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Antidepressant-like effects of CRF_1 receptor antagonist SSR125543 in an animal model of depression

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Abstract

Much interest has been expressed in the antidepressant potential of nonpeptide, orally active corticotropin-releasing factor (CRF) receptor antagonists in recent years. Therefore, the present investigation examined the antidepressant-like effects of the novel CRF_1 receptor antagonist SSR125543 on the exaggerated swim test immobility in the Flinders Sensitive Line rat, a genetic animal model of depression. Chronic treatment with SSR125543 (3, 10, 20, 30 mg/kg, i.p.) for 14 days significantly increased swimming in the Flinders Sensitive Line rats. The reference serotonin reuptake inhibitor fluoxetine (5 mg/kg, i.p.) and the tricyclic antidepressant desipramine (5 mg/kg, i.p.) also significantly increased swimming, as expected. The higher doses of SSR125543 (20 and 30 mg/kg) also significantly increased the abnormally low level of social interaction behavior in the Flinders Sensitive Line rats. Together, these findings indicate that the CRF_1 receptor antagonist SSR125543 has both antidepressant- and anxiolytic-like effects in the Flinders Sensitive Line rats.

Keywords: Flinders Sensitive Line rat model; Depression; CRF_1 receptor antagonist SSR125543; Fluoxetine; Desipramine, Forced swim test; Social interaction test

1. Introduction

There has been increased interest in the antidepressant potential of drugs that block receptors for corticotropin-releasing factor (CRF). A number of orally active, non-peptide CRF_1 receptor antagonists have recently been developed (Griebel et al., 2002; Holsboer, 1999; Keck and Holsboer, 2001; Okuyama et al., 1999; Seymour et al., 2003). There is preliminary evidence of antidepressant activity in an open-label trial with one of these agents (Zobel et al., 2000). In addition, these compounds have proved effective in certain tests of depressive-like behavior in normal rats, such as subacute treatment in the forced swim test and the chronic mild stress model (Griebel et al., 2002; Seymour et al., 2003). However, there is no information on how rats with an innate tendency towards depressive-like behavior respond to CRF_1 receptor antagonists. Therefore, it was decided to compare the effectiveness of the CRF_1 receptor antagonist SSR125543 [4-(Chloro-4-methoxy-5-methylphenyl)-*N*-[(1*S*)-2-cyclopropylfluoro-4-methylphenyl)ethyl]5-methyl-*N*-(2-propynyl)-1,3-thiazamine] with the selective serotonin reuptake inhibitor fluoxetine and the classical tricyclic desipramine in a genetic animal model of depression with high predictive validity, the Flinders Sensitive Line rat (Overstreet, 2002).

The Flinders Sensitive Line rat is innately more immobile in the forced swim test than its control counterpart, the Flinders Resistant Line rat, and exhibits a decrease in immobility following chronic, but not acute, treatment with desipramine and sertraline (Overstreet, 1993, 2002; Pucilowski and Overstreet, 1993). The Flinders Sensitive Line rat exhibits other features that are similar to those

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found in human depressives, such as increased rapid eye movement sleep (Benca et al., 1992, 1996; Shiromani et al., 1988), has elevated levels of serotonin in limbic brain regions that are corrected following chronic antidepressant treatments (Zangen et al., 1997), and responds to other antidepressants but not the psychomotor stimulants amphetamine and scopolamine (see Overstreet, 2002; Overstreet et al., 1995).

In this study, the Flinders Sensitive Line rat model of exaggerated immobility was used to compare the antidepressant and anxiolytic potential of the CRF_1 receptor antagonist SSR125543 with that of the serotonin reuptake inhibitor fluoxetine, and the tricyclic desipramine after chronic treatment for 14 days.

2. Methods and materials

2.1. Animals

The Flinders Sensitive and Resistant Line rats were selected from breeding colonies maintained at the University of North Carolina Bowles Center for Alcohol Studies at 70–75 days of age and 350–370 g. They were housed in groups of three in temperature- and humidity-controlled rooms under a 12:12-h light/dark cycle (lights on 0700–1900 h). Rats were randomly divided into eight groups and then given the treatments described below. Only a reference Flinders Resistant Line group treated with vehicle was used in this study because previous evidence indicated that these rats, which exhibit a relatively low degree of immobility, do not exhibit decreases in immobility following many antidepressant treatments (Overstreet, 2002), including a CRF_1 receptor antagonist (Overstreet et al., 2004).

2.2. Treatments

The following seven treatment groups were established for the Flinders Sensitive Line rats: carboxymethylcellulose (0.5%), the vehicle for SSR125543; SSR125543 (3, 10, 20, 30 mg/kg); fluoxetine (5 mg/kg); desipramine (5 mg/kg). The di-tosylate salt of SSR125543 was used and it was suspended in carboxymethylcellulose. The hydrocholoride salts of fluoxetine and desipramine were dissolved in distilled water and isotonic saline, respectively. The eighth group was a Flinders Resistant Line group treated with isotonic saline. The rats were injected i.p. once daily for 14 consecutive days between 0900 and 1100 h. On the day after the last injection, the rats were subjected sequentially to the social interaction test (approximately 18 h after the last treatment) and the forced swim test (approximately 22 h after the last treatment). Unpublished observations in other rats indicated that exposure to the social interaction test prior to being exposed to the swim test did not significantly alter swimming times.

2.3. Behavioral tests

Approximately 18 h after the last treatment, rats with the same treatment and similar body weights were placed in a square test arena (60×60 cm, marked with sixteen 15×15 cm squares on the floor) for the testing of social interaction. The tests were carried out under low (30 lx) light and unfamiliar conditions to generate an intermediate level of social interaction behavior (File, 1980; File and Seth, 2003; Overstreet et al., 2002, 2003). The amount of time spent in social interaction (grooming, licking, sniffing, 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, 1980; File and Seth, 2003). The total number of lines crossed during the session provided a measure of general activity.

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 bottom with its hindpaws. The rat was placed in the swim tank for a single 5-min session 21–23 h after the last treatment and the s of immobility were scored by an observer blind to the treatment condition and rat strain being tested (Overstreet, 1993; Zangen et al., 1997).

2.4. Statistical analyses

The data for the three measures were summarized into means±S.E.M. for each of the eight treatment groups. Graphical representations of the findings were compiled using Prism software. Initially, it was confirmed that the data were normally distributed and that the variances of the groups did not significantly vary (Cochran's C). Then the data for each measure were subjected to one-way analyses of variance. If these tests revealed significant group differences, follow-up Tukey's tests were carried out to elucidate the pattern of group differences. The GBstat software package was used for the statistical analyses.

3. Results

Fig. 1 illustrates the antidepressant-like effects of each of the compounds used in the Flinders Sensitive Line rats, as the immobility time was significantly less (swimming time was significantly greater) in all of the rats treated with active drugs compared to the rats that received vehicle. Note also that the immobility is much less in the Flinders Resistant Line rats compared to the Flinders Sensitive Line rats, confirming previous studies (see Overstreet, 2002, for review).

Test with a one-way analysis of variance indicated significant treatment effects ($F(7,70)=10.75$, $P<0.0001$). The letters above the bars indicate whether the groups are significantly different according to Tukey's protected t-test;

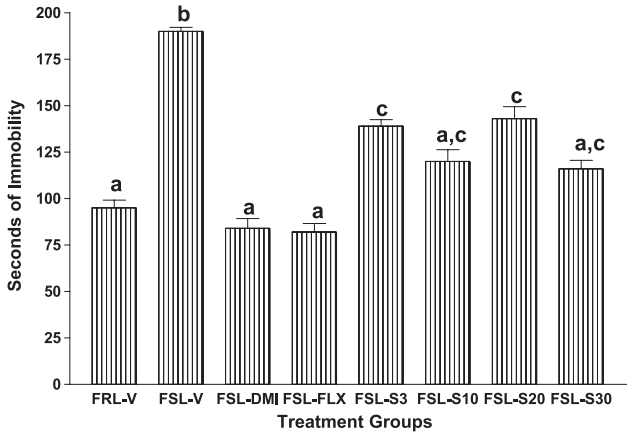

Fig. 1. Effects of SSR125543 (S), desipramine (DMI) and fluoxetine (FLX) on swim test immobility. FSL or FRL rats were treated for 14 consecutive days (i.p.) with the respective treatments and then tested in the swim tank approximately 21–23 h after the last injection. Bars that have different letters are significantly different, $P<0.01$, according to Tukey's protected t-test.

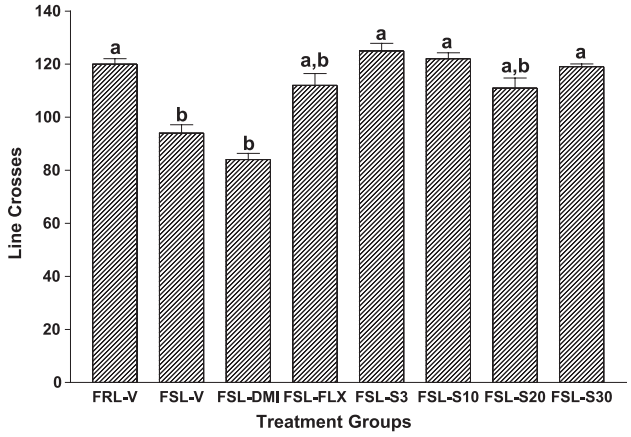

Fig. 3. Effects of SSSR125543 (S), desipramine (DMI) and fluoxetine (FLX) on line crosses in the social interaction test. FSL or FRL rats were treated for 14 consecutive days (i.p.) with the respective treatments and then tested in the social interaction arena approximately 18–20 h after the last injection. Bars that have different letters are significantly different, $P<0.01$, according to Tukey's protected t-test.

thus, all treated Flinders Sensitive Line rats were different from the vehicle-treated Flinders Sensitive Line rats, but not from each other (Fig. 1).

Fig. 2 illustrates the effects on social interaction. Several of the treatments increased the low social interaction in the Flinders Sensitive Line rats and the one-way analysis of variance was significant ($F(7,70)=4.95$, $P<0.01$). Interestingly, desipramine increased the time spent in social interaction, but fluoxetine did not. SSR125543 had a dose-dependent effect on time spent in social interaction, with significant increases being observed after 20 and 30 mg/kg (Fig. 2).

Fig. 3 illustrates the effects on line crosses. Neither desipramine nor fluoxetine increased line crosses, but SSR125543 increased activity at all doses except 20 mg/kg, enough to result in a small but significant outcome in the one-way analysis of variance ($F(7,70)=3.10$, $P<0.01$).

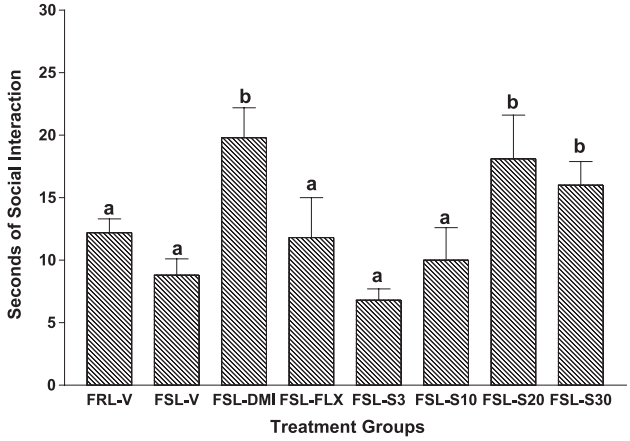

Fig. 2. Effects of SSSR125543 (S), desipramine (DMI) and fluoxetine (FLX) on time spent in social interaction. FSL or FRL rats were treated for 14 consecutive days (i.p.) with the respective treatments and then tested in the social interaction arena approximately 18–20 h after the last injection. Bars that have different letters are significantly different, $P<0.01$, according to Tukey's protected t-test.

4. Discussion

The findings for swim test immobility confirmed the large strain difference between the Flinders Sensitive and Resistant Line rats, with the Flinders Sensitive Line rats being immobile for almost twice as long as the Flinders Resistant Line rats (Fig. 1; see Overstreet, 1993, 2002; Overstreet et al., 1995, 1998; Zangen et al., 1997). All treatments reduced the immobility time in the FSL rats. These findings are consistent with previous literature indicating that antidepressants reduce immobility in the FSL rats (see Overstreet, 2002). In contrast, studies that have been performed with antidepressants in the Flinders Resistant Line rats have generally failed to demonstrate a significant change in immobility (Overstreet, 2002; Overstreet et al., 2004). Treatment of Flinders Resistant Line rats with fluoxetine and desipramine confirmed the lack of anti-immobility response in these rats (data not shown).

The CRF_1 receptor antagonist, SSR125543, was effective in counteracting the exaggerated immobility of the FSL rats. This outcome confirms its antidepressant-like effects in normal rats in the forced swim test and chronic mild stress (Griebel et al., 2002) and supports the potential utility of this compound in particular, and CRF_1 receptor antagonists in general, as antidepressants. Other studies with these and other CRF_1 antagonists support this conclusion as well (see Griebel et al., 2002; Holsboer, 1999; Keck and Holsboer, 2001; Overstreet et al., 2004; Seymour et al., 2003; Zobel et al., 1999). However, the pattern of results with SSR125543 on the swim test was less than optimal, as there was not a clear dose-dependent effect. Although immobility times of the groups treated with 10 and 30 mg/kg were not

significantly different from that of the control Flinders Resistant Line group, they were also not significantly different from those of the groups treated chronically with 3 or 20 mg/kg. This "partial" normalization of immobility scores has been observed with low doses of other treatments (Overstreet et al., 2004), suggesting that twice daily dosing may be more effective.

Although all of the drugs predicted to reduce immobility did so, there were substantial differences among the drugs in regard to their effects on the measures in the social interaction test. While desipramine produced a robust increase in time spent in social interaction, fluoxetine did not. Both of these findings replicated previously reported results (File et al., 1999; Overstreet et al., 2000; 2004). The lack of effect with fluoxetine may be related in part to the fact that only a two-week treatment period was used, as others have reported significant effects of selective serotonin reuptake inhibitors on social interaction behavior with longer treatment regimens (Bristow et al., 2000). Nevertheless, the present results indicate that desipramine has more robust effects on social interaction behavior than fluoxetine under the conditions used here (once daily dosing with 5 mg/kg for 14 days).

There was clearer evidence for a dose-related effect of SSR125543 on time spent in social interaction (Fig. 2). The two higher doses increased time spent in social interaction significantly, but the two lower doses did not. Thus, for these higher doses, there is both an antidepressant- and an anxiolytic-like effect of SSR125543. This outcome also suggests that SSR123343A has a more rapid onset of its anxiolytic activity than fluoxetine.

For locomotor activity, the Flinders Sensitive Line rats were slightly, but significantly, less active than the Flinders Resistant Line rats (Fig. 3). This supports previous findings on these strains in novel environments (see Overstreet, 2002). All doses of SSR125543, except 20 mg/kg, significantly increased locomotor activity above the level seen in the FSL rats given vehicle (Fig. 3). However, neither desipramine nor fluoxetine significantly increased activity, suggesting that the decrease in immobility produced by these treatments cannot be explained by their effects on locomotor activity. To obtain further information about the relationship between activity and immobility, an analysis of covariance was carried out. When the number of line crosses was used as the covariate, there were still significant differences among the immobility times ($F(6,104)=32.37$, $P<0.0001$). This analysis is not conclusive, as a more appropriate test would be to examine the effects of chronic SSR125543 treatment on swimming behavior. Nevertheless, the fact that the dose of 20 mg/kg did not alter activity but did increase swimming suggests that the effects of SSR125543 on swim test immobility cannot be explained simply as a stimulant effect. Furthermore, the changes in activity are quite small (10–20%) compared to the changes in immobility (40–50%).

The increases in social interaction induced by SSR125543 also appear to be independent of its effects on locomotor activity. All doses of SSR125543 except 20 mg/kg increased activity, but only the two higher doses increased time spent in social interaction. Analysis of covariance using the number of line crosses as the covariate showed that there were still significant group differences for time spent in social interaction ($F(7,70)=5.66$, $P<0.01$). This conclusion of an independence between social interaction and line crosses is consistent with the views expressed by others (File and Seth, 2003; Overstreet et al., 2002, 2003).

In conclusion, the CRF_1 receptor antagonist SSR125543 has antidepressant-like effects in the Flinders Sensitive Line rats as reflected by the decreases in the exaggerated swim test immobility. In addition, it has anxiolytic-like effects as reflected by an increase in the abnormally low social interaction behavior of the Flinders Sensitive Line rats. Clinical testing of this compound as an antidepressant and/or anxiolytic is warranted.

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References

Benca, R.M., Obermier, W.H., Thisted, R.A., Gillin, J.C., 1992. Sleep and psychiatric disorders. A meta-analysis. Arch. Gen. Psychiatry 49, 651–670.

Benca, R.M., Overstreet, D.H., Gilliland, M.A., Russell, D., Bergmann, B.M., Obermeyer, W.H., 1996. Increased basal REM sleep but no difference in dark induction or light suppression of REM sleep in Flinders Rats with cholinergic supersensitivity. Neuropsychopharmacology 15, 45–51.

Bristow, L.J., O'Connor, D., Watts, R., Duxon, M.S., Hutson, P.H., 2000. Evidence for accelerated desensitisation of $5\text{-}HT_{2C}$ receptors following combined treatment with fluoxetine and the $5\text{-}HT_{1A}$ receptor antagonist, WAY 100635, in the rat. Neuropharmacology 39, 123–132.

File, S.E., 1980. The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. J. Neurosci. Methods 2, 219–238.

File, S.E., Seth, P., 2003. A review of 25 years of the social interaction test. Eur. J. Pharmacol. 463, 35–53.

File, S.E., Quagazzal, A.M., Gonzalez, L.E., Overstreet, D.H., 1999. Chronic fluoxetine in tests of anxiety in rat lines selectively bred for differential $5\text{-}HT_{1A}$ receptor function. Pharmacol. Biochem. Behav. 62, 695–701.

Griebel, G., Simiand, J., Steinberg, R., Jung, M., Gully, D., Roger, P., Geslin, M., Scatton, B., Maffrand, J.P., Soubrie, P., 2002. 4-(2-Chloro-4-methoxy-5-methylphenyl)-*N*-[(1*S*)-2-cyclopropyl-1-(3-fluro-4-methyl)ethyl]5-methyl-3-thiazol-2-amine hydrochloride (SSR125543A), a potent and selective corticotropin-releasing factor (1) receptor antagonist: II. Characterization in rodent models of stress-related disorders. J. Pharmacol. Exp. Ther. 301, 333–345.

Holsboer, F., 1999. The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. J. Psychiat. Res. 33, 181–214.

Keck, M.D., Holsboer, F., 2001. Hyperactivity of CRH neuronal circuits as a target for therapeutic interventions in affective disorders. Peptides 22, 835–844.

Okuyama, S., Chaki, S., Kawashima, N., Suzuki, Y., Ogawa, S., Nakazato, A., Kumagai, T., Okubo, T., Tomisawa, K., 1999. Receptor binding, behavioral, and electrophysiological profiles of nonpeptide corticotropin-releasing factor subtype1 receptor antagonists CRA1000 and CRA1001. J. Pharmacol. Exp. Ther. 289, 926–935.

Overstreet, D.H., 1993. The flinders sensitive line rats: a genetic animal model of depression. Neurosci. Biobehav. Rev. 17, 51–68.

Overstreet, D.H., 2002. Behavioral characteristics of rat lines selected for differential hypothermic responses to cholinergic or serotonergic agonists. Behav. Genet. 32, 335–348.

Overstreet, D.H., Pucilowski, O., Rezvani, A.H., Janowsky, D.S., 1995. Administration of antidepressants, diazepam and psychomotor stimulants further confirms the utility of flinders sensitive line rats as an animal model of depression. Psychopharmacology 121, 27–37.

Overstreet, D.H., Daws, L.C., Schiller, G.D., Orbach, J., Janowsky, D.S., 1998. Cholinergic/serotonergic interactions in hypothermia: implications for rat models of depression. Pharmacol. Biochem. Behav. 59, 777–785.

Overstreet, D.H., Knapp, D.J., Breese, G.R., 2000. Mechanisms involved in the acute anxiogenic effects of fluoxetine. Presented at the Satellite Symposium "Serotonin: from the Molecule to the Clinic" held in New Orleans, LA, Nov. 1–3, 2000.

Overstreet, D.H., Knapp, D.J., Breese, G.R., 2002. Accentuated decreases in social interaction in rats subjected to repeated ethanol withdrawals. Alcohol., Clin. Exp. Res. 26, 1259–1269.

Overstreet, D.H., Knapp, D.J., Moy, S.S., Breese, G.R., 2003. A 5-HT_{1A} agonist and a 5-HT_{2C} antagonist reduce social interaction deficit induced by multiple ethanol withdrawals in rats. Psychopharmacology 167, 344–352.

Overstreet, D.H., Keeney, A.D., Hogg, S., 2004. Antidepressant effects of citalopram and CRF receptor antagonist CP-154,526 in a rat model of depression. Eur. J. Pharmacol. 4929, 195–201.

Pucilowski, O., Overstreet, D.H., 1993. Effect of chronic antidepressant treatment on responses to apomorphine in selectively bred rat strains. Pharmacol. Biochem. Behav. 32, 471–475.

Seymour, P.A., Schmidt, A.W., Schultz, D.W., 2003. The pharmacology of CP154,526, a non-peptide antagonist of the CRH_1 receptor. A review. CNS Drug Rev. 9, 57–96.

Shiromani, P.J., Overstreet, D.H., Levy, D., Goodrich, C.A., Campbell, S.S., Gillin, J.C., 1988. Increased REM sleep in rats selectively bred for cholinergic hyperactivity. Neuropsychopharmacology 1, 127–133.

Zangen, A., Overstreet, D., Yadid, G., 1997. High serotonin and 5-hydroxyindoleacetic acid levels in limbic brain regions in a rat model of depression: normalization by chronic antidepressant treatment. J. Neurochem. 69, 2477–2483.

Zobel, A.W., Yassouridis, A., Frieboes, R.M., Holsboer, F., 1999. Prediction of medium-term outcome by cortisol response to the combined dexamethasone-CRH test in patients with remitted depression. Am. J. Psychiatry 156, 949–951.

Zobel, A.W., Nickel, T., Kunzel, H.E., Ackl, N., Sonntag, A., Ising, M., Holsboer, F., 2000. Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. J. Psychiatr. Res. 34, 171–181.