in rat pups). Also, evidence for saredutant's ability following a chronic administration to counteract physical degradation of mice caused by chronic stress will be presented.

2. Material and methods

2.1. Animals

Male rats (Iffa Credo, Saint Germain sur l'Abresle, France, or CERJ, Le Genest St-Isle, France) or male BALB/c mice (CERJ, Le Genest St-Isle, France) were kept in temperature- and humidity-controlled rooms (22 °C, 50%) under a normal light/dark 12/12 h cycle [light on from 07:00 AM] (or otherwise specified), with water and food available *ad libitum* (or otherwise specified).

All procedures have been approved by the Animal Care and Use Committee of Sanofi-Aventis Recherche and Développement and fully comply with French legislation (decree 87–848, October 19, 1987; and order from April 19, 1988), which implemented the European directive (86/609/EEC) on research involving laboratory animals.

2.2. Social interaction

2.2.1. Animals

Male Sprague–Dawley rats (CERJ, Le Genest St-Isle, France), 7- to 8-week-old, weighing 200–220 g at their arrival, were housed 4/cage.

2.2.2. Material

The social interaction test arena consisted of a grey Plexiglas box (75 × 75 × 42 cm high) with four bulbs generating 235 lx (white light) at the level of the floor of the arena. A camera fixed above the arena was connected to a computer that controlled the Ethovision® Pro 2.3 software (Noldus, Wageningen, The Netherlands), and was used to track each animal of the dyad. To that end, the system recorded the x–y coordinates of the isobaric center of each rat. The system considered that a social interaction episode was taking place between the two rats whenever the distance between the two isobaric centers was less than 14 cm (user-defined distance for rats weighing 180–200 g).

2.2.3. Pharmacological studies

Pairs of unfamiliar rats were treated (identical treatment for both rats) and individually housed for 30 min. Saredutant (10–20 mg/kg), fluoxetine (3–10 mg/kg) and diazepam (0.5–1 mg/kg) were administered by i.p. route, whereas buspirone (0.3–1 mg/kg) was administered by s.c. route (5 ml/kg of body weight). The pair of rats was then placed into the arena during 10 min for recording. The different compounds have been tested within independent experiments.

2.2.4. Data analysis

Data are expressed as the average duration of social interaction/dyad (sum of the time spent in socially interacting) and expressed in seconds/experiment (compound) a one way ANOVA, followed by *post-hoc* Dunnet's tests (comparison to the vehicle-treated control group), was performed.

2.3. Differential reinforcement of low rate—72s operant schedule

2.3.1. Animals

Male Wistar rats (Iffa Credo, Saint Germain sur l'Abresle, France) were singly housed. Their weight was kept at 450 + 50 g by feeding with 20 g of food chow given at the end of the day and over the week-end.

2.3.2. Material

The experiments were carried out in 8 rat operant chambers (Med Associates, East Fairfield, VT, USA), each fitted with a 2.8 W overhead house light and a stainless steel rods floor. A 4.8 × 1.9 cm lever was positioned on the right side of a food tray that was connected to a food pellets (45 mg, Formula P, Noyes, Research Diets, New Jersey, USA) dispenser. Each operant chamber was enclosed in a ventilated and sound-attenuating cubicle; all events were recorded and controlled by the "Med-PC software".

2.3.3. Acquisition of the operant behaviour

Rats were first trained (5 days a week) in daily 30 min sessions to press a lever to obtain a food pellet. When rats emitted at least 100 responses/training session, they were subjected to a differential reinforcement of low rate (DRL) 15 s schedule (i.e., a lever-press occurring before a delay of 15 s had elapsed was not rewarded and the timer was reset to 0 s for a further 15 s cycle; session duration: 60 min). Across successive DRL sessions, the timing of the DRL schedule was progressively increased from

15 s to the final timing of 72 s. Rats were injected with saline 30 min pre-session once they attained the DRL-72s stage. Once performance had stabilized (i.e. less than 10% variation of total number of responses during 6 consecutive DRL-72s vehicle sessions, and less than 7 reinforcers/session), rats were subjected to pharmacological challenge sessions.

2.3.4. Pharmacological studies

Thirty minutes before the start of the operant session, rats received i.p. (1 ml/kg of body weight) different doses of saredutant (3 and 10 mg/kg) or imipramine (2.5–5–10–20 mg/kg), administered in a mixed order. For a given drug treatment, control values were calculated by averaging the performance of all vehicle sessions immediately preceding all drug sessions. Furthermore, a stability criterion (less than 10% variation of total number of responses between the vehicle session immediately before the drug session, and the 6 vehicle sessions preceding the start of the pharmacological study) was in effect in-between each drug session.

2.3.5. Data

The following parameters were automatically recorded by the Med-PC software: the total number of lever-presses emitted during the session, the number of food pellets obtained (equivalent to the number of reinforced responses) and the inter-response time (IRT), i.e., the time elapsed between two lever-presses (Richards et al., 1993). IRT's were subsequently split into 9 bins (IRT bin [0–12 s], IRT bin [13–24 s],..., IRT bin [85–96 s] and IRT bin [>96 s]). From these raw data, the percentage of lever-presses emitted in each of the nine 12 s-bins and the percentage of lever-presses reinforced were calculated. The percentages of lever-presses during two IRT bins in particular were analyzed: 1) the IRT bin [0–12 s], corresponding to the "burst responses" and shown to be sensitive to benzodiazepine-like compounds (Richards et al., 1993) 2) the IRT bin [49–96 s], as it has been shown to be particularly sensitive to antidepressant drugs (Cohen et al., 1997).

2.3.6. Data analysis

The percentage of lever-presses emitted in bins [0–12 s] and [49–96 s], the percentage of lever-presses reinforced and the total number of lever-presses were analyzed with a Friedman's test, followed by *post-hoc* tests for a comparison between drug and mean of control sessions (vehicle treatment). A Cochran–Mantel–Haenzsel test was applied to analyze the effects of drug treatments on the distribution of responses across the IRT bins, followed by *post-hoc* tests with a Bonferroni–Holm correction factor for a comparison between drug and control sessions (vehicle treatment).

2.4. Acquisition of passive avoidance in olfactory bulbectomized rats

2.4.1. Animals

Male Wistar rats (Iffa Credo, Saint Germain sur l'Abresle, France) were first housed 4/cage before surgery and then were singly housed after the lesion of olfactory bulbs.

2.4.2. Surgery

Rats were first administered with diazepam (0.8 mg/kg, i.p.). Etamsylate (Hemoced®) an anti-hemorrhagic, was administered (125 mg/kg, i.p.) before anaesthetics to avoid lethal bleeding. Rats were anaesthetized with tiletamine and xylazine (both 30 mg/kg, i.p.), mounted on a stereotaxic frame. After incision of the skin, the skull was exposed and two 2 mm holes drilled through the skull 8 mm anterior to bregma and ±2 mm lateral to median suture. The olfactory peduncles were cut and removed by aspiration, by using a thin pipette connected to a vacuum pump. Three small hemostatic sponges (Sun Medical Co, Japan) were introduced in each olfactory cavity. Sham lesioned rats were treated in an identical manner except that the peduncles were not cut nor the bulbs removed. The skin of the skull was stitched up with a resorbable thread (Dexon II®).

After surgery, rats were isolated in individual cages and placed under a heat source until awakening.

After the passive avoidance test completion, bulbectomized animals were killed with pentobarbital overdose and decapitated. The brain was removed from the skull and the extent of the lesions verified by visual inspection. Bulbectomized rats were removed from the study if less than approximately 80% of the bulbs had been removed or if damage extended into the frontal cortex.

2.4.3. Acquisition of passive avoidance

Four weeks after surgery, rats were tested in a step-down passive avoidance task. After receiving saredutant (10–30 mg/kg, i.p.) or fluoxetine (30 mg/kg, i.p.), rats were retained in a red dim light anteroom during 30 min.

Each rat was introduced for 1 min of free exploration in a cage (30 × 30 × 28 cm) provided with a grid floor connected to a shock scrambler (Coulbourn Instrument, PA, USA). Then, the rat was placed on a platform (7.5 × 30 cm) mounted 4.5 cm above the grid floor with a white light located 21 cm above the platform. When the animal stepped off the platform with four paws, it received an electric shock (0.4 mA for 1 s) delivered through the grid floor. The rat was then returned to its home cage for 1 min after which it was given a second trial. If the rat stayed 120 s on the platform, it was returned to its home cage for 1 min without receiving a shock.

This procedure was continued until a total of 5 trials had been given.

2.4.4. Data analysis

The latency to step off the platform, expressed in s, was noted for each of the 5 trials. If the animal stayed 120 s, a value of 120 was attributed for this trial. To perform the statistical analysis, the time spent on the platform from the 2nd to the 5th trial was summed. A Kruskal–Wallis analysis was performed, followed by a *post-hoc* analysis, taking the vehicle group as the reference group.

2.5. Separation-induced ultrasonic vocalizations

2.5.1. Animals

Female Sprague–Dawley rats with 3–4-day-old (PND) male pups were obtained from Iffa Credo (Saint Germain sur

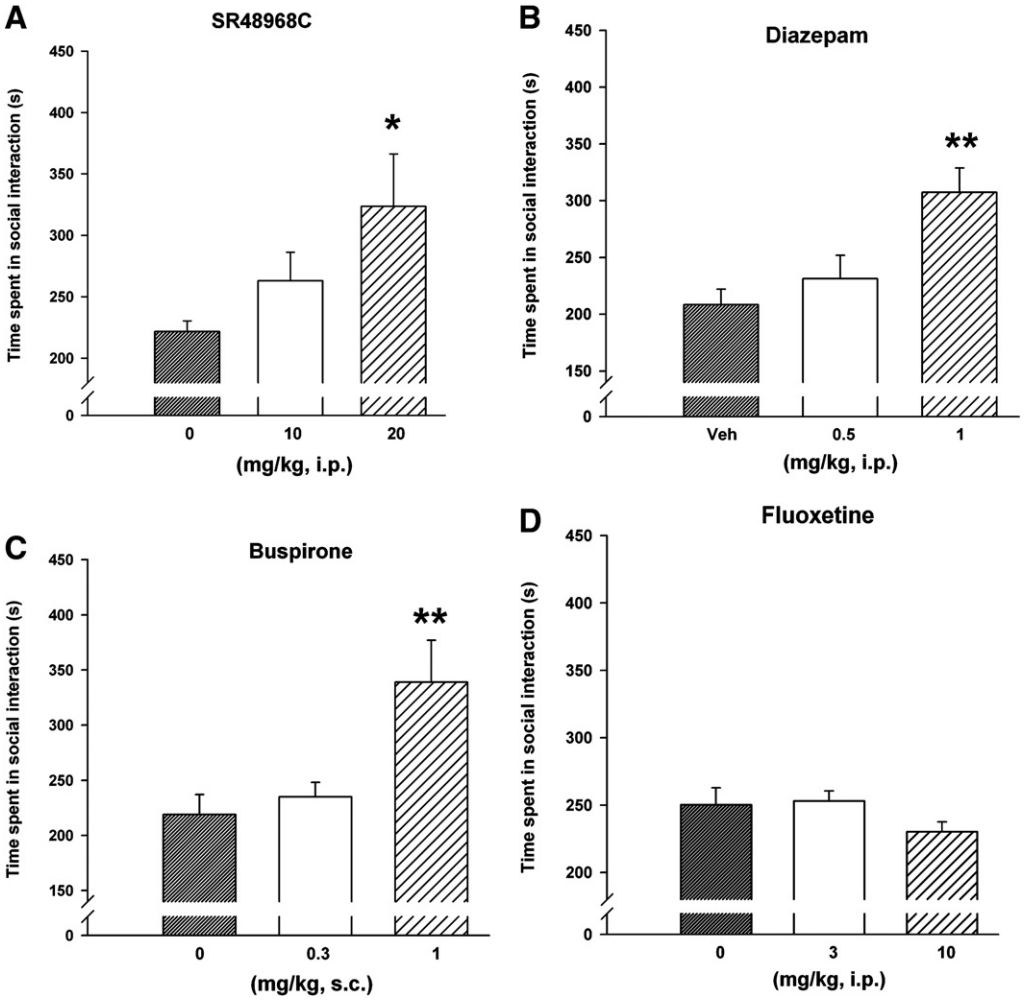

Fig. 1. A: Effect of saredutant on the time spent in social interaction in dyad of rats placed in a high illuminated and unknown open-field during 10 min. Drug or vehicle (same treatment for both rats) was injected i.p. 30 min s pre-test. Data are expressed as the mean duration of social interaction + SEM. $N = 12$ dyads. $^{*}p < 0.05$ vs vehicle group (0), Dunnett's t test after significant ANOVA. B: Effect of diazepam on the time spent in social interaction in dyad of rats. Refer to A legend. $N = 8$ dyads. $^{**}p < 0.01$ vs vehicle group (0), Dunnett's t test after significant ANOVA. C: Effect of buspirone on the time spent in social interaction in dyad of rats. Refer to A legend. $N = 8$ dyads. $^{**}p < 0.01$ vs vehicle group (0), Dunnett's t test after significant ANOVA. D: Effect of fluoxetine on the time spent in social interaction in dyad of rats. Refer to A legend. $N = 6$–7 dyads.

l'Abresle, France). They were housed in the animal facility for 3–4 additional days.

2.5.2. Procedure

Each pup (PND 7) was first separated from its mother and littermates, injected s.c. (0.1 ml/pup) with saredutant (1–10 mg/kg), fluoxetine (10 mg/kg) or vehicle, and returned to its mother. Thirty minutes later, the pup was placed in a soundproof cage. The Ultravox system (Noldus, Wageningen, The Netherlands) was used to record ultrasonic vocalizations emitted in the 40 kHz range. First, a modified ultrasound detector (Mini-3 bat model) connected to a microphone (positioned next to the pup) was used to transform ultrasonic sound into audible sound. The signal was sent to a PC, where the UltraVox software recorded each bout of ultrasonic vocalizations during the 3-minute test session.

2.5.3. Data analysis

Data are expressed as the mean number of distress calls and were analyzed with a Kruskal–Wallis test followed by one-sided lower Dunn test, taking the vehicle-treated group as the reference group.

2.6. Chronic mild stress-induced physical degradation in mice

2.6.1. Animals

Male BALB/c (6-week-old) mice, weighing 25–29 g at the start of the experiment and 25 to 34 g at the time of testing, were supplied by CERJ (Le Genest St-Isle, France) and were housed singly for testing.

2.6.2. Procedure

The CMS protocol is based on a protocol originally designed by Willner (Willner et al., 1992) for rats and adapted by Ducottet (Ducottet et al., 2003) for mice. The protocol consists of the sequential application of a variety of mild stressors, including restraint, forced swim, water and/or food deprivation, pairing with another stressed animal, each for a period between 2 and 24 h, in a schedule that lasts for six weeks. The

consequence of such exposure to stress is the progressive degradation of the physical state of the coat, consisting of a loss of fur and dirty fur that can be measured using the following scale: 3 points: clean and well groomed coat; 2 points: disorganized (poorly groomed) coat on the back; 1 point: dirty coat with loss of patches of fur. Physical state was measured at six occasions on a weekly base over the CMS period and on the day immediately after. Unlike any other measure reported in the CMS using BALB/c mice, the coat state has proven to be a reliable and valid measure of the depressive-like state of animals.

The mice submitted to the chronic stress procedure ("stressed groups") received one injection (20 ml/kg of body weight) of vehicle, saredutant (10 mg/kg, i.p.) or fluoxetine (10 mg/kg, i.p.)/day, the treatment being initiated two weeks after the beginning of the CMS, then lasting for 29 days. The no stress group was housed in a different room to avoid contamination by the stressed groups (e.g., distress ultrasonic vocalization, stress odors) and was not submitted to injections.

2.6.3. Data analysis

Data are expressed as the mean physical state scores, and were analyzed for each of the seven measures with a Wilcoxon test (non-stressed vs stressed controls) and with a Kruskal–Wallis test (stressed controls vs stressed treated groups) followed by *post-hoc* comparison test.

2.7. Drugs

Saredutant was synthesized by the Medicinal Chemistry Department of Sanofi-Aventis, fluoxetine, imipramine and diazepam were purchased from Sigma-Aldrich®, (Saint Quentin Fallavier, France), and buspirone from Bristol Myers Company (Princeton, New Jersey, USA). Drugs were prepared as a suspension in saline plus Tween 80® (approximately 2% v/v). Doses are expressed as the weight of base of the compounds.

2.8. Statistical analysis

For each behavioural experiment, refer to the appropriate section "data analysis". All statistical analyses were performed with the SAS system 8.2 (SAS Institute Inc. Cary, NC, USA).

3. Results

3.1. Social interaction

Saredutant dose-dependently increased the time spent in social interaction [$F(2,33)=3.2$, $p=0.05$], with the 20 mg/kg i.p. dose producing a one-third increase of this behaviour [$p<0.015$] (Fig. 1A). The two anxiolytic compounds diazepam and buspirone increased social interaction [$F(2,21)=7.55$, $p<0.01$ and $F(2,21)=6.75$, $p<0.01$, respectively], with the 1 mg/kg i.p. dose producing a one-third increase of this behaviour for both compounds [both $p<0.01$] (Fig. 1B and C). By contrast, fluoxetine did not increase social interaction in dyads of rats [$F(2,14)=1.54$, $p=0.24$] (Fig. 1D).

3.2. DRL-72s schedule

3.2.1. Saredutant

The NK2 receptor antagonist saredutant (3 and 10 mg/kg) significantly increased the percentage of responses occurring in the IRT bin [49–96 s] and of reinforced responses [Friedman's $H(2)=13.0$, $p<0.01$ and $H(2)=9.25$, $p<0.01$, respectively] (Table 1). The Cochran–Mantel–Haenzsel analysis revealed that saredutant significantly [$QSMH(8)=119.7$, $p<0.0001$] affected the distribution of responses across the IRT bins, with a rightward shift of the curve (Fig. 2A). Saredutant significantly reduced the total number of lever-presses [Friedman's $H(2)=14.3$, $p<0.001$] without affecting the percentage of responses in the IRT bin [0–12 s], also named "burst responses" [Friedman's $H(2)=3.25$, *ns*] (Table 1).

3.3. Imipramine

Global analyses show that imipramine (10 and 20 mg/kg) significantly [Friedman's $H(4)=11.50$, $p<0.05$] increased the percentage of responses occurring in the IRT bin [49–96 s], which translated into an augmentation of the percentage of reinforced responses [Friedman's $H(4)=19.50$, $p<0.001$]. It significantly [Friedman's $H(4)=17.60$, $p<0.01$] decreased the percentage of burst responses (Table 1). Imipramine dose-dependently shifted the IRT distribution curves towards longer duration, starting with the dose of 2.5 mg/kg, in a significant manner [Cochran's $QSMH(8)=125.40$, $p<0.0001$] (illustrated in Fig. 2B). Overall, imipramine significantly [$H(4)=13.90$, $p<0.01$] reduced the total number of responses (Table 1).

3.4. Acquisition of a passive avoidance task in olfactory bulbectomized rats

Four weeks after lesion, bulbectomized rats exhibited a clear deficit in acquiring the passive avoidance task, as revealed by the shorter duration spent on the platform (sum of the trials 2 to

Table 1
Effects of imipramine and saredutant on the DRL-72s schedule in rats

Drugs	Doses (mg/kg, i.p.)	% of responses in IRT bin [48–96 s] [a]	% of reinforced lever-presses [a]	% of responses in IRT bin [0–12 s] [a]	Total number of lever-presses
Imipramine ($n=8$)	0	26.3±2.4	6.1±0.4	12.8±1.8	95.7±2.9
	2.5	31.8±4.6	7.7±1.5	8.7±2.1	86.5±4.9
	5	39.1±5.9	11.9±4.0	10.7±3.3	83.8±7.2
	10	40.8±4.1*	13.0±0.8*	5.5±0.9*	77.4±4.4*
	20	43.3±3.7*	20.0±3.0**	4.1±1.0**	68.5±3.0**
Saredutant ($n=8$)	0	15.9±2.1	2.7±0.7	15.0±1.6	117.4±5.3
	3	29.8±4.2*	6.5±1.7*	16.2±4.6	97.9±6.4*
	10	34.7±4.4**	8.5±2.0*	11.6±1.8	87.5±4.5**

*$p<0.05$, **$p<0.01$ vs control performances (0): Friedman analysis followed by multiple comparison test.

[a] Percentages are expressed relatively to the total number of lever-presses +/− SEM.

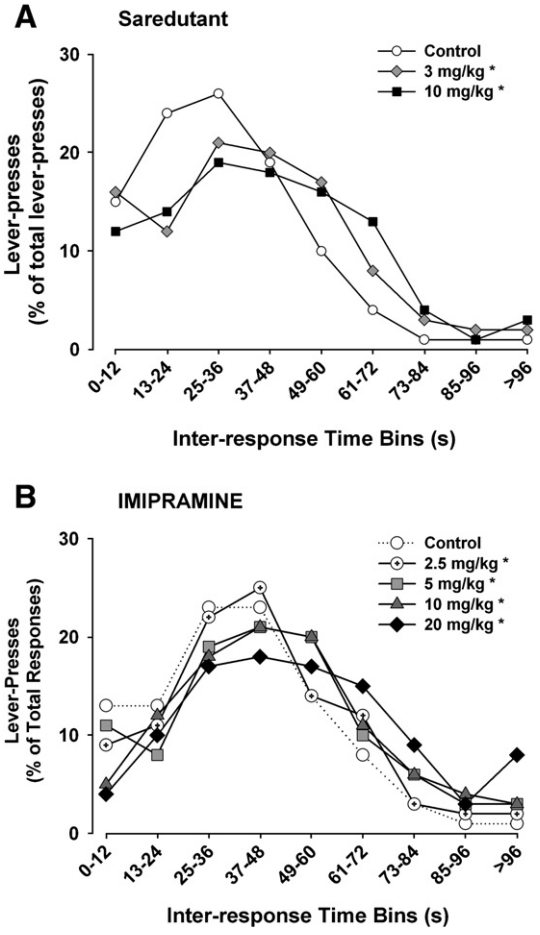

Fig. 2. A: Effect of saredutant on the inter-response time (IRT) distribution on a DRL-72s operant schedule in rat. Data are expressed as the average percentage of lever-presses emitted during each IRT bin (with respect to the total number of lever-presses). Error bars are omitted for clarity. Drug or vehicle was injected i.p., 30 min pre-session. *$p<0.05$ *post-hoc* test (Bonferroni–Holm's correction factor) vs vehicle-treated group, following a significant Cochran–Mantel–Haenszel analysis. $N=8$ rats/group. B: Effect of imipramine on the IRT distribution on a DRL-72s operant schedule in rat. Refer to legend of A for details. $N=8$ rats/group.

5: 147.3±44.5 s), compared to the time spent by sham rats (393.5±41.5 s) (Fig. 3A). The time spent on the platform for each of the 5 trials is illustrated by the curves in the Fig. 3B. A treatment with saredutant (10 and 30 mg/kg, i.p.) clearly improved the acquisition of the task as shown by the increase of the time spent on the platform (279.2±40.9 s and 341.8±25.8 s, respectively). This improvement in acquisition is statistically significant when compared to vehicle group performance [Kruskal–Wallis (2)=10.59; $p<0.01$] for both doses of saredutant [$p<0.05$ and $p<0.01$, respectively]. Such an improvement is also observed after a treatment with fluoxetine (30 mg/kg, i.p.) [388.1±26.7 s vs 147.3±44.5 s; Wilcoxon's test: $S=38$; $p<0.001$].

3.5. Separation-induced distress vocalizations in rat pups

There was a significant effect of the treatment [Chi2(4)=14.6, $p<0.01$]. Saredutant (3 and 10 mg/kg, s.c.) significantly decreased ultrasonic distress calls emitted by rat pups separated from their mother and littermates [$p<0.01$ vs vehicle group, for both doses]. The effect was of the same magnitude to that observed after a treatment with fluoxetine (10 mg/kg, s.c.) [$p<0.01$ vs vehicle group] (Fig. 4).

3.6. Chronic mild stress

There was a significant degradation of the coat state of mice due to stress as it is revealed by the wilcoxon's test when taking into account NOSTRESS and STRESS-vehicle-treated groups [measure 1: Wilcoxon's test $S=0$, n.s.; measure 2, Wilcoxon's test $S=3.938$, $p<0.0001$; from measures 3 to 7, p_s inferior to 0.0001]. The effects of the compounds on the coat of the mice appear on the measure 5 [Chi2(2)=12.81, $p<0.01$], after two weeks of chronic treatment. Both saredutant and fluoxetine-treated groups are statistically different from vehicle-treated group [$p=0.0055$ and $p=0.0269$, respectively]. This observation is maintained on measures 6 and 7 [Chi2(2)=29.11 and

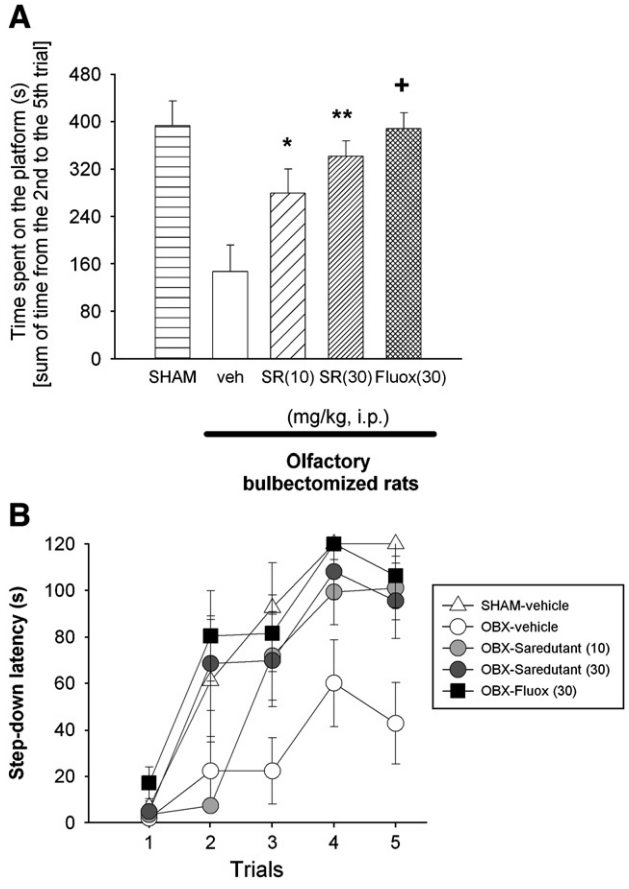

Fig. 3. A: Effect of saredutant and fluoxetine in olfactory bulbectomized (OBX) rats acquiring passive avoidance task, four weeks after the lesion. Drug or vehicle was injected i.p., 30 min pre-test. Data are expressed as the cumulative time spent on the platform from the 2nd trial to the 5th trial+SEM. Each trial is limited to 120 s. $N=6–9$ rats/group. *$p<0.05$, **$p<0.01$ vs vehicle group, *post-hoc* tests following significant Kruskal–Wallis analysis. +$p<0.05$, vs vehicle group (veh), Wilcoxon's test. B: Effect of saredutant and fluoxetine in olfactory bulbectomized (OBX) rats acquiring passive avoidance task: Curves illustrate the temporal timing of the acquisition by plotting trial/trial, the mean time stayed on the platform+SEM. $N=6–9$ rats/group.

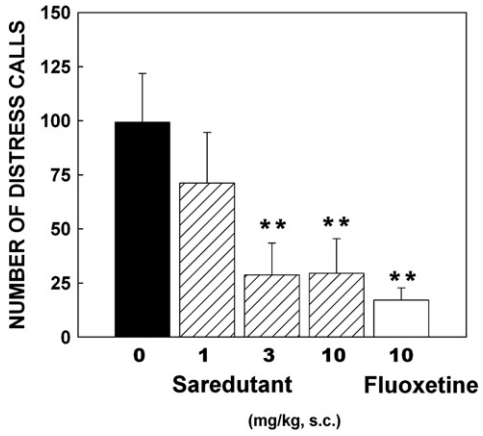

Fig. 4. Effect of saredutant and fluoxetine on ultrasonic distress vocalizations emitted during 3 min by 7-day-old rat pups separated from their mother and littermates. Drug or vehicle was injected s.c., 30 min pre-test. Data are expressed as mean number of distress calls+SEM. $N=10$ rat pups/group. $^{**}p<0.01$ vs vehicle group (0), Dunn's test.

21.41, both $p<0.0001$, respectively]. No difference exists between fluoxetine and saredutant groups (Fig. 5).

4. Discussion

The present series of experiments demonstrated that the selective NK2 receptor antagonist, saredutant, displayed anxiolytic- and antidepressant-like effects in a variety of animal models of stress-related disorders.

In the social interaction task, initially described by File (File and Hyde 1978), saredutant produced anxiolytic-like effects as shown by an increased social interaction in dyads of unfamiliar rats. This action was of the same magnitude as the one observed with anxiolytic drugs, such as diazepam and buspirone. By contrast, the antidepressant fluoxetine was inactive, demonstrating the good selectivity of this model. This positive result obtained with saredutant in rat is consonant with the anxiolytic-like activity observed in another species, the gerbil (Salomé et al., 2006). However, in gerbils, the minimal effective dose was 3 mg/kg (p.o.), whereas in rat, it was 20 mg/kg (i.p.). This difference in the minimal effective dose may not be explained by a species-difference at the level of the receptor since there is no relevant difference in the affinity at NK2 receptors among rats, gerbils or guinea-pigs but may be due to experimental peculiarities, as, for example, gerbils but not rats had been pre-exposed to the arena and/or the route of administration (p.o. and i.p. in gerbils and rats, respectively.

A number of previous studies have reported anxiolytic-like effects saredutant in the light/dark exploration test (Stratton et al., 1993b; Walsh et al., 1995), the elevated plus-maze (Griebel et al., 2001a,b; Teixeira et al., 1996) and in the Mouse Defense Test Battery (Griebel et al., 2001a). Moreover, saredutant significantly increased the time spent by marmosets at the front of the cage following confrontation with a human 'threat', an effect which is consistent with an anxiolytic action (Walsh et al., 1995). However, saredutant (0.1–3 mg/kg, i.p.) failed to increase punished responses in conflict-based procedures (Griebel et al., 2001b), suggesting a specific profile of activity of NK2 antagonists compared to drugs such as benzodiazepines, which are active in all these procedures.

Little is known about the neurobiological substrate responsible for the anxiolytic-like activity observed after NK2 receptor blockade. It can be hypothesized that the preferential ligand for NK2 receptor, neurokinin A (NKA), is released in stressful conditions and may play a role in the expression of anxiogenic-like behaviour. Accordingly, the central administration of the preferential NK2 receptor agonist NKA and/or the selective agonist [β-Ala^8]NKA(4–10), a fragment of NKA, has been reported to produce anxiogenic-like effects in the murine elevated plus-maze (De Lima et al., 1995; Teixeira et al., 1996).

In addition to a potential reversal of NKA agonist activities, it has been suggested that tachykinin NK2 receptor antagonists may produce some of their psychotropic effects via the dorsal raphe nucleus (DRN), by interacting with neurotransmitters such as 5-HT or GABA (Walsh et al., 1995). This suggestion is based notably on the finding that intra-DRN infusion of the NK2 receptor antagonists GR100679, GR115211 and GR159897 produced anxiolytic-like effects in the social interaction and elevated plus-maze tests in rats (Stratton et al., 1993a, 1994). Moreover, the NK2 receptor agonist GR64349 was found to increase anxiety-like behaviour in the rat social interaction test following infusion into the DRN (Stratton et al., 1993a). However, additional brain areas may participate in the action of saredutant on emotional processes, especially hippocampal, septal, and cortical structures where NK2 receptors have been mainly identified (Saffroy et al., 2001, 2003).

In addition to its anxiolytic-like activity, saredutant was shown previously to display antidepressant-like effects in several behavioural models (forced-swim test in rats, maternal separation-induced vocalization in guinea-pigs) (Steinberg et al., 2001) and tonic immobility in gerbils (Salomé et al., 2006) thus exceeding the limited therapeutic activity of the classical benzodiazepines being devoid of antidepressant properties. Here the antidepressant potential of saredutant was confirmed in several paradigms, including a differential reinforcement of low

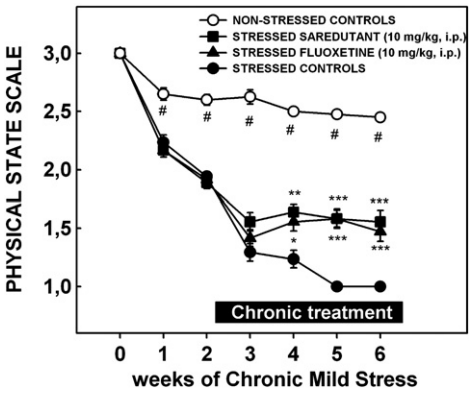

Fig. 5. Effect of saredutant and fluoxetine on physical degradation in chronically stressed mice compared to non-stressed controls. Data are expressed as mean physical state score±SEM. $N=17–20$ mice/group. $\#p<0.001$ vs stressed controls, Wilcoxon's test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs stressed controls, pairwise *post-hoc* comparisons after significant Kruskal–Wallis.

rate DRL-72s schedule, a passive avoidance task in OB-lesioned rats and in the maternal separation-induced vocalization test in rat pups. Furthermore, we report that chronic administration of saredutant also partially reversed the physical degradation of mice submitted to chronic mild stress. Qualitatively, the behavioural effects seen following saredutant were generally comparable to those following clinically effective antidepressants such as fluoxetine or imipramine.

In the DRL-72s schedule paradigm, saredutant induced an increase of reinforcement rates accompanied by a slight decrease of total number of responses. The study of the distribution of the responses across the inter-response time bins showed a rightward shift in the fitted response curve toward longer inter-response time. This change in the pattern of operant responses in the DRL-72s is similar to that obtained with prototypical antidepressants such as tricyclics and selective serotonin reuptake inhibitors (O'Donnell and Seiden 1983), or as with more recent compounds endowed with antidepressant-like activities (e.g., corticotrophin-releasing factor receptor antagonist or vasopressin V1b receptor antagonist (Louis et al., 2006; Richards et al., 1993).

It is the first time that an antidepressant-like activity of a NK2 receptor antagonist is described in the deficit of passive avoidance acquisition in bulbectomized rodent. This model has been proposed as a reliable procedure to investigate depressive-like behaviours in rats, which relies on robust and validated neuroanatomical and pharmacological criteria (for reviews, see (Kelly et al., 1997; Song and Leonard 2005)). This lesion induces a number of behavioural changes, including increased locomotor activity and deficits in active and passive avoidance tasks (Cairncross et al., 1979). This latter deficit is reversed following acute (Broekkamp et al., 1980; Joly and Sanger 1986) or repeated (Broekkamp et al., 1980; Noreika et al., 1981; Richardson and Tiong 1999; van Riezen et al., 1977) administrations of antidepressants.

The mechanism(s) by which saredutant displays antidepressant-like activity in the DRL-72s paradigm and passive avoidance remain(s) to be clarified. In these procedures (and in many others sensitive to acute antidepressant treatment), the activity of classical antidepressants has been proposed to rely on their ability to modulate 5-HT and NE neurotransmissions (Balcells-Olivero et al., 1998; Britton and Koob 1989; Broekkamp et al., 1980; Dekeyne et al., 2002; Garrigou et al., 1981; Jolly et al., 1999; Richards et al., 1993; Richards and Seiden 1991). It has been shown that peripheral administration of saredutant (0.3 mg/kg, i.p.) did not alter on its own the firing of noradrenergic neurons in the locus coeruleus nor have extracellular levels of NE been modified in the cortex (Steinberg et al., 2001). However, since saredutant was tested at higher doses (3 up to 30 mg/kg i.p.) in our conditions, an effect on NE, and also on 5-HT neurotransmission remains to be proven.

Saredutant also reversed maternal separation-induced vocalization, an effect observed also after an acute treatment with fluoxetine. Although very little is known about the ontogeny of the NK2 receptor and the time-course of its expression through the first postnatal days, autoradiographic studies in neonate rat brain have demonstrated the existence of NK2 receptor binding sites in the thalamus, hippocampus and cortex (Hagan et al., 1993). The present data suggest that at the tested postnatal age of rats, the NK2 receptor is functional. Interestingly, saredutant has been found to reduce maternal separation-induced increase in the number of neurons displaying NK1 receptor internalization in the amygdala of guinea-pig pups (Steinberg et al., 2001), suggesting that NK2 receptor blockade counteracts tachykininergic activation induced by stress in this cerebral structure.

The chronic mild stress (CMS) paradigm has the benefit of a largely approved face validity model (Willner et al., 1992). The progressive emergence of the dirty fur aspect along the CMS procedure (Ducottet et al., 2003) and the improvement of the physical degradation by a chronic treatment with antidepressants (Alonso et al., 2004; Ducottet et al., 2003) demonstrate the relevance and validity of the physical state parameter. As for DRL-72s schedule and passive avoidance, it is also the first description of a beneficial effect of a NK2 receptor antagonist in this paradigm. Very little is known about the modulation of tachykinin neurotransmission in such CMS procedures. In rats exposed for 3 weeks to a CMS, the substance P mRNA levels were increased in the anterodorsal part of the medial amygdaloid nucleus, in the ventromedial and dorsomedial hypothalamic nuclei and the lateral hypothalamic area (Sergeyev et al., 2005), suggesting an activation of the tachykininergic transmission in these cerebral regions. Interestingly, chronic treatment with imipramine has been found to decrease SP concentrations in the amygdala, the hippocampus, the substantia nigra and the striatum (Shirayama et al., 1996). Thus, it can be hypothetized that chronic antidepressants may normalize stress-induced tachykininergic activation. However, the significance of these results is limited to substance P and its preferential target, NK1 receptor. Nevertheless, it should also be noted that substance P and neurokinin A are encoded by the same precursor, preprotachykinin A, suggesting that when the level of Substance P is modified, that of NKA could also be regulated. SP can coexist with neurokinin A within the same neuronal population, and may be co-released with the latter peptide as it is for example observed in the spinal cord (Duggan and Furmidge 1994).

There are some indications that chronic blockade of NK2 receptors have a similar impact on neuronal plasticity compared to classic antidepressants. Indeed, saredutant increased the expression of the brain cAMP response element binding (CREB) protein messenger ribonucleic acid (mRNA) in the rat hippocampus after repeated treatment (1 mg/kg, i.p., 21 days) but not acute administration (Steinberg et al., 2001). Similar effects were observed after repeated treatment with fluoxetine in this study and were consonant with other results obtained in non-stressed animals, with different classes of antidepressants, including 5-HT and NE selective reuptake inhibitors (Thome et al., 2000). Since the CMS procedure decreases the CREB protein mRNA levels in the mouse hippocampus (Song et al., 2006) and since this effect is partially reversed by repeated treatment with imipramine and fluoxetine, the beneficial effect of chronic treatment with saredutant in chronically stressed mice could also rely on a partial reversal of

the stress-induced decrease of CREB in some brain regions, such as the hippocampus, leading to a sustained neuronal plasticity.

5. Conclusion

The studies summarized here provide strong, additional evidence that a pharmacological blockade of tachykinin NK2 receptors results in a clear anxiolytic- and antidepressant-like effect in rodents. These additional preclinical findings therefore underpin the therapeutic potential of NK2 receptor antagonists for the treatment of both depression and anxiety disorders.

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