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# Behavioral effects of saredutant, a tachykinin NK2 receptor antagonist, in experimental models of mood disorders under basal and stress-related conditions

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ABSTRACT

The present study was made to investigate the role of tachykinin NK2 receptors in the expression of stress-related behaviors in animals. Under basal conditions, intraperitoneal (i.p.) administration of the selective tachykinin NK2 receptor antagonist, saredutant (1 and 3 mg/kg) or diazepam (1 mg/kg) exerted anxiolytic-like effects in rodents, as they reduced grooming score of Wistar male rats tested in the novelty-induced grooming sampling test (NGT) and increased percentage of time and entries in open arms of Swiss male mice tested in the elevated plus maze (EPM) test. After previous exposure to stress-related conditions, as induced by a 2-min forced swim made 5 min prior to the EPM test, saredutant but not diazepam, exhibited anxiolytic-like effects in mice. To study the antidepressant-like activity of tachykinin NK2 receptor antagonist under basal conditions, different groups of rats were injected i.p. with saredutant (2.5, 5 and 10 mg/kg) or the tricyclic antidepressant, clomipramine (50 mg/kg) and tested in the forced swim test (FST), a widely used antidepressant-responsive test. The influence of stress-related conditions was studied in rats subjected to electric foot-shocks (1 mA, 1 s) 24, 5 and 1 h prior to FST, after drugs injection. In the FST, clomipramine decreased the immobility time only under basal conditions, but not after application of acute foot-shocks. To the contrary, saredutant-treated rats also exhibited more active behavior in FST after previous exposure to stressors. These results give further support to the hypothesis that tachykinin NK2 receptors may be a therapeutic target for pharmacological treatment of stress-related diseases, such as anxiety and depression.



## 1. Introduction

It is accepted that stress may be involved in the clinical manifestation of anxiety and depression as well as that a great number of stressful life events lead up to psychic disorders, through a dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis. Preclinical studies have highlighted both the effects of pre-training stressors such as drug injection procedure, inescapable foot-shocks, restraint, forced swim on the behavioral response of animals and the influence of these stressful stimuli on the action of anxiolytic and/or antidepressant drugs. Mechanisms underlying these effects remain still unclear but they might reflect changes occurring in the HPA axis or in neurotransmitter systems such as the γ-aminobutyric acid (GABA), serotonin (5-HT) and norepinephrine (NE) systems (Drago et al., 2001; Briones-Aranda et al., 2002; Teixeira and De Lima, 2003; Chaki et al., 2004; Consoli et al., 2005).

The biological actions of tachykinins substance P (SP), neurokinin A (NKA) and neurokinin B (NKB), are mediated by the activation of three G protein-coupled receptors identified as tachykinin-1 (NK1) receptor, tachykinin-2 (NK2) receptor and tachykinin-3 (NK3) receptor localized in brain regions involved in affective behavior and in the adaptive responses to stress (Regoli et al., 1994). Recent neurochemical and behavioral studies suggest a pivotal role of tachykinin NK2 receptors in the modulation of emotional processes. NKA, the endogenous ligand for tachykinin NK2 receptors, coexists with SP within the same neuronal population and is co-released with the latter peptide by stressful stimuli (Griebel et al., 2001a; Steinberg et al., 2001). In the tachykinin system, the tachykinin NK1 and NK2 receptors regulate the HPA axis in response to stressors (Nussdorfer and Malendowicz, 1998; Steinberg et al., 2001). Furthermore, NKA or its fragment NKA (4–10), injected centrally in rats, produced anxiogenic- and depressive-like effects counteracted by selective tachykinin NK2 receptor antagonists, such as GR100679, GR159879 or SR144190 (Griebel, 1999; Holmes et al., 2003). Saredutant is a non-peptide compound showing high affinity and selectivity for the tachykinin NK2 receptor (Emonds-Alt et al., 1992). Previous studies have reported that it exhibits anxiolytic-like effects in exploration-

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based procedures such as the light/dark test, social interaction, elevated plus maze (EPM) test, but not in conflict-based procedures as the punished drinking test (Griebel et al., 2001a). In addition to its anxiolytic-like effects, saredutant exhibits behavioral and neurochemical characteristics of "antidepressant-like activity" (Steinberg et al., 2001; Chardenot et al., 2002; Salomè et al., 2006).

Based on the above premises, this study was undertaken to confirm the anxiolytic and antidepressant profile of the tachykinin NK2 receptor antagonist, saredutant in the tests commonly used to assess potential anxiolytic and antidepressant treatments under basal conditions (no previous exposure to stressors). Since the real role of tachykinin NK2 receptors following previous exposure to stress are still unclear (Griebel et al., 2001a), this raises some questions on the effects of this drug after stressor applications. To assess the anxiolytic effects under basal conditions, different groups of mice were tested in the EPM test, a well validated test to search for new anxiolytic agents (Pellow et al., 1985; File, 1992). For the measurement of previous exposure to stress, different groups of mice were tested in the EPM procedure 5 min after a stress session of 2-min forced swim as described by Chaki et al. (2004). Since growing evidence indicates that measures of anxiety from different animal species could reflect both different states of anxiety and different species-specific anxiety levels (Lister, 1990), different groups of rats were subjected to the novelty-induced grooming sampling test (NGT). In order to assess the "antidepressant-like activity" of saredutant under basal conditions, different groups of rats were tested in the forced swim test (FST), a well validated antidepressant-responsive test (Porsolt et al., 1978). The influence of previous exposure to stress in different groups of animals was assessed by applying electric foot-shocks (1 mA, 1 s) 24, 5 and 1 h prior the FST, as described by Consoli et al. (2005). Comparative data for the anxiolytic diazepam and the tricyclic antidepressant (TCA) clomipramine, obtained under the same experimental conditions are also provided.

## 2. Materials and methods

### 2.1. Animals

Male rats of the Wistar strain weighing 220–240 g and male Swiss mice weighing 30–50 g (Charles River, Italy) were used throughout all experiments. For at least 1 week prior to the experiment, animals were housed five to a cage at a constant temperature of 21 °C, and under a 12-h light/dark cycle (lights on between 8.00 and 20.00), with food and water available ad libitum. Randomly assigned to any treatment group, animals were used only once in the behavioral experiments and then were sacrificed at the end of behavioral procedures. All behavioral tests took place in an experimental room with the same light–dark cycle and the environmental conditions, such as humidity and temperature levels inside the room, similar to those of the housing facility.

All experiments were carried out according to the European Community Council Directive 86/609/EEC and efforts have been made to minimize animal suffering and to reduce the number of animals used. The rationale, design and methods of this study have been approved by the Ethical Committee for Animal Research, University of Catania.

### 2.2. Behavioral tests

#### 2.2.1. Novelty-induced grooming sampling test (NGT)

Novelty-induced grooming behavior was observed between 15.00 and 18.00 h, under the same environmental conditions according to the method described elsewhere (Drago et al., 1980). The rats were placed individually into Plexiglas boxes (24×12×24 cm) in a low noise room. After a minute of adaptation, a point was scored if the animal displayed one of the following grooming behaviors: washing (vibrating movements of the fore paws in front of the snout and liking of the same paws leading to a series of strokes along the snout and semicircular movements over the top of the head), scratching (scratching of the body by one of the limbs), licking (licking of the body fur, limbs and tail), and genital grooming (licking of genital area). Stretching and yawning episodes were not recorded. The sum of the points in a session of 30 min is called grooming score. Grooming is regarded as a displacement behavior, occurring in contexts in which the animals experience behavioral conflicts as a reaction to stress situations. These general contexts are directly or indirectly related to anxiety (Spruijt et al., 1992). Grooming behavior of all animals was recorded on a tape using a video-camera (Hitachi Videocam) and then scored in monitor display by two independent observers. The mean score of the two observations was used for the statistical analysis. Inter-observer reliability was 98–100% for the various behavioral measures.

#### 2.2.2. Elevated plus maze (EPM) test

The apparatus consisted of two opposite open arms (30×5 cm) and two arms with walls (30×5×14 cm) that were attached to a central platform (5×5 cm) to form a cross. The maze was elevated 50 cm from the floor (Pellow et al., 1985). Illumination (40 lx) was provided at the center of the maze. After treatment, each animal was placed at the center of the maze with its nose in the direction of one of the closed arms, and observed for 5 min, according to the following parameters: number of entries in the open and closed arms, and time of permanence in each of them (i.e., the time spent by the animal in the open and closed arms). An entry was defined as all four paws having crossed the line between an arm and the central area. It is accepted that the anxiolytic effect of a drug treatment is illustrated by increased parameters in open arms (time and/or number of entries), although entries in closed arms and total entries reflect the motor component of the exploratory activity. From these values, both the percentage of time and of entries in open arms provided as the measures of anxiety was calculated for each animal. Ethological measure, as grooming score described in Section 2.2.1 was also evaluated. On removal of each mouse, the maze floor was carefully wiped with a wet towel. The behavior of all animals was recorded on a tape using a video-camera (Hitachi Videocam) and then scored in monitor display by two independent observers.

#### 2.2.3. Forced swim test (FST) procedure for rats

For the FST procedure, each rat was forced to swim inside a vertical Plexiglas cylinder containing 25 cm of water maintained at 25 °C (Porsolt et al., 1978). After 15 min in the water it was removed and allowed to dry for 15 min in a heated container before being returned to its home cage. It was then replaced in the cylinders 24 h later and the total duration of immobility was measured during a 5-min test. The rat was judged to be immobile whenever it remained passively floating in the water in a slightly hunched but upright position, its head just above the surface.

#### 2.2.4. Forced swim swim-stress procedure

The forced swim as described by Chaki et al. (2004), consisted of placing for 2 min each mouse into a glass cylinders (height, 25 cm; diameter, 10 cm) containing 10 cm water, maintained at 23–25 °C.

### 2.3. Drugs and experimental design

The tachykinin NK2 receptor selective antagonist, saredutant {(*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-ichlorophenyl)-butyl] benzamide} was provided by Sanofi-Aventis, France. It was dissolved in physiological saline containing 0.1% Tween 80. Diazepam and clomipramine hydrochloride (Sigma, USA) were prepared freshly daily as a suspension in physiological saline containing 0.1% Tween 80 and by solution in distilled water, respectively. All compounds were

**Table 1**
Number of grooming episode in rats treated with saredutant (1 and 3 mg/kg) or diazepam (1 mg/kg)

| Treatment | Grooming score |
|---|---|
| VHC (10) | 35.33±3.03 |
| Diazepam 1 mg/kg (10) | 13.51±1.18[a] |
| Saredutant 1 mg/kg (10) | 21.83±2.04[a] |
| Saredutant 3 mg/kg (10) | 19.70±3.07[a] |

Effects of saredutant, an antagonist of tachykinin NK2 receptors in rats tested in novelty-induced grooming sampling test (NGT). Diazepam, saredutant or VHC were administered i.p. 30 min prior the behavioral test. Values are mean±S.E.M. In parentheses the number of animals per group is indicated.

[a] Significant difference as compared to VHC-injected controls (VHC) [$F(3,36)=18.18$; $p<0.01$, Dunnett's test for multiple comparisons].

administered intraperitoneally (i.p.) in a total volume of 1 ml/kg for rats and of 10 ml/kg for mice.

Two different experiments were programmed and carried out. In the first experiment different groups of mice were injected i.p. with saredutant (1 and 3 mg/kg), diazepam (1 mg/kg) or the respective vehicle (VHC) 30 min prior to testing in the EPM test without stress application or after a stress-related condition given by the forced swim lasting 2 min. From now, non-stressed animals will be indicated as NST and stressed animals (either by application of forced swim or of foot-shocks as described later) will be indicated as STR. The EPM was performed 5 min after application of a forced swim. In rats subjected to NGT, saredutant (1 and 3 mg/kg), diazepam (1 mg/kg) or VHC were administered i.p. 30 min prior to behavioral testing. The doses of the compounds were selected based on results of previous experiments (Griebel et al., 2001a).

In the second experiment, in order to assess depressive-like behavior under basal conditions, different groups of rats were injected i.p. with saredutant (2.5, 5 and 10 mg/kg), with TCA clomipramine (50 mg/kg) or with VHCs 24, 5 and 1 h prior to the FST procedure. For the measurement of immobility time in the FST after foot-shock exposure, as described by Consoli et al. (2005), other groups of rats were subjected to electric foot-shocks (1 mA, 1 s), after drugs injection in order to ensure a specific and constant treatment/stressor interaction. Rats received three such shocks through an electric grid put in a box with transparent walls. In this experiment, two groups of control animals were injected i.p. with saredutant or clomipramine VHCs, following the same procedure. The doses of the compounds were selected based on results of previous experiments (Drago et al., 2001; Steinberg et al., 2001; Micale et al., 2006, 2008).

### 2.4. Statistical analysis of data

Data were analyzed using unpaired *t*-test, one- or two-factor ANOVA, followed by the post-hoc Dunnett's test for multiple comparisons. A *p*-value of 0.05 or less was considered as indicative of a significant difference.

## 3. Results

As described in Table 1, the systemic administration of saredutant (1 and 3 mg/kg) decreased the grooming score of rats tested in the NGT. One-way ANOVA analysis revealed a significant drug effect for saredutant-treated animals in comparison to VHC-injected controls ($p<0.01$). This effect was similar to that induced by diazepam (1 mg/kg). Thirty min after acute treatment, saredutant (1 and 3 mg/kg) showed dose-dependent anxiolytic-like effects in mice tested in the EPM procedure under basal condition. One-way ANOVA analysis revealed, in fact, a main effect for percentage of time ($F_{3.36}=9.2$; $p<0.01$) and entries ($F_{3.36}=13.3$; $p<0.01$) in open arms for saredutant-treated

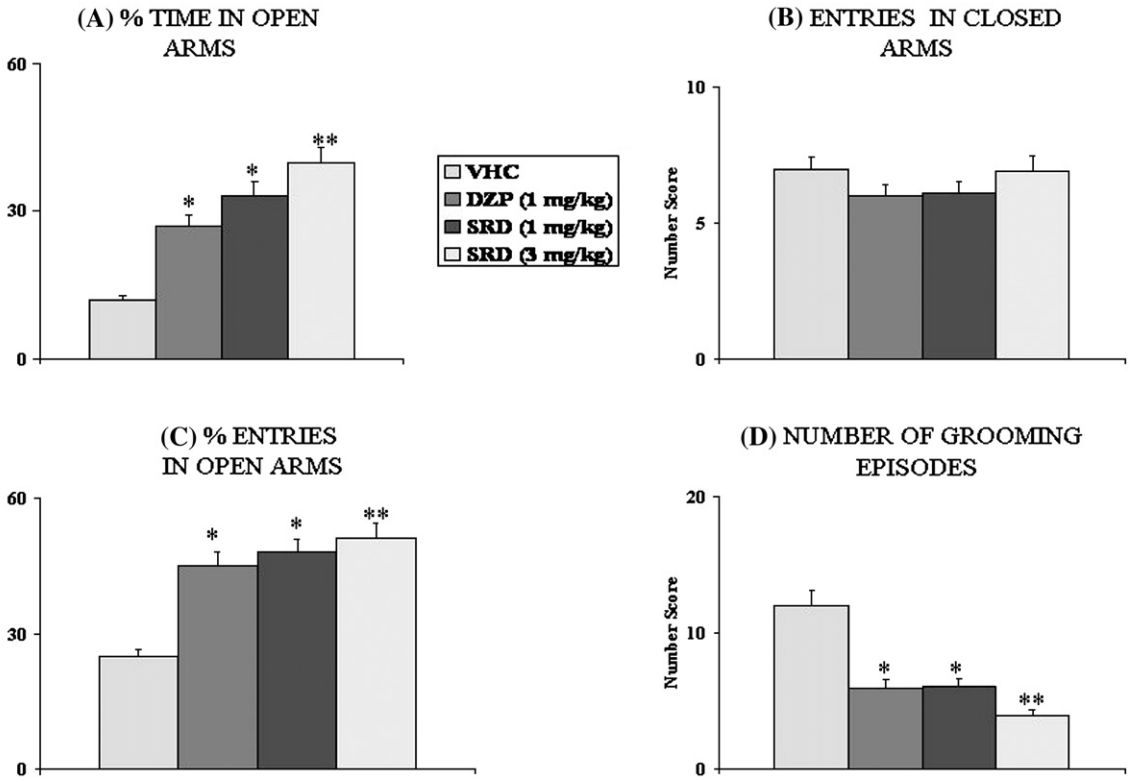


**Fig. 1.** Effects of saredutant, a tachykinin NK2 receptor antagonist, in elevated plus maze (EPM) test under basal conditions in mice. Saredutant (SDR; 1 and 3 mg/kg), diazepam (DZP; 1 mg/kg) or VHC were administered i.p. 30 m prior to behavioral testing. Data are presented as means±S.E.M for the percentage of time spent in open arms (A), the number of closed arms entries (B), the percentage of entries in open arms (C) and the grooming episodes (D) from 10 mice. *Significantly different as compared to VHC-injected controls (VHC) ($p<0.05$, Dunnett's test for multiple comparisons). **Significantly different as compared to VHC-injected controls (VHC) ($p<0.01$, Dunnett's test for multiple comparisons).

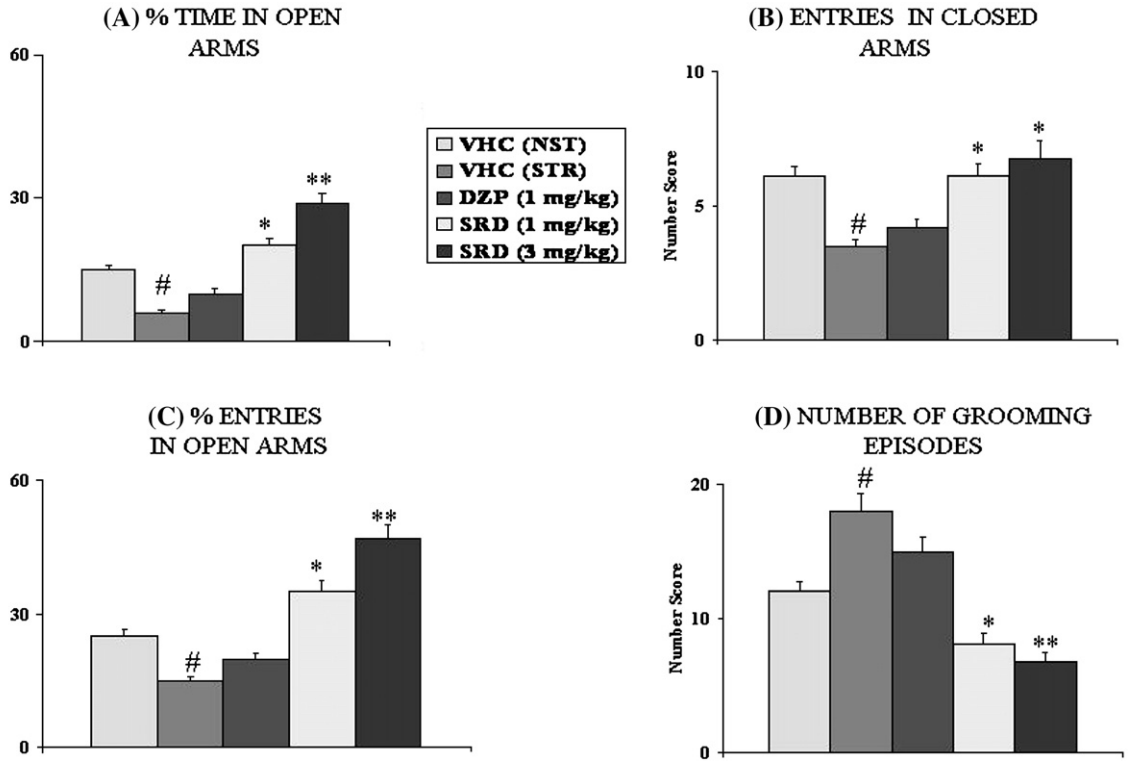


**Fig. 2.** Effects of saredutant, a tachykinin NK2 receptor antagonist, in mice tested in elevated plus maze (EPM) test under stress conditions (STR). Saredutant (SDR; 1 and 3 mg/kg), diazepam (DZP; 1 mg/kg) or VHC were administered i.p. 30 m prior to behavioral test made 5 min after a swim stress. Data are presented as means ± S.E.M for the percentage of time spent in open arms (A), the number of closed arms entries (B), the percentage of entries in open arms (C) and the grooming episodes (D) from 10 mice. #Significantly different as compared to VHC-injected non-stressed controls (VHC + NST) ($p<0.05$, $t$-test). *Significantly different as compared to VHC-injected stressed controls (VHC + STR) ($p<0.05$, Dunnett's test for multiple comparisons). **Significantly different as compared to VHC-injected stressed controls (VHC + STR) ($p<0.01$, Dunnett's test for multiple comparisons).

animals in comparison to VHC-injected controls. Furthermore, the analysis of ethological parameter showed a significant decrease of grooming score in saredutant-treated animals ($F_{3.36}=8.1$; $p<0.01$), confirming the anxiolytic-like effect. This effect was statistically significant both at the dose of 1 mg/kg ($p<0.05$) and 3 mg/kg ($p<0.01$). Diazepam (1 mg/kg) also increased percentage of time and entries in open arms and decreased grooming score ($p<0.05$). No difference was found for any treatment in the number of closed arm entries, a presumed index of locomotion (Fig. 1).

Fig. 2 shows the acute stress influence on saredutant effects on the behavior of mice tested in the EPM procedure. The forced swim application impaired the behavioral performance of VHC-injected controls (VHC + STR). Unpaired $t$-test analysis revealed a significant decrease of percentage of time ($t=3.1$; $p<0.05$) and entries ($t=3.9$; $p<0.05$) in open arms, number of entries in closed arms ($t=2.2$; $p<0.05$) and an increase of grooming score ($t=2.9$; $p<0.05$) as compared to VHC-injected non non-stressed (VHC + NST) animals. Both doses of saredutant reversed swim-stress induced impaired performance of mice. One-way ANOVA analysis revealed, in fact, a main effect for percentage of time ($F_{3.36}=7.3$; $p<0.01$) and entries ($F_{3.36}=6.1$; $p<0.01$) in open arms, and number of entries in closed arms ($F_{3.36}=3.7$; $p<0.05$) as compared to VHC-injected stressed (VHC + STR) animals. Furthermore, the analysis of ethological parameter showed a significant decrease of grooming score in saredutant-treated animals ($F_{3.36}=5.3$; $p<0.01$). This effect was statistically significant both at the dose of 1 mg/kg ($p<0.05$) and 3 mg/kg ($p<0.01$). In contrast, diazepam (1 mg/kg) failed to induce any change of the behavioral performance in STR mice.

In the second experiment, two-way ANOVA (factor 1: stress, factor 2: drug treatment) revealed a main effect of stress ($F_{1.55}=3.43$ $p<0.05$), treatment ($F_{5.55}=10.21$ $p<0.01$) and a stress-treatment interaction ($F_{5.55}=3.21$ $p<0.05$). The systemic administration of saredutant produced a decrease of immobility time in the FST paradigm in NST rats. This effect was apparently not dose-dependent since the doses of 2.5 and 5 mg/kg induced similar effects (with a slight, non-significant higher effect for the 2.5 mg/kg dose) ($p<0.05$). Furthermore, this effect was similar to that induced by clomipramine 50 mg/kg. However, at the dose of 10 mg/kg NST rats exhibited the best behavioral performance in the FST paradigm as compared to VHC-injected NST controls ($p<0.01$).

**Table 2**
Effects of saredutant (2.5, 5 and 10 mg/kg) or clomipramine (50 mg/kg) on immobility time of rats in forced swim test (FST) in basal condition (NST) or after application of acute stress (STR)

| Treatment | NST | STR |
|---|---|---|
| VHCs (20) | 170.3 ± 7.1 | 246.3 ± 10.2[a] |
| Clomipramine 50 mg/kg (10) | 118.5 ± 9.2[a] | 208.6 ± 11.4 |
| Saredutant 2.5 mg/kg (10) | 90.3 ± 10.4[a] | 162.8 ± 13.1[b] |
| Saredutant 5 mg/kg (10) | 101.4 ± 6.5[a] | 186.7 ± 9.8[b] |
| Saredutant 10 mg/kg (10) | 62.4 ± 12.7[c] | 140.8 ± 7.7[d] |

Saredutant, clomipramine or their VHCs were administered i.p. 24, 5 and 1 h prior to behavioral testing under basal conditions (NST). For the measurement of stress-related behavior different groups of rats (STR) were subjected to acute stress (electric foot-shock 1 mA, 1 s) after each drug injection. In this experiment, two groups of control animals were injected i.p. with saredutant VHC or clomipramine VHC. As similar results were obtained from these two control groups, data were combined.
Values are mean ± S.E.M. of time measures expressed in s. In parentheses the number of animals per each group is indicated.
[a] Significant difference as compared to VHC-injected NST controls ($p<0.05$, Dunnett's test for multiple comparisons).
[b] Significant difference as compared to VHC-injected STR controls ($p<0.05$, Dunnett's test for multiple comparisons).
[c] Significant difference as compared to VHC-injected NST controls ($p<0.01$, Dunnett's test for multiple comparisons).
[d] Significant difference as compared to VHC-injected STR animals ($p<0.01$, Dunnett's test for multiple comparisons) and clomipramine-treated STR animals ($p<0.05$, Dunnett's test for multiple comparisons).

Application of foot-shocks impaired the performance of VHC-injected STR animals in comparison to NST group in the FST, inducing an increased immobility time ($p<0.05$). In contrast, all saredutant-treated animals showed a decreased immobility time as compared to VHC-injected STR animals ($p<0.05$, $p<0.01$). However, no difference in immobility time was found between clomipramine and VHC-injected STR groups. In this experiment, two groups of control animals were used, i.e. those injected i.p. with the saredutant VHC and those injected with the clomipramine VHC. As similar results were obtained from these two control groups, data were combined (Table 2).

## 4. Discussion

The present study clearly shows that the selective tachykinin NK2 receptor antagonist, saredutant given systemically to rodents is effective to improve the behavioral performance in tests widely used to screen anxiolytic- or antidepressant drugs under basal and stress-related conditions. More specifically, NST saredutant-treated mice or rats either walked out onto open arms for a longer duration without locomotor activity impairment or showed a decreased grooming score in EPM procedure and NGT, respectively. Both these behavioral tests are considered for a measure of the anxiolytic-like effect of drugs.

Furthermore, the saredutant profile was comparable in terms of magnitude of the effects to that of the classical anxiolytic drug, diazepam. It should be recalled that growing evidence indicates that measures of anxiety from different tests could reflect different states of anxiety. This prompted us to use two different tests as indicated by other authors (Lister, 1990; File, 1992). Thus, while the EPM procedure reflects the conflict between exploration and avoidance of a novel environment in a void and the inhibition of exploratory behavior is commonly associated with high emotionality or anxiety, in the NGT the novel environment only influences the emotionality of rodents (Archer, 1973). Grooming is a "maintenance" behavior, a common species-characteristic movement pattern with readily definable components (Bolles, 1960). In rodents, spontaneous grooming behavior may occupy as much as 25%–40% of the wakeful time, but is specifically elicited in situations in which an animal is in stress-induced conflict or frustration. A typical condition of such type is NGT. Under this situation, grooming may play a deactivating role in restoring homeostasis (Gispen and Isaacson, 1981). Interestingly, the anxiolytic-like effect of saredutant was found in two different experimental tests and in two different animal species, suggesting that this effect is not species- or test-specific.

The present findings are in agreement with previous studies showing that tachykinin NK2 receptors blockers elicited anxiolytic-like activity in different species such as rodents, marmosets and gerbils tested (Stratton et al., 1993; Walsh et al., 1995; Teixeira et al., 1996; Griebel, 1999; Griebel et al., 2001a; Salomè et al., 2006). In particular, a number of previous studies have reported anxiolytic-like effects of saredutant in the light/dark exploration test (Stratton et al., 1993; Walsh et al., 1995), the EPM procedure (Teixeira et al., 1996; Griebel et al., 2001a) and in the mouse defense test battery (Griebel et al., 2001b). 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). More recently, saredutant was also shown to increase social behavior in gerbils (Salomè et al., 2006). However, saredutant did not exhibit any effect in conflict-based procedures (Griebel et al., 2001a), suggesting a specific profile of activity of NK2 antagonists compared to drugs such as benzodiazepines, which are active in all these procedures.

The mechanisms underlying the anxiolytic-like effects of saredutant are unclear, but they could involve the localization of tachykinin NK2 receptors and their interaction with other neurotransmitter systems. The distribution of tachykinin NK2 receptors in rodents, guinea pigs, monkey and humans has been described in those brain areas that are involved in emotional processes such as cortex, amygdala, hippocampus, thalamus and dorsal raphe nucleus (DRN) (Saffroy et al., 2001, 2003). Thus, it is likely that tachykinin NK2 receptor antagonists may produce their psychotropic effects via the DRN, since intra-DRN infusion of the NK2 receptor antagonists GR100679, GR115211 and GR159897 produced anxiolytic-like effects in the social interaction and EPM tests in rats. (Stratton et al., 1993, 1994; Walsh et al., 1995). Moreover, the NK2 receptor agonist GR64349 was found to increase anxiety-like behavior in the rat social interaction test following infusion into the DRN (Stratton et al., 1993).

In the present study, in addition to an anxiolytic-like activity, saredutant induced a decrease of immobility time in NST rats tested in the FST, an antidepressant-responsive test widely used for preclinical studies on novel antidepressant drugs (Porsolt et al., 1978). A wide variety of antidepressants and compounds with potential antidepressant activity reduce the duration of immobility in the FST procedure. However, one major drawback of the FST (as with many antidepressant-sensitive paradigms) is the fact that short-term antidepressant treatments reverse the immobility, whereas in the clinic it can take weeks for the same antidepressants to elevate mood (Cryan et al., 2002). Unlike EPM, in FST we did not find any dose–response effect, since the doses of 2.5 and 5 mg/kg induced similar effects (with a slight, non-significant higher effect for the 2.5 mg/kg dose). The reason for this difference is unclear, but may at least partially be due to the different doses, animal species and tests (mice in EPM and rats in FST). Furthermore, the effect of saredutant on immobility time in FST was similar to that induced by the TCA, clomipramine. Saredutant has been reported to exert antidepressant-like effects in animal models after acute or chronic administration to rodents, gerbils, and guinea pigs, suggesting also for its antidepressant activity a non-species-specific activity (Steinberg et al., 2001; Dableh et al., 2005; Salomè et al., 2006). Interestingly, the spectrum of potential therapeutic activity of this drug seems to be broader than classical benzodiazepines, which are not endowed with antidepressant properties.

Recent studies in rodents have suggested the involvement of post-receptor components of the cyclic AMP (cAMP) second messenger cascade in the action of different classes of antidepressants, including selective serotonin reuptake inhibitors (SSRIs), NE-selective reuptake inhibitors (SNRIs) and monoamine oxidase inhibitors (Is-MAO). The results of these studies showed that chronic but not acute treatment with antidepressants up-regulates the cAMP system at several levels, including CREB mRNA and PRAX-1 mRNA expression in the cerebral cortex and hippocampus (Nibuya et al., 1996; Thome et al., 2000). In line with these effects, also saredutant induced an up-regulation both of CREB mRNA and of PRAX-1 mRNA expression in hippocampus of rats after repeated treatment, suggesting that its antidepressant-like effects could be due to these neurochemical processes of adaptive significance in specific brain areas. Interestingly, these effects were not observed after repeated treatment with its R-enantiomer SR48965, which displays a 2000-fold lower affinity for the NK2 receptors, suggesting the selective involvement of the NK2 receptors in the effects of saredutant (Steinberg et al., 2001; Chardenot et al., 2002). These neurochemical effects are in agreement with the lag time of several weeks requisite for the onset of the clinical efficacy of antidepressants and they are also considered as intracellular targets to elucidate the mechanisms by which antidepressant act in brain function (Malberg et al., 2000).

Other point in our results deserving attention is the lack of inhibitory action of acute stressors on the behavioral effects of saredutant. This is, in fact, the first evidence of this type. Growing evidence indicate the involvement of stress in the clinical manifestations of mood disorders. Risk factors for depression can include, in fact, stressful life events in the period leading up to the depressive episode (Tennant, 2002). In our experimental design we used acute stress paradigm (forced swim or foot-shocks) in order to distinguish the stress procedure from the experimental models. In fact, a chronic

shock paradigm such as chronic mild stress might affect per se the response of animals to antidepressant drugs and it is considered as an experimental model of depression. Furthermore, acute stress induces widespread activation of the brain monoamine systems, while repeated exposure to stress can result in either sensitization or tolerance to a subsequent stressful experience (Hajós-Korcsok et al., 2003). Forced swim is a stressful situation rats might encounter in nature and is a mixed psychological (novelty, water) and physiological (exercise, temperature) stressor. Furthermore, this stressor induces behavioral and endocrine changes in line with those found in patients with mood disorders (Dal-Zotto et al., 2000). Foot-shock, in the present study made three times along 24 h prior to behavioral testing, was chosen because no adaptive response of HPA axis has been found in previous experiments (Consoli et al., 2005).

While exposure to stressors (forced swim or foot-shocks) may lead to an impaired performance of control animals and to reduced anxiolytic- and antidepressant-like effects of diazepam and clomipramine in EPM and FST respectively, in both tests the effects of saredutant were not counteracted by the acute stressors. Furthermore, the exposure to forced swim in control group induced a reduction of closed arm entries in EPM, suggesting a possible impairment of locomotor activity in this test. The effects of stressors on locomotor activity in tests as open field test and EPM commonly used to assess the anxiety in rodents are controversial; since it has been seen that single or repeated restraint stress reduced exploration in rodents, even though no change have been also reported (Carli et al., 1989; Mercier et al., 2003; Hsu et al., 2007; Klenerová et al., 2007). Our results are in agreement with other preclinical studies, showing that exposure of rodents to various stressful stimuli can induce changes both in the emotional state of the animals and in the effects of anxiolytic and/or antidepressant drugs (Sanchez, 1997; Takeda et al., 1998; Drago et al., 2001; Griebel et al., 2001a; Briones-Aranda et al., 2002; Chaki et al., 2004; Consoli et al., 2005; Avgustinovich et al., 2007). It should be mentioned, however, that some discrepancy exists with other studies, and the mechanisms underlying the different findings are not well established. However, they could be due to stressor variables (type, duration and or intensity), behavioral test variables (type, latency between stress and testing time), species or strain variables (different sensitivity to stressors). Other explanation could be that different stressors activate different neurotransmitter pathways and that the animal tests are differentially sensitive to modulation by different neurotransmitters (File, 1996).

Stress can influence central nervous system (CNS) functions by complex neuroendocrine changes. Among them, dysregulation of the HPA axis and of the sympathetic system with increased plasma corticosterone and catecholamine levels have been implicated in stress-related disorders (Koob, 1999; De Kloet, 2003). Interestingly, saredutant counteracts the firing rate of NE neurons in locus coeruleus (LC) induced by corticotropin-releasing factor (CRF) that plays a primary role in coordinating the response of the body to stressors. Thus, an interaction between tachykinergic neurotransmission and CRF, through its involvement in the regulation of HPA axis has been suggested (Steinberg et al., 2001). It should be considered, however, that the mechanism mentioned above cannot explain the behavioral effects of clomipramine or diazepam in animal models of stress-related disorders observed in the present study. In conclusion, our findings provide further support to the anxiolytic- and antidepressant-like effects of the tachykinin NK2 antagonist saredutant, suggesting that its effects are not changed under stress conditions. The mechanisms underling the similar amplitude of saredutant effects in NST or STR rodents remain to be clarified. Since stress stress-induced tachykinergic activation, the same amplitudes of saredutant effect between no pre-exposed and pre-exposed animals could be due to its capacity to counteract the NKA agonist activity in experimental conditions with low (FST or EPM) or high stress levels, as those induced by previous exposure to stressors.

The clinical extension of these findings may be relevant for the pharmacological treatment of stress-related disorders, since current treatments given by SSRIs for depression and along with benzodiazepines for anxiety disorders, show limits such as adverse side effects, delayed onset of therapeutic action and non-responsiveness by a large number of patients. Other advantage represented by the clinical application of this kind of drugs is using them in situations in which depressive and anxious symptoms are mixed together (Maubach et al., 1999; Nemeroff, 2003).

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