



Saredutant, an NK2 receptor antagonist, has both antidepressant-like effects and synergizes with desipramine in an animal model of depression

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ARTICLE INFO

Article history:

Received 23 October 2009

Received in revised form 4 May 2010

Accepted 6 May 2010

Available online 12 May 2010

Keywords:

Saredutant

FSL model of depression

Forced swim test

Social interaction test

Desipramine

ABSTRACT

Previous work established that saredutant, an NK2 receptor antagonist, has antidepressant and anxiolytic-antistress effects in a variety of rodent models. The purpose of the present investigation was two-fold: to confirm the antidepressant-like effects of saredutant using a genetic animal model of depression, the Flinders Sensitive Line (FSL) rat, and to assess whether saredutant might synergize with desipramine to produce antidepressant-like effects at doses not seen with the individual compounds. For the main study the FSL rats and the control Flinders Resistant Line (FRL) rats were treated with various doses of saredutant (1, 3, and 10 mg/kg in FSL, 3 mg/kg in the FRL), the tricyclic desipramine (5 mg/kg) as a positive control, or vehicle for 14 consecutive days and then tested in the social interaction and forced swim tests about 22 h later. For the synergism study, the FSL rats were treated with subeffective doses of saredutant (1 mg/kg) or desipramine (2.5 mg/kg) or both for 14 consecutive days and then the behavior tests were performed. Saredutant, like desipramine, increased social interaction (at 10 mg/kg) reduced immobility (at 3 and 10 mg/kg), and had no effect on locomotor activity in the FSL rats, but did not affect any of these variables in the FRL rat. Neither saredutant (1 mg/kg) nor desipramine (2.5 mg/kg) affected any variable by themselves; however, their combination significantly lowered swim test immobility. These findings confirm the antidepressant-like effects of saredutant in a genetic animal model of depression. Moreover, they suggest that saredutant might also act as an add-on therapy for individuals who are not fully responding to their antidepressant treatment.

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1. Introduction

A lot of interest has been generated recently in the tachykinin receptors as potential targets for antidepressant drugs. This family of neuropeptides (i.e., substance P [SP], neurokinin A and neurokinin B) is widely distributed within the mammalian peripheral and central nervous systems (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 alpha-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). The interest in tachykinins for mood disorders has been boosted by the finding that antagonists at all three subtypes of neurokinin receptors have antidepressant-like activity in rodent models (Dableh et al., 2005). The present paper will focus on a selective NK2 receptor antagonist, saredutant (SR48968), because of the following significant findings: Saredutant reduced immobility in the

forced swim test in both rats and mice (Steinberg et al., 2001); the antidepressant-like effects was confirmed by Griebel et al. (2001), who also reported anxiolytic-like effects. Salome' et al. (2006) reported similar effects in gerbils. Micale et al. (2008) reported that the antidepressant and anxiolytic-like effects of saredutant were seen under both basal and stress-induced conditions. Finally, Louis et al. (2008) used a variety of other anxiety (social interaction) and depression (chronic mild stress) tests to verify that saredutant had antidepressant-like and anxiolytic effects. All of these studies used normal rats whose environment was varied in some way to induce the behavioral pathology. In the present study we sought to determine whether saredutant would also exhibit antidepressant-like effects in a genetic animal model of depression.

The Flinders Sensitive Line (FSL) rat has been suggested as a genetic animal model of depression because it has a number of features that resemble human depressives, including a type of psychomotor retardation and increased REM sleep (Benca et al., 1996; See Overstreet et al., 2002, 2005 for reviews; Shiromani et al., 1988). The Flinders Lines were selectively bred for differences in cholinergic function (Overstreet, 1986, 1993). Because of the evidence that depressed individuals are more sensitive to cholinergic agonists (Janowsky et al., 1994), the behavior of the FSL rats was more fully characterized. They were more immobile in the forced swim test (Overstreet, 1993, 2002) but not in the

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elevated plus maze (Schiller et al., 1991) or prepulse inhibition of startle (Markou et al., 1994). The FSL rats, compared to their control Flinders Resistant Line (FRL) rats, appeared to exhibit depressed-like but not anxiety- or schizophrenia-like behavior (Overstreet et al., 1995). Many studies have shown that the FSL rats respond to classical or novel antidepressant agents by decreasing their exaggerated immobility (Overstreet and Griebel, 2004, 2005; Overstreet et al., 1998, 2004a,b, 2008; Pucilowski and Overstreet, 1993; Zangen et al., 1997, 1999). Thus, they appear to be the ideal rats to confirm the antidepressant-like effects of saredutant.

Another recent development in the field is the addition of other, different medicines in the treatment regime of individuals who do not respond fully to current antidepressants. A case in point has been the use of aripiprazole as an adjunctive therapy (e.g., Nelson et al., 2010; Bourin et al., 2009). To examine whether saredutant might be an add-on therapy, we investigated the effects of subthreshold doses of saredutant and desipramine.

2. Methods

2.1. Animals

The male FSL and FRL rats were selected from the breeding colonies maintained in the Bowles Center for Alcohol Studies at about 75 days of age. Rats were housed in standard rooms with temperatures about 72 °C and humidity about 30% under a 12:12 light:dark cycle (lights on from 07:00 to 19:00). They had free access to food and water. These experiments were approved by the University of North Carolina Institutional Animal Care and Use Committee.

2.2. Drugs

Saredutant was supplied by Sanofi-Aventis. It was made up in carboxymethylcellulose at concentrations of 1, 3, and 10 mg/ml. Desipramine was purchased from Sigma and dissolved in saline solution at 2.5 mg/ml. Saredutant was injected at 1 ml/kg, while desipramine was injected at 2 ml/kg.

2.3. Research design – Experiment 1

In the first experiment the dose-related effects of saredutant were investigated. The FSL rats ($n=8$) were treated with one of the following: vehicle (carboxymethylcellulose – CMC) or 1, 3, or 10 mg/kg saredutant, or 5 mg/kg desipramine as a positive control. The FRL rats were given CMC or 3 mg/kg saredutant. All injections were i.p. and were given for 14 consecutive days. Approximately 22 h after the last injections the rats were placed in the social interaction chamber with similarly treated rats for a 5-min recording. Then about 2 h later the rats were individually tested in the forced swim test for a single 5-min session.

2.4. Research design – Experiment 2

The objective of this study was to determine whether subthreshold doses of saredutant and desipramine might combine to produce an antidepressant-like effect. An FRL rat ($n=8$) treated with CMC was used in this study to provide a reference group for the FSL rats. The FSL rats were treated with one of the following: CMC; saredutant (1 mg/kg) only, desipramine (2.5 mg/kg) only, saredutant (1 mg/kg) and desipramine (2.5 mg/kg) combined. As with the previous study, treatments were given for 14 consecutive days and the testing began about 22 h after the last treatment.

2.5. Social interaction test

Approximately 22 h after the last injection rats with the same treatment and similar body weights were placed in a square test arena (60×60 cm, marked with sixteen 15×15 cm² on the floor) for the testing of social interaction under low ambient light (30 lx). The amount of time spent in social interaction (grooming, licking, sniffing, and crawling over or under) was recorded during a 5-min session by an experienced observer who was blind to the treatment condition. This measure provides one index of anxiety-like behavior, with more “anxious” rats spending less time in social interaction (File and Seth, 2003; Overstreet et al., 2002). In addition, a motor activity measure was collected. The total number of lines crossed during the session provided the measure of general activity.

2.6. Forced swim test

Approximately two hr after the social interaction test, rats were tested in the forced swim test. The swim tank was 18 cm in diameter and 40 cm tall. The tank was filled with enough 25 °C water so the rat could not touch the bottom. The rat was placed in the swim tank for a single 5-min session approximately 24 h after the last treatment and the time (seconds) of immobility was scored by an observer blind to the treatment condition and rat strains being tested (Overstreet, 1993; Zangen et al., 1997). Immobility was recorded if three of the rat's four paws were not moving; otherwise, the rat was regarded as mobile.

2.7. Statistical analysis

Data were analyzed by one-way ANOVAs, with Tukey's tests being carried out if the ANOVA was significant.

3. Results

3.1. Saredutant has dose-dependent effects

As can be seen in Fig. 1, saredutant had dose-dependent effects on swim test immobility, with the low dose of 1 mg/kg having no effect and the doses of 3 and 10 mg/kg significantly reducing immobility. In contrast, saredutant (at 3 mg/kg) did not significantly alter the lower immobility seen in the control FRL rats. Finally, the positive control desipramine also significantly reduced swim test immobility. The overall ANOVA was significant ($F[6,48] = 15.55, p < 0.0001$) and Tukey's tests confirmed that the differences outlined above were statistically significant.

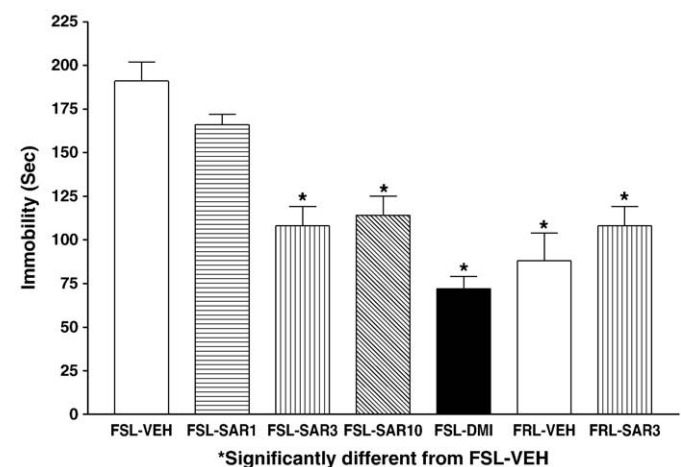


Fig. 1. The effects of saredutant and desipramine on swim test immobility in the FSL and FRL rats. Rats were injected i.p. for 14 consecutive days and the swim test was carried out about 24 h after the last injection. Data represent the means \pm s.e.m. for 8 rats.

The pattern of results for saredutant on social interaction was similar to that for immobility, as revealed in Fig. 2. The lowest dose (1 mg/kg) did not increase social interaction but the two higher doses (3 and 10 mg/kg) did, with the highest dose more effective. Once again, saredutant was ineffective in the FRL rats, failing to change social interaction. The overall ANOVA was significant ($F[6,48] = 19.13$, $p < 0.0001$).

There were no drug-related effects on activity, as shown in Fig. 3. There were significant group differences ($F[6,48] = 9.03$, $p < 0.0001$), but these differences were exclusively related to the differences between the FSL and FRL rats, with FRL rats being more active. There were no significant differences ($p < 0.1$) in weight change suggesting that the drugs did not have any side effects.

3.2. Saredutant and desipramine are synergistic

As shown in Fig. 4, neither saredutant (1 mg/kg) nor desipramine (2.5 mg/kg) was effective in reducing the exaggerated immobility in the forced swim test. However, when the two drugs were combined, there was a significant reduction in immobility (Fig. 4) and the overall ANOVA was significant ($F[4,32] = 12.40$, $p < 0.0001$). Tukey's protected t tests confirmed the significant differences of the combination of doses in the FSL rats and vehicle in the FRL rats from vehicle in the FSL rats. Clearly, desipramine and saredutant have a synergistic effect on immobility.

In the social interaction test, there were no significant differences in social interaction ($F[4,32] = 1.67$, NS; data not shown), but there were for activity ($F[4,32] = 5.39$, $p < 0.001$). As shown in Fig. 5, the treatments involving desipramine were less active than the other treatments.

4. Discussion

This is the first report on the effects of the NK2 receptor antagonist saredutant in a genetic animal model of depression. The results for swim test immobility in the study almost exactly replicate previous findings with other rodent models demonstrating that the compound is endowed with antidepressant-like properties (Steinberg et al., 2001; Micale et al., 2008; Louis et al., 2008). In these previous studies saredutant was found to be active in both acute (i.e., forced swimming and DRL-72 tests) and chronic (i.e., chronic mild stress) models of depression. In the present experiment an antidepressant-like effect was seen following repeated administration with both 3 and 10 mg/kg, but not 1 mg/kg. Just as significant is the fact that saredutant (3 mg/kg) did

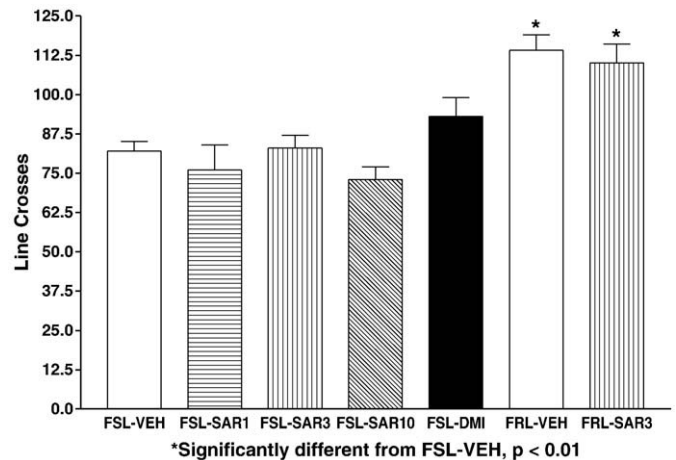


Fig. 3. The effects of saredutant and desipramine on line crossings in the FSL and FRL rats. See legend to Fig. 2.

not have an antidepressant-like effect in the FRL rats, confirming that this control strain does not respond to antidepressants (Overstreet and Griebel, 2004; Overstreet et al., 2004a, 2008). These two sets of data suggest that the antidepressants are producing an adaptive change in the FSL rats that normalizes their abnormal brain chemistry (e.g., Zangen et al., 1997, 1999), thus normalizing their behavior.

Interestingly, FSL rats have been shown to display altered concentrations of neurokinin A immunoreactivity in the frontal cortex and striatum compared to FRL rats (Husum et al., 2000). These differences were abolished by lithium treatment, suggesting that normalizing neurokinin A-like immunoreactivity concentrations may represent one of the therapeutic mechanisms of mood stabilizers (Husum et al., 2000) and, by extension, antidepressants. It is possible that saredutant, by blocking NK2 receptors, may similarly normalize the concentration of neurokinin A in brain structures of FSL rats known to be involved in mood disorders, thereby exerting its antidepressant-like activity.

The abnormally low social interaction behavior in the FSL rats was also counteracted by chronic treatment with the high (10 mg/kg) dose of saredutant. This anxiolytic-like action is comparable to other effects reported for saredutant in anxiety models (e.g., Griebel et al., 2001; Salome' et al., 2006; Louis et al., 2008; Micale et al., 2008). This NK2 receptor antagonist attenuated anxiety-related behaviors in a wide range of rodent and primate models based on the exposure of subjects to

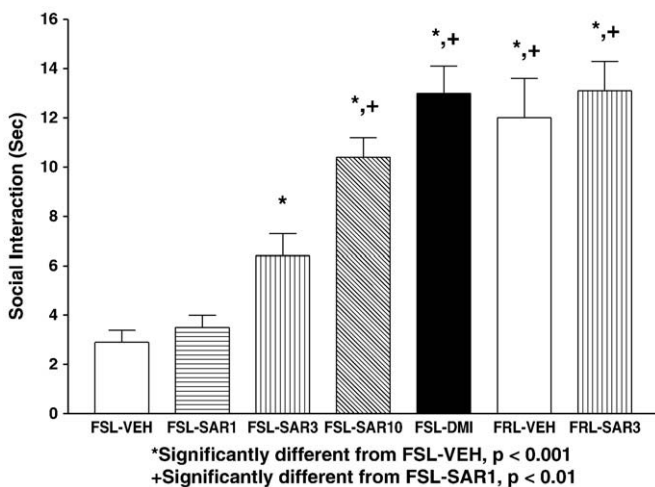


Fig. 2. The effects of saredutant and desipramine on social interaction behavior in the FSL and FRL rats. Rats were injected i.p. for 14 consecutive days and the social interaction test was carried out about 22 h after the last injection. Data represent the means \pm s.e.m. for 8 rats.

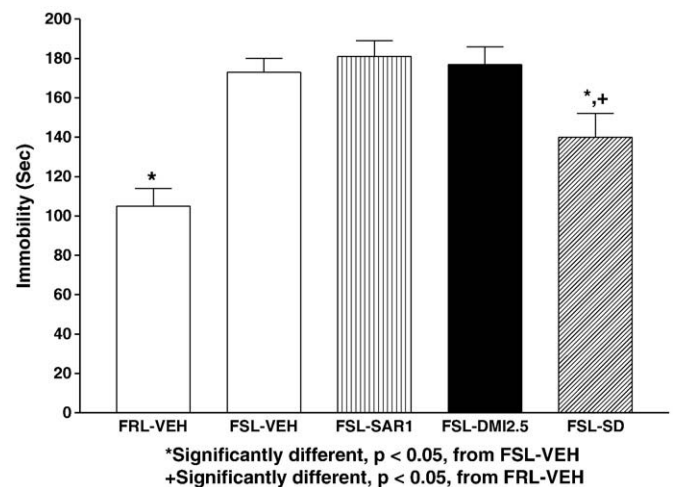


Fig. 4. The effects of subthreshold doses of saredutant and desipramine, alone and in combination, on swim test immobility in the FSL and FRL rats. Rats were injected i.p. for 14 consecutive days and the swim test was carried out about 24 h after the last injection. Data represent the means \pm s.e.m. for 8 rats.

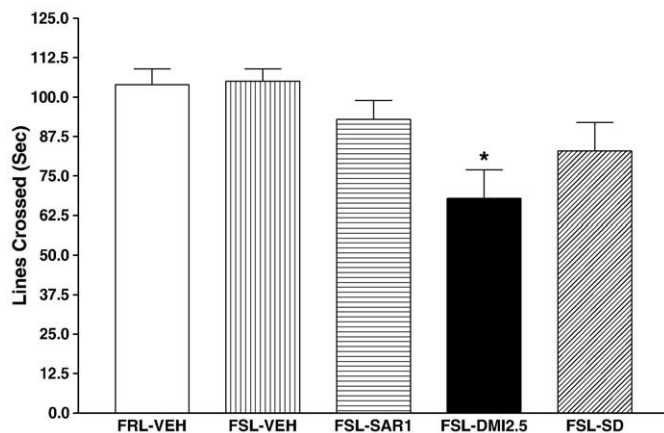


Fig. 5. The Effects of subthreshold doses of saredutant and desipramine, alone and in combination, on line crosses in the FSL and FRL rats. Rats were injected i.p. for 14 consecutive days and the line crossings were carried out about 22 h after the last injection. Data represent the means \pm s.e.m. for 8 rats.

predatory of social stress. These latter are of particular relevance in the current context of normalization of low levels of social interaction in the FSL rats by saredutant. But, unlike previous studies, the design was different. Because the drug treatment was for 14 consecutive days and the behavior test carried out 22 after the last treatment. The effects may be due to adaptive changes that permit a more normal social interaction behavior. Previous findings tended to examine the acute anxiolytic effects of saredutant, so the present findings extend the understanding of its anxiolytic effects.

Another important finding of this work is that the combined treatment with ineffective doses of saredutant (1 mg/kg) and desipramine (2.5 mg/kg) were able to reduce swim test immobility, but not increase social interaction. To our knowledge, this represents the first preclinical report suggesting that selective blockade of the NK2 receptor may have a synergistic effect with noradrenaline (NA) reuptake inhibition. Although drug levels for saredutant and desipramine were not analyzed to support a pharmacokinetic mechanism underlying this behavioral drug–drug interaction, it is reasonable to argue for a pharmacodynamic action of this synergy as previous studies demonstrated that neurokinin A and saredutant were able to modulate NA neurotransmission under certain circumstances (Steinberg et al., 2001; For example, saredutant was shown to block neural firing in the locu coeruleus and NA release in the prefrontal cortex following uncontrollable stress. The increase of extracellular levels of NA following desipramine produces, when the drug is given chronically, a down-regulation of beta-adrenergic receptors (e.g., Bradford et al., 1987), which in turn results in a decrease in the functioning of the brain NA system (e.g., Mason and Angel, 1983). It can be speculated that this latter mechanism combined with the action of saredutant on the NA system may explain the synergistic effects between desipramine and the NK2 receptor antagonist.

Varying the conditions for the drug doses, such as adding saredutant to an effective dose of desipramine, was not attempted because the interest was in using subeffective doses of both compounds. If this combination worked, as it did here, one could argue that lower doses could be used to treat depressed patients, resulting in fewer side effects.

The reduction in line crosses induced by 2.5 mg/kg desipramine is puzzling because it was not observed in rats treated with 5 mg/kg. Similarly, previous studies that have used desipramine did not report any effects on activity (Overstreet and Griebel, 2004, 2005; Overstreet et al., 2004a,b, 2008). Unpublished reports suggest that desipramine may have time-dependent effects on activity, with decreases seen up to 18 h after the last injection. However, there were no time differences in the studies reported here, so the reduction in activity after 2.5 mg/kg desipramine remains a mystery.

In conclusion, these data extend previous findings that saredutant has anxiolytic- and antidepressant-like properties. More important, they suggest that saredutant might be used in antidepressant-resistant patients, both as a primary drug but also as an add-on therapy with other antidepressant drugs.

Acknowledgements

Funding for this project was provided by Sanofi-Aventis. We thank Robert A. Angel for technical assistance.

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