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The neurokinin NK2 antagonist, saredutant, ameliorates stress-induced conditions without impairing cognition

Nancy Rogacki [a,*], Matilde Lopez-Grancha [b], Vanessa Naimoli [a], Lisa Potestio [a], Rachel J. Stevens [a], Philippe Pichat [b], Olivier E. Bergis [b], Caroline Cohen [b], Geoffrey B. Varty [a], Guy Griebel [b]

[a] Sanofi-Aventis, CNS Department, 1041 Route 202/206, Bridgewater, NJ 08807, USA
[b] Sanofi-Aventis, CNS Department, 31 avenue Paul Vaillant-Couturier, Bagneux, France

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ABSTRACT

The current work extends our previous findings in stress-related disorders, but also addresses the impact of a neurokinin-2 (NK2) antagonist on cognition. Besides efficacy in mood disorders, an NK2 antagonist may have the potential to lack the disinhibitory components and adverse side effects associated with existing clinical treatments. Saredutant (3–30 mg/kg, per os, p.o.) was tested for anxiolytic-like potential in three mouse models: holeboard, stress-induced hyperthermia (SIH) and four-plate. In the holeboard model saredutant (30 mg/kg) showed a trend to increase head dipping without affecting general activity. In the SIH model, saredutant demonstrated a significant reduction in stress-induced temperature at 30 mg/kg, while the number of punished crossings in the four-plate was increased at all doses tested (3–30 mg/kg). While chlordiazepoxide (CDP) demonstrated anxiolytic-like effects in these models, the adverse side effects of benzodiazepines, such as sedation, disinhibition and cognitive deficits are well-documented. Saredutant produced no detrimental effect in three models of cognition: Morris Water Maze (MWM) in rats, spontaneous alternation in a Y-maze in mice and novel objection recognition in mice. In contrast, the benzodiazepine, diazepam (DZM), produced cognitive impairments. NK2 receptor antagonists like saredutant may therefore yield beneficial effects for mood disorders without the adverse effects of current treatments.

1. Introduction

The neurokinin-2 (NK2) receptor has been implicated in the modulation of stress-related disorders such as anxiety and depression (Griebel et al., 2001; Louis et al., 2008; Micale et al., 2008; Steinberg et al., 2001; Walsh et al., 1995). Localization studies have shown that NK2 receptors are found in brain regions involved in emotional processes such as the amygdala, hippocampus, cortex, dorsal raphe nucleus and thalamus (Bensaid et al., 2001; Hagan et al., 1993; Saffroy et al., 2001, 2003). The endogenous NK2 ligand, neurokinin A (NKA), is co-localized with the NK1 receptor ligand, Substance P, and induction of stress results in co-release of these ligands (Griebel et al., 2001; Steinberg et al., 2001). In addition, a relationship between NK2 and the stress hormone corticotrophin-releasing factor (CRF) has been evidenced via blockade of CRF-induced firing of locus coeruleus neurons by the peripherally administered non-peptide NK2 receptor antagonist, saredutant (Steinberg et al., 2001). Additionally, saredutant has demonstrated central activity in a wide variety of neurochemical, electrochemical and behavioral assays that relate to stress disorders (Emonds-Alt, 2004; Ebner et al., 2009).

The magnitude of stress may affect both the clinical manifestations of mood disorders, as well as the therapeutic response to treatment. A previous study compared the behavioral effects of saredutant under basal (no prior application of stressor) and acute stress-induced conditions in models of anxiety and depression (Micale et al., 2008). Unlike the benzodiazepine anxiolytic, diazepam (DZM), and the tricyclic antidepressant, clomipramine, the effects of saredutant were not counteracted by prior stressful conditions. Also, in contrast to those agents, saredutant demonstrated dual efficacy in numerous models sensitive to anxiolytics or antidepressants (Griebel et al., 2001; Louis et al., 2008; Steinberg et al., 2001). In addition, saredutant evidenced continued activity in chronic settings (Louis et al., 2008). The following are examples of such acute and chronic paradigms: the elevated plus-maze, light/dark box, mouse defense test battery, forced swim test, differential reinforcement of low rate-72s, and chronic mild stress, thereby addressing the different aspects of affective and mood disorders. Furthermore, the anxiolytic-like effects of saredutant have been extended to a primate model, the marmoset intruder test (Walsh et al., 1995).

Additional proposed key features of saredutant, besides the dual efficacy for anxiety and depression, may be the clinical potential to lack the disinhibitory components and adverse side effects associated with benzodiazepines and tricyclic antidepressants (Micale et al., 2008), as well as the serotonin selective re-uptake inhibitor (SSRI)

* Corresponding author. Tel./fax: +1 908 231 4415.
E-mail address: nancy.rogacki@sanofi-aventis.com (N. Rogacki).

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antidepressants (Schmitt et al., 2001). Chronic stress may lead to sensitization or tolerance to future stress (Hajós-Korcsok et al., 2003). Saredutant remained effective under those conditions as demonstrated by efficacy in chronic mild stress and the restoration of the deficit in acquisition of passive avoidance in bulbectomized rats (Louis et al., 2008). Another concern is that the current mood disorder treatments may induce cognitive impairments (Ballenger, 2000; Stewart, 2005). No study to date has investigated the cognitive profile of an NK2 antagonist.

The current work is an extension of our previous profiling of saredutant for its therapeutic potential in stress-related disorders. More importantly, this is the first report of an NK2 antagonist's lack of effect on models of cognitive functions. Saredutant was tested for anxiolytic-like potential in the holeboard, stress-induced hyperthermia (SIH) and four-plate assays. The benzodiazepine chlordiazepoxide (CDP) was used as a classic anxiolytic comparator compound for those same models. In the same fashion, the SSRI antidepressant paroxetine was used in the SIH assay. In addition, different aspects of learning and memory were addressed with the Morris Water Maze (MWM), spontaneous alternation in a Y-maze task and episodic memory in a novel object recognition task. Two of these models of cognition included the anxiolytic compound DZM due to its cognitive impairment potential (Bertaina-Anglade et al., 2006). The present investigation therefore provides additional efficacy for saredutant regarding anxiety disorders, but more critically, elucidates its potential for the lack of adverse side effects, such as disinhibition and cognitive impairments that characterize the current therapeutic regimen.

2. Materials and methods

2.1. Animals

All procedures were performed in accordance with the 'Guide and Care and Use of Laboratory Animals' (National Institutes of Health) and were approved by the Institutional Animal Care & Use Committee of Sanofi-Aventis. Mice and rats were kept under temperature- and humidity-controlled (22 °C, 50%) conditions on a 12:12 light:dark cycle with food and water available ad libitum. All tests were conducted within the time frame of 7:00–17:00 h. Random assignment to treatment groups was made in all studies.

2.2. Drugs

Saredutant (SR48968), ((S)-N-methyl-N-[4-(4-acetylamino-4-phenylpiperidino-2-(3,4-dichlorophenyl) butyl] benzamide), was synthesized by the CNS Medicinal Chemistry Department of Sanofi-Aventis (Montpellier, France). The saredutant dose range tested (3–30 mg/kg) was based on previous work (Louis et al., 2008). Preliminary data indicated the selection of positive controls and their appropriate dosages. Paroxetine was obtained from Apin Chemicals LTD (Abingdon, Oxfordshire, UK). DZM was purchased from Sigma-Aldrich (St Louis, MO, USA) or Francochim (Paris, France). CDP was procured from Sigma-Aldrich (St Louis, MO, USA). The cognition work for saredutant utilized 0.6% methylcellulose combined with 0.5% Tween 80 in distilled water for per os (p.o.) administration. DZM was solubilized in 0.9% physiological saline. The anxiety assays were conducted as follows: saredutant was dissolved in 0.5% Tween 80 alone or in a combination with 0.6% carboxymethylcellulose for the four-plate experiment. CDP was solubilized in 0.9% physiological saline or distilled water (four-plate). Paroxetine was prepared in distilled water. Routes of administration were i.p. or p.o. as noted in the procedures. Injection volumes for mice were 10 ml/kg, while those for rats were 1 ml/kg (anxiety assays) or 5 ml/kg (cognition assays). Doses refer to the free base.

2.3. Procedures

2.3.1. Holeboard

Male C57BL/6 mice (Taconic Farms, Germantown, NY) weighing approximately 18–20 g were used. The holeboard apparatus consisted of open-field boxes (Med-Associates, St Albans, VT) that were contained in ventilated, sound-attenuating cubicles (36.5×63.5×42 cm; l×w×h). Each chamber (28.5×28.5×20 cm) was equipped with a house light (40 lx) and a stainless steel holeboard (27.5×27.5 cm) 2.54 cm above the bottom with 16 holes (2 cm diameter) arranged in a 4×4 pattern. Mice were dosed with vehicle, saredutant (3, 10, 30 mg/kg, p.o.) or CDP (3 mg/kg, i.p.) 30 min prior to testing. Following the pre-treatment time, mice were placed in the center of the apparatus and permitted free exploration for 10 min. Head dips (number of beam breaks below floor level) and locomotor activity (distance traveled in cm) were measured by infrared beams located above and below the platform, respectively. Data was recorded automatically on a computer with Med-PC activity monitor software (Med Associates, St. Albans, VT, USA). Data were analyzed with either a one-way ANOVA followed by Dunnett's *t* test for treatment vs. the vehicle control group or with a Student's *t* test.

2.3.2. Stress-induced hyperthermia (SIH)

Male C57BL/6 (Taconic Farms, Germantown, NY) mice weighing 18–22 g were used. The SIH test procedure was a modification of the method of van der Heyden et al. (1997). Mice were transferred from group housing to individual housing 24 h before the test. The SIH paradigm includes routinely the administration of compounds 60 min before the first temperature measurement to allow for any stress effect created by the injection to dissipate completely. Vehicle or saredutant (3, 10, and 30 mg/kg, p.o.) were both administered in this fashion. In separate experiments, this also applied to the dosing of CDP (1, 3, and 10 mg/kg, i.p.) or paroxetine (1, 3, and 10 mg/kg, i.p.). Each was assessed relative to their respective vehicles. Individually housed mice were subjected to two sequential rectal temperature measurements taken within a 10 min interval. The first measurement captured the animal's basal core temperature (Time 1 = T1), and served as the stressor that elicits the SIH response. The second temperature (Time 2 = T2) recorded the SIH response and the difference between the first and second temperatures (T2 − T1 or ΔT) defined the SIH measure. Temperature measurements were made to the nearest 0.1 °C with a glycerol lubricated thermistor probe (Physitemp TH-5 Thermalert Monitoring Thermometer and RET-3 rectal probe for mice, Clifton, NJ, USA) inserted 2 cm into the rectum of each subject and held in place until temperature reading stabilized for approximately 20 s. The mouse was returned to its individual housing between measurements. Compounds which reduce ΔT, without affecting T1, were proposed to exert anxiolytic-like effects (Olivier et al., 2003). Data were analyzed as appropriate by either a one-way ANOVA followed by Dunnett's *t* test for treatment vs. the vehicle control group or with a non-parametric Kruskal–Wallis followed by Holm–Bonferroni multiplicity adjustment comparisons as applicable.

2.3.3. Four-plate

Male Swiss Webster mice (Charles River Laboratories, Raleigh, North Carolina) weighing 20–27 g were used. The custom made four-plate apparatus (Phenome Technologies, Inc., Skokie, IL) consisted of a clear plastic box in a rectangular shape (24×18×28 cm) with a transparent lid. The floor was covered with four identical rectangular steel plates (8×11 cm) separated from one another by a gap of 4 mm. The plates were connected to a programmable animal shocker (model H13-16, Coulbourn Instruments, Whitehall, PA) that can generate electric shocks. Mice were placed into a large holding cage for 30 min prior to dosing. Mice were then administered either vehicle or saredutant (3, 10, 18, or 30 mg/kg, p.o.) with a 60 min pre-treatment

time or CDP (10 mg/kg, i.p.) with a 30 min pre-treatment time and then placed into individual, smaller holding cages for that duration of the pre-treatment period. Following the elapsed pre-treatment time, mice were placed in the four-plate apparatus and given a 15 s exploration period. Mice were then subjected to a 0.5 mA foot shock with a 0.5 s duration when crossing from one plate to another (defined as two legs on one plate and two legs on another). The number of punished crossings was recorded for a period of 60 s. Between subjects the apparatus was cleaned with 70% alcohol between each animal to remove odor cues. Compounds with anxiolytic-like activity are capable of increasing the number of punished passages. Data were analyzed with a non-parametric Kruskal–Wallis followed by Holm–Bonferroni multiplicity adjustment comparisons or a non-parametric Wilcoxon test.

2.3.4. Spatial reference memory in the rat Morris Water Maze (MWM)

Male Wistar rats (CERJ, Le Genest-Saint-Isle, France) weighing 250–300 g on arrival were used. The MWM task was performed as previously described (Morris, 1984) and utilized a circular arena (150 d × 60 h cm) made of gray polyvinyl chloride (PVC) filled with water (23 ± 2 °C) to a height of 35 cm. A Plexiglas escape platform (12 × 12 × 32 cm) was placed into the pool, 1 cm below the water surface and 10 cm from the wall. The water was made opaque by the addition of a white artificial colorant (Rohm and Haas, Sweden) rendering the platform invisible. The test room contained several permanent extra-maze cues such as posters, a flag and other objects on the walls. A video-tracking camera (placed 200 cm above the center of the pool surface) monitored the trajectory of the rat. The video signal was transmitted to a computer in an adjacent room and analyzed using the VIDEOTRACK© system (View Point Ltd, Champagne au Mont d'Or, France). The platform was placed at one of four possible cardinal locations: NW, SE, NE, and SW; this remained the same for all trials but starting positions changed from one trial to another. Vehicle or saredutant (3, 10 or 30 mg/kg, p.o.) were administered 60 min before each testing session. Learning consisted of three consecutive daily acquisition sessions, each of them consisting of three trials, with a maximum trial duration of 120 s with an inter-trial interval of 30 s. Latency times (in s) to find the hidden platform were recorded during each trial of each learning session. If the rat located the platform within the maximum trial time allowed, it was left on the platform for 30 s. If the rat did not locate the platform within the time limit, it was gently placed on it for a 30 s period. Two-way ANOVA with repeated measures analysis was employed for the acquisition data (latency times to reach the platform, averaged across all three sessions for each of the three trials). This was performed on the ranked data of the variable 'latency', with 'treatment' as the between factor, and 'trial' as the within factor. Post hoc analysis was performed with Dunnett's multiple comparison analyses using the vehicle as the comparator.

2.3.5. Spatial working memory in the spontaneous alternation task in the Y-maze in mice

Male NMRI mice (CERJ, Le Genest-Saint-Isle, France) weighing 16–18 g on arrival were used. Activity in a Y-maze was used to measure spontaneous alternation performance (spatial working memory) and locomotor activity (Heo et al., 2003; Mamiya et al., 2004; Sarter et al., 1988). The apparatus used was made of gray PVC and consisted of three equally spaced arms (28 × 6 × 16 cm) at equal angles (120°). Spontaneous alternation behavior involves responsiveness to novelty that relies on the need to remember which of the two choice arms were recently visited to enable selection of the novel alternative. Vehicle, saredutant (3, 10 or 30 mg/kg, p.o.) or DZM (4 mg/kg, p.o.) were administered 60 min before each testing session. Following the appropriate pre-treatment times, each mouse was placed in one of the arms and allowed to move freely for 5 min without food reward. The recorded parameters were: (1) the total arm entries and (2) the spontaneous alternation. An arm entry occurred when the body of the mouse completely entered into an arm compartment (tail optional). The sequence of the arm entries (alternations) referred to three successive visits to the three separate arms of the maze (i.e., ABC, ACB, BAC, BCA, CAB or CBA). The percent spontaneous alternation (SA%) was calculated by multiplying the number of alternations by 100, divided by the total number of possible alternations (total arm entries subtracted by 2). The apparatus was cleaned with water between sessions to remove the previous animal odor and permitted to dry. The percentage of spontaneous alternations and the total arm entries were analyzed by the non-parametric Kruskal–Wallis test followed by Holm–Bonferroni multiplicity adjustment comparisons.

2.3.6. Short-term visual episodic memory in mice using the object recognition task

Male Swiss mice (CERJ, Le Genest-Saint-Isle, France) 16–18 g on arrival were employed. Mice were tested using a visual recognition memory task similar to that described by Ennaceur and Delacour (1988). The apparatus consisted of uniformly lit (20 lx) gray PVC enclosure (52 l × 52 w × 40 h cm) with a video camera positioned 160 cm above the bench top. The observer was located in an adjacent room fitted with a video monitoring system. The experiment consisted of three separate sessions: context habituation, an acquisition trial (learning) and a test trial (recall). During the habituation period, the animals were allowed to freely explore the apparatus for 2 min. Time spent in locomotor activity was manually registered. Saredutant (3, 10 or 30 mg/kg, p.o.), DZM (4 mg/kg, p.o.) or vehicle (p.o.) were injected 60 min before the acquisition trial. 24 h later animals were placed into the open field for the acquisition trial now in the presence of two identical objects (either 7 × 3 × 8 cm metal triangles representing object A or 9 × 3 × 7 cm plastic yellow and blue pyramids representing object B), placed 10 cm away from the two opposite corners of the back wall. Mice were left in the enclosure for the amount of time necessary to spend maximally 15 s exploring these two objects, within a 5 min time limit. Animals were removed from the enclosure once they reached the maximum exploration time. Exploration of an object was defined as the animal's head being located within 2 cm of the object while either looking at, sniffing or touching the object. Combinations of the location and content of the familiar object were balanced to reduce potential biases due to spatial or object preferences.

A test trial was conducted 1 h after the acquisition trial. In this session, one of two objects in the acquisition trial was replaced with a new object. Then the mouse was again placed in the open field for 4 min and the time spent exploring each object was recorded to the whole second (precision ± 1 s). Two identical sets of objects were used to allow for cleaning between animals to eliminate potential olfactory cues. Normal animals exhibit a high discrimination level between the two objects with that inter-session interval; that is, the animal spends more time exploring the novel object rather than the familiar object, which is suggestive of an intact short-term episodic-like memory. Data (time exploring each of the two objects) in seconds were analyzed with a two-way ANOVA with repeated measures, with the treatment as the between factors and the object as the within factor. Post hoc analysis was performed with Dunnett's multiple comparisons test for comparing the time exploring the familiar vs. the novel object for each treatment.

2.4. Statistics

Statistical analyses for all of the behavioral tasks executed were performed using the SAS system 8.2 software (SAS Institute, Inc., Cary, NC, USA). Normality and equal variance were checked before determining whether to use parametric or non-parametric analyses.

Confidence limits at the 5% level were considered statistically significant.

3. Results

3.1. Holeboard

Saredutant demonstrated a trend to increase head dips at 30 m/kg; [$F(3,32)=2.35$; $p=0.09$], but did not effect locomotor response; [$F(3,32)=1.54$; n.s.] (Fig. 1). CDP also demonstrated a trend to increase head dips; [$t(16)=1.92$; $p<0.07$], but in contrast to saredutant, it also increased locomotion; [$t(16)=4.00$;$p<0.001$].

3.2. Stress-induced hyperthermia (SIH)

Saredutant reduced the SIH response as measured by the stress-induced temperature elevation minus the basal temperature (Fig. 2) [$F(3,36)=3.25$; $p<0.05$] with a significant reduction at a dose of 30 mg/kg ($p<0.01$). No effect of saredutant occurred on basal temperature (data not shown) [$F(3,36)=1.33$; n.s.].

CDP and paroxetine were examined in separate experiments (Table 1). CDP reduced the SIH response in a highly significant manner (DF = 3,Chi2 = 19.50, $p<0.005$). A multiple comparisons test for treatment vs. control group revealed that the 10 mg/kg dose reduced T2 ($p<0.001$). In contrast, paroxetine had no effect on the SIH response (DF = 3, Chi2 = 2.82, n.s.). Neither CDP nor paroxetine impacted the basal temperature for any treatment group (DF = 3, Chi2 = 1.02, n.s.; DF = 3,Chi2 = 2.12, n.s.; respectively).

3.3. Four-plate

Saredutant significantly increased the number of punished crossings compared to the vehicle (DF = 4, Chi2 = 18.7, $p<0.001$) with significant effects at all doses tested (Fig. 3): 3 mg/kg ($p<0.001$), 10 mg/kg ($p<0.001$), 18 mg/kg ($p<0.01$) and 30 mg/kg ($p<0.001$). CDP (10 mg/kg) also induced a significantly increased number of punished crossings [Wilcoxon's test S = 66, $p<0.001$].

3.4. Spatial reference memory in the Morris Water Maze (MWM) test

Saredutant did not affect the escape latencies in the MWM task at any dose tested [$F(3,339)=0.99$; n.s.]. As depicted in Fig. 4, all groups showed a significant reduction in escape latency (s) over the trials [$F(2,339)=17.12$; $p<0.001$]. Latencies to reach the hidden platform (trial 1 vs. trial 3) were significantly improved in control animals ($p<0.05$) and in saredutant-treated rats at 3 mg/kg ($p<0.01$), 10 mg/kg ($p<0.05$)

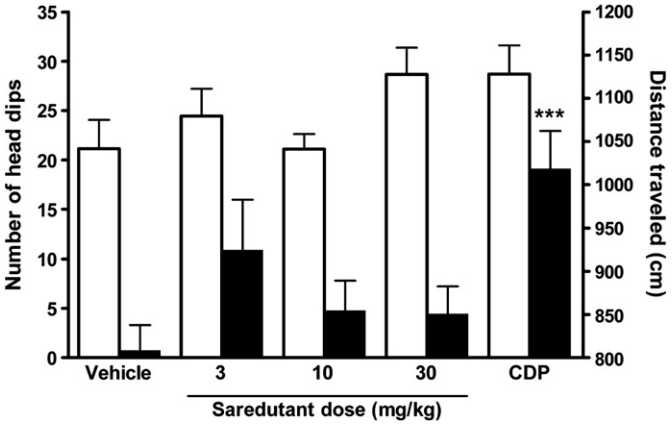

Fig. 1. The effects of saredutant and CDP on the total number of head dips is plotted with white bars on the left x axis (mean ± sem). The effects of saredutant and CDP on distance traveled (cm) is plotted with black bars on the right axis (mean ± sem) with $n=8$–10 per group. ***$p<0.001$ indicates significance from vehicle group.

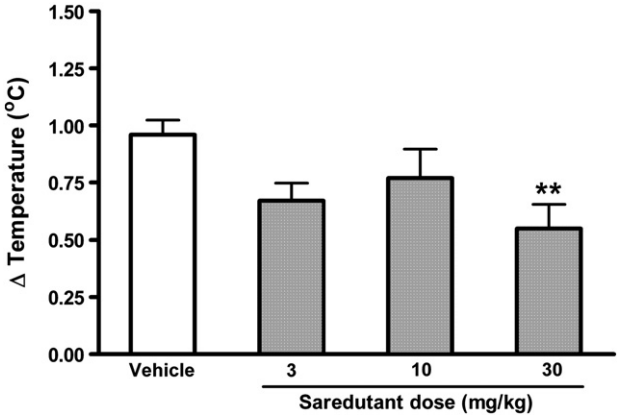

Fig. 2. The effects of saredutant on temperature change (Time 2 − Time 1; T2 − T1 or ΔT in °C, mean ± sem) with $n=9$–10 per group. **$p<0.01$ indicates significance from vehicle group.

and 30 mg/kg ($p<0.05$). None of the treatment groups displayed alterations in swimming speed (data not shown), indicating that latency is an adequate measure of the ability of the rats to find the platform and correctly address the spatial remembering of platform location.

3.5. Spatial working memory in the spontaneous alternation task in the Y-maze

A significant treatment effect occurred for spontaneous alternation behavior in the Y-maze (DF = 4, Chi2 = 12.45, $p<0.05$). Spontaneous alternation in the DZM-treated group was significantly lower than that of the vehicle-treated control ($p<0.05$) (Fig. 5a). Unlike DZM, the administration of saredutant did not affect alternation behavior at any dose tested. The number of arm entries did not change among all experimental groups except for DZM (Fig. 5b). A significant increase of the number of entries was observed in mice treated with DZM (DF = 4, Chi2 = 12.17, $p<0.05$).

3.6. Short-term visual episodic memory in mice using the object recognition task

Neither the locomotor activity recorded during the context habituation session, nor the total time spent in exploring both objects during the acquisition and the learning session, was significantly modified by the treatment with saredutant (data not shown). Following a short forgetting delay, vehicle-treated mice and saredutant-treated mice spent significantly more time exploring the novel object than the familiar one [$F(1,33)=58.08$, $p<0.001$] (Fig. 6). This was confirmed by a Dunnett's test in control animals ($p<0.001$) and in saredutant-treated mice at 3 mg/kg ($p<0.001$), 10 mg/kg ($p<0.01$), and 30 mg/kg ($p<0.01$). In contrast, animals treated with DZM (4 mg/kg) were unable to show a significant discrimination between the novel and familiar

Table 1
The effects of CDP and paroxetine on basal and stress-induced temperature change (°C).

Treatment	Temperature (°C)	
	T1	ΔT (T2−T1)
Vehicle	36.08 ± 0.19	1.24 ± 0.17
CDP 1 mg/kg	35.99 ± 0.14	1.09 ± 0.18
CDP 3 mg/kg	36.13 ± 0.14	0.73 ± 0.24
CDP 10 mg/kg	35.91 ± 0.13	0.07 ± 0.08***
Vehicle	36.06 ± 0.18	0.97 ± 0.04
Paroxetine 1 mg/kg	36.10 ± 0.18	0.93 ± 0.06
Paroxetine 3 mg/kg	36.29 ± 0.17	0.98 ± 0.10
Paroxetine 10 mg/kg	36.04 ± 0.29	0.84 ± 0.07

*** $p<0.001$.

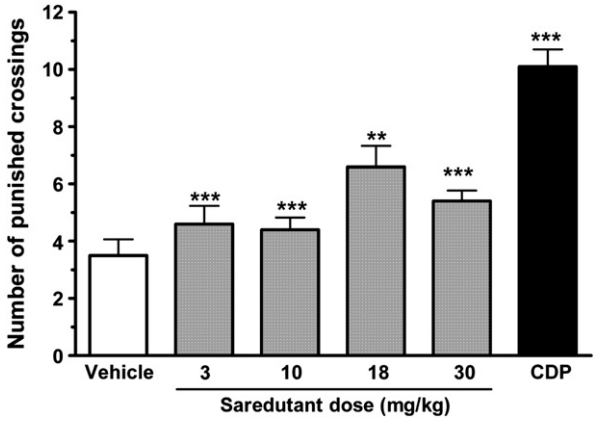

Fig. 3. The effects of saredutant and CDP on number of punished crossings (mean ± sem) with $n = 10–11$ per group. $^{**}p<0.01$, $^{***}p<0.001$ indicates significance from vehicle group.

object ($p = 0.11$, n.s.) without modifying exploration of the familiar object, suggesting a short-term episodic memory impairment.

4. Discussion

The present study provided further evidence that the NK2 antagonist saredutant has anxiolytic potential in assays that model differing aspects of anxiety. These include a model of basal stress (holeboard), induced stress (SIH) and conflict (four-plate). In addition, saredutant may differ from the existing anxiolytic treatments CDP and paroxetine that possess known side effects, such as sedation, disinhibition and cognitive impairments (Ballenger, 2000; Schmitt et al., 2001; Stewart, 2005). More critically, this is the first investigation to demonstrate that an NK2 antagonist may be devoid of cognitive impairment properties in three models of cognition: spatial learning and reference memory in the MWM, working memory using spontaneous alternation in the Y-maze task and short-term visual episodic memory in the object recognition task, unlike the existing benzodiazepine anxiolytic DZM.

4.1. Evaluation of the anxiolytic potential of saredutant

In a modified holeboard assay, the results for the head dip measure of exploration showed a trend towards anxiolytic potential, but not statistically significant. This trend for effect could not be explained by any disinhibitory impact of saredutant as locomotor activity was not altered at the highest dose tested. Exploration was therefore independent of motor effects of the compound. In addition, facilitation of head dipping behavior without impact on general activity concurred with findings from an elevated plus maze study conducted in rats in which saredutant significantly increased that measure without altering total arm entries at the highest dose tested

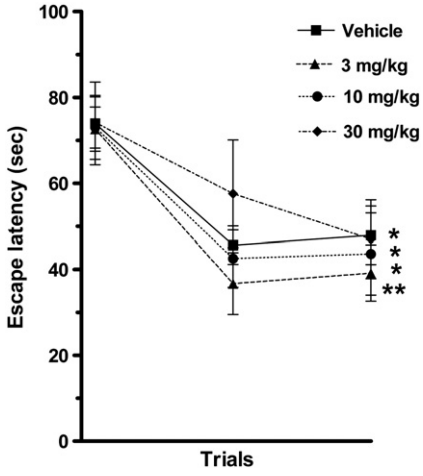

Fig. 4. The effects of saredutant on escape latency (sec) or over three acquisition trials (mean ± sem) with $n = 9–10$ per group. Squares represent the vehicle group; triangles represent saredutant 3 mg/kg; circles represent saredutant 10 mg/kg; diamonds represent saredutant 30 mg/kg. $^{*}p<0.05$;$^{**}p<0.01$ indicates significance from vehicle group (trial 1 vs. trial 3).

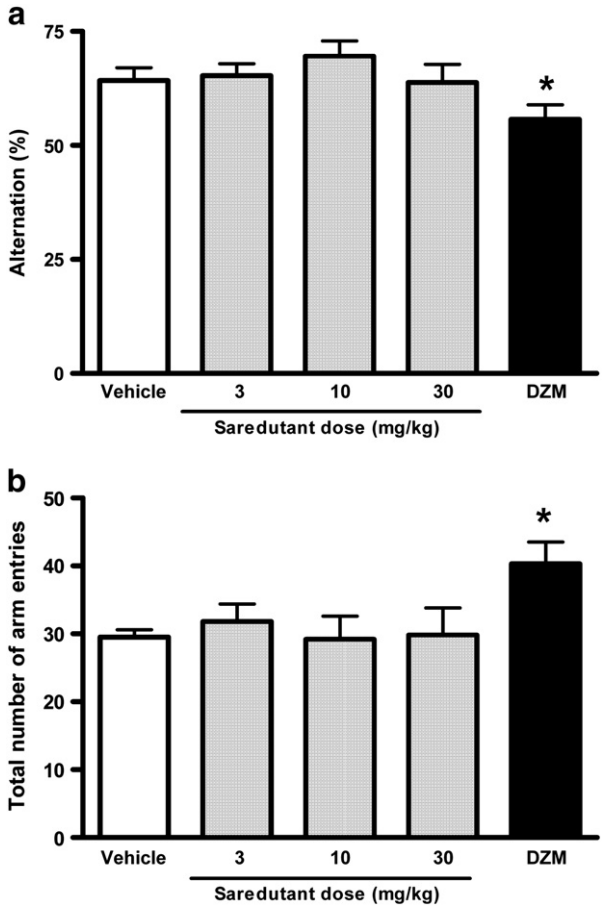

Fig. 5. (a) The effects of saredutant and DZM on percentage of spontaneous alternation (mean ± sem) with $n = 12$ per group. $^{*}p<0.05$ indicates significance from vehicle group. (b) The effects of saredutant and DZM on the total number of entries (mean ± sem). $^{*}p<0.05$ indicates significance from vehicle group.

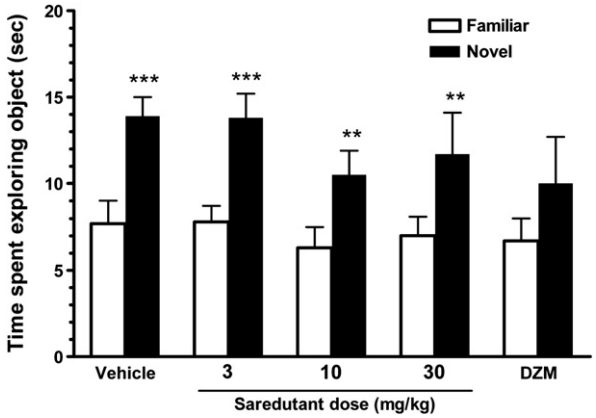

Fig. 6. The effects of saredutant and DZM on time (sec; mean ± sem) exploring two independent objects with $n = 9–10$ per group. White blocks represent the familiar object; black blocks represent the novel object. $^{**}p<0.01$;$^{***}p<0.001$ indicates significance compared to the familiar vs. novel object for each treatment group.

of 3 mg/kg (Griebel et al., 2001). While CDP produced a similar trend to increase head dipping this was also accompanied by a significant increase in locomotion at the highest dose, thus confounding the interpretation of the exploration being truly anxiolytic-like activity. Saredutant yielded more directed exploration without the confounding generalized activity or hyperactivity.

Hyperthermia is an objective, reproducible measure of autonomic nervous system activation in response to stress that has direct clinical translation (Vinkers et al., 2008). Recent reviews have discussed the pharmacological effects of benzodiazepines, but no validation for SSRI's, in the SIH model (Bouwknecht et al., 2007; Olivier et al., 2003). None of the compounds tested had any impact on basal temperature which eliminates any possible confounds of compound effect on basal core temperature and the concern that an anxiolytic compound possesses inherent hypothermic properties that may contaminate measures even prior to stress exposure.

Although the SSRI paroxetine had no effect on temperature, both saredutant and CDP significantly reduced stress-induced temperature change at 30 mg/kg and 10 mg/kg, respectively. These findings regarding the acute lack of effect for an SSRI confirms other work with the SIH model that demonstrated the need for chronic treatment to produce a hypothermic response in mice with fluoxetine (Conley and Hutson, 2007). While the benzodiazepine CDP evidenced greater potency as compared to saredutant, the alpha (α) subunits of the GABAA receptor are well-documented for their detrimental clinical effects such as amnesia, tolerance, dependence and alcohol potentiation (Conley and Hutson, 2007).

The four-plate assay is a model of anxiety by which the motivation to explore all regions of the apparatus comes in conflict with receiving shocks for traversing across the four plates (Aaron et al., 1971). Antinociceptive drugs have no activity in this paradigm. A recent study has confirmed that this assay can be characterized as an anxiolytic paradigm as opposed to an analgesic screen (Ripoll et al., 2006); for example, benzodiazepines such as alprazolam and DZM were active in the four-plate model, but had no effect in the hot plate test for analgesics. In contrast, the potent analgesic morphine had no effect in the four-plate assay in concordance with previous studies (Aaron et al., 1971; Ripoll et al., 2006). Saredutant was especially sensitive in this procedure with several doses tested increasing significantly the number of punished crossings.

Taken together, saredutant showed a trend for effect in one model and positive effects in two models of anxiety, including the SIH model which is characterized as insensitive to SSRI's (Conley and Hutson, 2007). That was confirmed in the present study with the lack of effect for paroxetine in that model. Previously saredutant has been shown as effective in a wide variety of models of both anxiety and depression (Griebel et al., 2001; Louis et al., 2008), as well as under conditions of previous exposure to acute stress (Micale et al., 2008) and chronic stress (Louis et al., 2008). This is indicative of the compound's clinical potential for dual efficacy in both affective diseases in populations undergoing various levels of stress. “Traditional” anxiolytics like the benzodiazepines demonstrate acute effects in models of anxiety, but the SSRI antidepressants often require chronic treatment in these same models (Borsini et al., 2002). In addition, while the benzodiazepines are limited clinically to specific anxiety states such as generalized anxiety disorder or acute panic attacks, the SSRI's are active in those diseases, as well as major depression, obsessive compulsive disorder, seasonal affective disorder and posttraumatic stress disorder. Saredutant therefore could differ from existing treatments on the market in that it has demonstrated dual efficacy with acute treatment across multiple assays, including those that traditionally have required chronic treatment for antidepressants that possess anxiolytic potential.

In addition, saredutant at higher doses has not shown any impact on general activity that could generate false negatives or positives in either the holeboard or the four-plate assays, unlike CDP's disinhibitory properties that may have contributed to impaired expression of head dipping exploration or enhanced the number of punished crossings made by the mice in each respective assay. Acute administration of SSRI's may even produce an anxiogenic profile (Conley and Hutson, 2007). Saredutant thereby potentially exhibits an antidepressant profile with more rapid onset of anxiolytic properties for perhaps more comprehensive facets of anxiety with the key benefit of reduced side effects.

4.2. Evaluation of potential cognitive deficits with saredutant

Although no prior report exists for the impact of an NK2 antagonist on cognition, previous studies have indicated that the intracerebroventricular (icv) administration of the NKA peptide may enhance learning under basal conditions (Flood et al., 1990) or it may ameliorate induced deficits (Kameyama et al., 1998). In the former study, NKA improved memory retention with an icv injection immediately following training in a T-maze. In the latter study, NKA (0.3 μg) reversed scopolamine-induced impairment of spontaneous alternation performance in a Y-maze, but did not alter the increase in total arm entries by scopolamine. The opioid antagonist naloxone, which was able to block Substance P's improvement of spontaneous alternation, had no effect on NKA's impact on spontaneous alternation. It was hypothesized that NKA may activate the cholinergic system, which has been confirmed by Steinberg et al. (1998).

Both of these studies conducted with the endogenous peptide would predict that an NK2 antagonist may disrupt cognitive processes (Flood et al., 1990; Kameyama et al., 1998). In the present study, we therefore investigated the impact of the NK2 antagonist saredutant in three models of cognition (MWM, spontaneous alternation in a Y-maze task and visual episodic memory in an object recognition task) to determine whether the compound would demonstrate any cognitive deficits. In addition, in the latter two models DZM was included as a comparator.

Saredutant was found to affect neither working memory nor reference memory in the MWM, thus spatial memory remained unaltered in the presence of saredutant at any dose tested. All treatment groups were found to rapidly acquire the task by the second day and maintain that acquisition on the third day similar to the vehicle treatment group, thereby indicative of intact learning and memory skills. Also, swim speed was unaffected by saredutant. Overall, saredutant therefore showed no deficits of cognition or motor side effects in the MWM. This is in contrast to our previous findings with DZM (1, 3 and 10 mg/kg) that impaired escape latencies with high significance on day three of acquisition at all doses tested (Stemmelin et al., 2008), suggestive of a severe deficit in spatial reference memory.

Saredutant had no effect on spontaneous alternation behavior in the Y-maze at all doses tested, indicative of no detrimental impact of the compound on spatial working memory. Based on the total number of arm entries, saredutant did not alter locomotor activity in the assay. This was in direct contrast to DZM which demonstrated a significantly lower percentage of alternation within the same study, accompanied by a significant increase in the number of total arm entries. DZM therefore displayed a significant memory impairment evidenced by errors committed as the mice were unable to assess what arms had been entered previously. DZM may have shown disinhibitory properties that impacted memory processes negatively. The results for the NK2 antagonist saredutant were in direct contrast to that predicted by the Kameyama et al. (1998) work utilizing the NKA peptide to ameliorate spontaneous alternation performance in a Y-maze task.

Saredutant had no effect on short-term visual episodic memory at any dose tested as measured by the object recognition task. Mice were able to discriminate that they had interacted with the familiar object during the learning session and therefore spent more time, approximate to that of the vehicle treatment group, examining a novel

object. In contrast, the DZM group could not distinguish between the two objects at all, thereby evidencing complete abolishment of object recognition.

Taken together, the three models of cognition employed in the current investigation all demonstrated that saredutant is devoid of any detrimental effect on cognition. These models assessed differing aspects of learning and memory, including spatial working and reference memory and short-term visual episodic memory. The findings for an NK2 antagonist were in direct contrast to those determined with the endogenous NKA peptide which was found to enhance learning in a T-maze and ameliorate a scopolamine-induced memory deficit of spontaneous alternation performance in a Y-maze task (Flood et al., 1990; Kameyama et al., 1998). DZM, however, produced impaired learning and memory in all of the assays employed. Another critical finding was that saredutant had no effect on any locomotor or activity measures within the assays; however, DZM evidenced some potentially disinhibitory activity in the spontaneous alternation behavioral model.

The regulation of acetylcholine (ACh) by NKA has been investigated by in vivo microdialysis in the septohippocampal region under both basal and stress-induced conditions (Steinberg et al., 1998). Under control conditions, ACh levels in the hippocampus were unaffected by blockade with saredutant applied systemically, suggesting no role for the tonic modulation of the cholinergic system by NKA. Under conditions of tactile-induced stress (stroking the fur on the back and neck of rats), shown to produce hippocampal ACh release, ACh levels were restored to normal by saredutant. The interpretation was made that NKA may regulate a potent and selective gating mechanism that facilitates information processing in the septohippocampal regarding emotional function. This indicates that the relationship between ACh and NKA is not a simplistic one.

In conclusion, we have shown that saredutant is active in two additional models of anxiety, with a trend for effect in a third. These models measure differing aspects of this stress-induced disorder. Taken together with our past results in numerous models of mood disorders, these results further elucidate the potential role of an NK2 antagonist in the treatment of anxiety and depression applied both acutely and chronically in clinical settings. In addition, saredutant appears to lack the sedative, disinhibitory and acutely anxiogenic aspects of the current therapeutic regimen. Most importantly, and for the first time, it was demonstrated that an NK2 antagonist had no impact on three models of cognition in the present study. In contrast to the previous findings with the NKA peptide that would predict an NK2 antagonist to induce cognitive deficits, saredutant was evidenced as completely devoid of these negative effects. Saredutant therefore exhibits several aspects of an advantageous profile over existing treatments for mood disorders.

References

Aaron C, Simon P, Larousse C, Boissier JR. Evaluation of a rapid technique for detecting minor tranquilizers. Neuropharmacology 1971;10:459–69.

Ballenger JC. Benzodiazepine receptors agonists and antagonists. In: Sadock VA, Sadock BJ, Kaplan HI, editors. Kaplan and Sadock's Comprehensive Textbook of Psychiatry. 7th edition. Philadelphia: Lippincott Williams and Wilkins; 2000. p. 2317–23.

Bensaid M, Faucheux BA, Hirsch E, Agid Y, Soubrié P, Oury-Donat F. Expression of tachykinin NK2 receptor mRNA in human brain. Neurosci Lett 2001;303(1):25–8.

Bertaina-Anglade V, Morillon E, Drieu la Rochelle C. The object recognition task in rats and mice: a simple and rapid model in safety pharmacology to detect amnesic properties of a new chemical entity. J Pharmacol Toxicol Methods 2006;54(2): 99-105.

Borsini F, Podhoma J, Marazatti D. Do animal models of anxiety predict anxiolytic-like effects of antidepressants? Psychopharmacology 2002;163(2):121–41.

Bouwknecht JA, Olivier B, Paylor RE. The stress-induced hyperthermia paradigm as a physiological animal model for anxiety: a review of pharmacological and genetic studies in the mouse. Neurosci Biobehav Rev 2007;31:41–59.

Conley RK, Hutson PH. Effects of acute and chronic treatment with fluoxetine on stress-induced hyperthermia in telemetered rats and mice. Eur J Pharmacol 2007;564(1–3):138–45.

Ebner K, Sartori SB, Singewald N. Tachykinin receptors as therapeutic targets in stress-related disorders. Curr Pharm Des 2009;15:1647–74.

Emonds-Alt X. Tachykinin NK2 receptor antagonists. . editor-in-chiefIn: Starke K, editor. Handbook of Experimental Pharmacology, vol. 164. New York: Springer-Verlag; 2004. p. 220–44.

Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. Behav Brain Res 1988;31(1):47–59.

Flood JF, Baker ML, Hernandez EN, Morley JE. Modulation of memory retention by neuropeptide K. Brain Res 1990;520(12):284–90.

Griebel G, Perrault G, Soubrié P. Effects of SR48968, a selective non-peptide NK2 receptor antagonist on emotional processes in rodents. Psychopharmacology 2001;158(3):241–51.

Hagan RM, Beresford IJM, Stables J, Dupere J, Stubbs CM, Elliott PJ, et al. Characterisation, CNS distribution and function of NK2 receptors studied using potent NK2 receptor antagonists. Regul Pept 1993;46:9-19.

Hajós-Korcsok E, Robinson DD, Yu JH, Fitch CS, Walker E, Merchant KM. Rapid habituation of hippocampal serotonin and norepinephrine release and anxiety-related behaviors, but not plasma corticosterone levels, to repeated footshock stress in rats. Pharmacol Biochem Behav 2003;74:609–16.

Heo HJ, Park YJ, Suh YM, Choi SJ, Kim MJ, Cho HY, et al. Effects of oleamide on choline acetyltransferase and cognitive activities. Biosci Biotechnol Biochem 2003;67(6): 1284–91.

Kameyama T, Ukai M, Shinkai N. Ameliorative effects of tachykinins on scopolamine-induced impairment of spontaneous alternation performance in mice. Methods Find Exp Clin Pharmacol 1998;20(7):555–60.

Louis C, Stemmelin J, Boulay D, Bergis O, Cohen C, Griebel G. Additional evidence for anxiolytic- and antidepressant-like activities of saredutant (SR48968), an antagonist at the neurokinin-2 receptor in various rodent-models. Pharmacol Biochem Behav 2008;89:36–45.

Mamiya T, Asanuma T, Kise M, Ito Y, Mizukuchi A, Aoto H, et al. Effects of pre-germinated brown rice on beta-amyloid protein-induced learning and memory deficits in mice. Biol Pharm Bull 2004;27(7):1041–5.

Micale V, Tamburella A, Leggio GM, Mazzola C, Li Volsi V, Drago F. Behavioral effects of saredutant, a tachykinin NK2 receptor antagonist, in experimental models of mood disorders under basal and stress-related conditions. Pharmacol Biochem Behav 2008;90(3):463–9.

Morris R. Developments of a water maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984;11(1):47–60.

Olivier B, Zethof T, Pattij T, van Boogaert M, van Oorschot R, Leahy C, et al. Stress-induced hyperthermia and anxiety: pharmacological validation. Eur J Pharmacol 2003;463(1–3):117–32.

Ripoll N, Hascoët M, Bourin M. The four plates test: anxiolytic or analgesic paradigm? Prog Neuropsychopharmacol Biol Psychiatry 2006;30(5):873–80.

Saffroy M, Torrens Y, Glowinski J, Beaujouan JC. Presence of NK2 binding sites in the rat brain. J Neurochem 2001;79(5):985–96.

Saffroy M, Torrens Y, Glowinski J, Beaujouan JC. Autoradiographic distribution of tachykinin NK2 binding sites in the rat brain: comparison with NK1 and NK3 binding sites. Neuroscience 2003;116(3):761–73.

Sarter M, Bodewitz G, Stephens DN. Attenuation of scopolamine-induced impairment of spontaneous alternation behaviour by antagonist but not inverse agonist and agonist fl-carbolines. Psychopharmacology 1988;94:491–5.

Schmitt JAJ, Kruizinga MJ, Riedel WJ. Non-serotonergic pharmacological profiles and associated cognitive effects of serotonin reuptake inhibitors. J Psychopharmacol 2001;15(3):173–9.

Steinberg R, Marco N, Voutsinos B, Bensaid M, Rodier D, Souilhac J, et al. Expression and presence of septal neurokinin-2 receptors controlling hippocampal acetylcholine release during sensory stimulation in rat. Eur J Neurosci 1998;10(7):2337–45.

Steinberg R, Alonso R, Griebel G, Bert L, Jung M, Oury-Donat F, et al. Selective blockade of neurokinin-2 receptors produces antidepressant-like effects asscociated with reduced corticotrophin-releasing factor function. Pharmacol Exp Ther 2001;299 (2):449–58.

Stemmelin J, Cohen C, Terranova JP, Lopez-Grancha M, Pichat P, Bergis O, et al. Stimulation of the beta3-adrenoceptor as a novel treatment strategy for anxiety and depressive disorders. Neuropsychopharmacology 2008;33(3):574–87.

Stewart SA. The effects of benzodiazepines on cognition. J Clin Psychiatry 2005;66 (Suppl 2):9-13.

van der Heyden JAM, Zethof TJJ, Olivier B. Stress-induced hyperthermia in singly housed mice. Physiol Behav 1997;62(3):463–70.

Vinkers CH, van Bogaert MJV, Klanker M, Korte SM, Oosting R, Hanania T, et al. Translational aspects of pharmacological research into anxiety disorders: the stress-induced hyperthermia (SIH) paradigm. Eur J Pharmacol 2008;585:407–25.

Walsh DM, Stratton SC, Harvey FJ, Beresford IJM, Hagan RM. The anxiolytic-like activity of GR159897, a nonpeptide NK2 receptor antagonist, in rodent and primate models of anxiety. Psychopharmacology 1995;121:186–91.