Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 492 (2004) 195–201

www.elsevier.com/locate/ejphar

Antidepressant effects of citalopram and CRF receptor antagonist CP-154,526 in a rat model of depression

David H. Overstreet[a,*], Adam Keeney[b], Sandra Hogg[b]

[a] *Center for Alcohol Studies, CB #7178, 3009 Thurston-Bowles Building, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7178, USA*
[b] *Psychopharmacological Research, H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Copenhagen, Denmark*

Received 29 September 2003; received in revised form 5 April 2004; accepted 9 April 2004

Abstract

Due to the interest in the antidepressant potential of nonpeptide corticotropin-releasing factor $(CRF)_1$ receptor antagonists, the present investigation examined the antidepressant-like effects of the CRF_1 receptor antagonist CP-154,526 on the exaggerated swim test immobility in the Flinders Sensitive Line (FSL) rat, a genetic animal model of depression. Chronic treatment with CP-154,526 (10 mg/kg; 2 × day) for 14 days increased swimming in the Flinders Sensitive Line rats. Citalopram (5 and 10 mg/kg; 2 × day) and desipramine (5 mg/kg; 1 × day) also significantly increased swimming in the Flinders Sensitive Line rats, as expected. However, neither CP-154,526 nor citalopram (10 mg/kg) altered swimming times in the control Flinders Resistant Line (FRL) rats. Citalopram (10 mg/kg) and CP-154,526 also increased the abnormally low level of social interaction behavior in the Flinders Sensitive Line rats. These findings indicate that citalopram and CP154,526, a CRF_1 receptor antagonist, have both antidepressant and anxiolytic effects that can be detected in an experimental model of depression only and not in "normal" control animals.

Keywords: Flinders sensitive line rat model; Depression; CRF_1 receptor antagonist; Citalopram; 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, including CP-154,526 (Holsboer, 1999; Keck and Holsboer, 2001; Okuyama et al., 1999; Seymour et al., 2003). These compounds have proved effective in certain animal tests of depressive-like behavior. Classical tricyclics (e.g., desipramine) and selective serotonin reuptake inhibitors (e.g., fluoxetine and citalopram) are also known to be effective in some animal tests of depressive-like behavior. Therefore, it was decided to compare the effectiveness of a CRF_1 receptor antagonist with citalopram and 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 (Pucilowski and Overstreet, 1993). Originally selected for its increased cholinergic sensitivity (Overstreet, 1993), the Flinders Sensitive Line rat exhibits other features that are similar to those found in human depressives, such as increased rapid eye movement sleep (Benca et al., 1992; Shiromani et al., 1988), has serotonergic abnormalities that are corrected following chronic antidepressant treatments (Zangen et al., 1997), and responds to other antidepressants (see Overstreet, 2002). The FSL rats also exhibit reduced levels of CRF in the median eminence, locus coeruleus, and prefrontal cortex compared to the FRL rats (Owens et al., 1991).

Therefore, the Flinders Sensitive Line rat model of exaggerated immobility in the swim test was used in this study to evaluate the antidepressant potential of a CRF_1 receptor antagonist CP-154,526, the selective serotonin reuptake inhibitor citalopram, and the tricyclic desipramine.

* Corresponding author. Tel.: +1-919-966-1159; fax: +1-919-966-5679.
E-mail address: dhover@med.unc.edu (D.H. Overstreet).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.04.010

The social interaction test is a widely used and accepted procedure for detecting anxiety-like behavior in rodents (e.g., File, 1980; File and Seth, 2003; Guy and Gardner, 1985; Irvine et al., 2001). This task is particularly sensitive to manipulation of the serotonergic system, unlike the elevated plus maze (e.g., File et al., 1996; Gonzalez et al., 1998). Rats that were selectively bred for increased sensitivity to the 5-HT_{1A} receptor agonist, 8-hydroxy-2-di-*N*-propylaminotetralin (8-OH-DPAT; the High DPAT Sensitive rats) exhibited anxiety-like behavior in the social interaction test, but not in the elevated plus maze (File et al., 1999; Gonzalez et al., 1998). The Flinders Sensitive Line rats are also more sensitive to the effects of 8-OH-DPAT than their Flinders Resistant Line counterparts (Overstreet, 2002; Overstreet et al., 1998). Therefore, the Flinders Sensitive and Resistant Line rats were tested in the social interaction test after vehicle treatment to determine whether there were strain differences and after chronic treatment with citalopram and CP154,526 to determine whether there might be anxiolytic effects, as several reports indicate that chronic treatment with selective serotonin reuptake inhibitors may have anxiolytic properties (e.g., Bristow et al., 2000; File et al., 1999; Lightowler et al., 1994).

2. Methods and materials

2.1. Animals

These experiments adhered to the European Community guidelines for the use of experimental animals and were approved by the UNC Institutional Animal Care and Use Committee. 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 an average age of 75 days old and an average body weight of 375 grams for the FSL rats and 401 g for the FRL rats. They were housed in groups of three in temperature-and humidity-controlled rooms under a 12:12 light/dark cycle (lights on 0700–1900). Rats were randomly divided into five (Flinders Sensitive Line) or three (Flinders Resistant Line) groups containing 8–11 rats and then given the treatments described below. Fewer Flinders Resistant Line groups were used because previous evidence indicated that the Flinders Resistant Line rats, which exhibit a relatively low degree of immobility, do not exhibit decreases in immobility following many antidepressant treatments (see Overstreet, 2002).

2.2. Treatments

The following treatment groups were established: vehicle or isotonic saline; CP-154,526 (synthesized by Lundbeck A/S as Lu02613C; 10 mg/kg of the base), an orally active, nonpeptide CRF_1 antagonist; Citalopram (synthesized by Lundbeck A/S), a selective serotonin reuptake inhibitor at doses of 5 and 10 mg/kg of the hydrobromide salt; desipramine (Sigma, St. Louis, MO; 5 mg/kg of the hydrochloride salt). Desipramine was dissolved in isotonic saline (vehicle) and CP-154,526 and citalopram were dissolved in distilled water. The Flinders Sensitive Line rats were subjected to all of these five treatments. However, the Flinders Resistant Line rats were subjected to vehicle, CP-154,526, and 10 mg/kg citalopram only because desipramine is known to be ineffective (Overstreet, 2002) and the lower dose of citalopram was expected to be ineffective also. Rats were injected i.p. daily either once (desipramine) or twice (vehicle, CP-154,526, 5 and 10 mg/kg citalopram). Doses and frequency of injection were selected on the basis of reports from the literature (Papp et al., 2002; Pucilowski and Overstreet, 1993; Seymour et al., 2003; Sanchez et al., 2003).

Treatments were given for 14 consecutive days. On the day after the last injection the rats were subjected sequentially to the social interaction test (approximately 22 h after the last treatment) and the forced swim test (approximately 24 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 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 16 15 × 15 cm squares 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, 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. In addition, two motor activity measures were collected. The total number of lines crossed during the session provided a measure of general activity. The number of entries into the central squares of the arena provided another, probably independent, measure of anxiety-like behavior. Rats that spend less time in the center of the open field arena are regarded as more ‘‘anxious’’.

Approximately 2 h 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 bottom. The rat was placed in the swim tank for a single 5-min session approximately 24 h after the last treatment and the seconds of immobility was 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 four 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, the data for each measure were subjected to one-way analysis of variance separately for the Flinders Sensitive and Resistant Line rats after confirming that the data were normally distributed (Shapiro-Wilk Test) and that the intergroup variances were similar (F test on variances). If the analysis of variance revealed significant group differences, post hoc Tukey's tests were carried out to elucidate the pattern of group differences. The GBstat software package was used for the statistical analyses. Two-way analyses of variance were conducted to compare treatment responses in the Flinders Sensitive and Resistant Line rats for the three treatments in common.

3. Results

3.1. Forced swim test

Fig. 1 illustrates the antidepressant-like effects of each of the compound used in the Flinders Sensitive Line rats, as the immobility was significantly less (Swimming time was significantly greater) in all of the rats treated with active drugs compared to the Flinders Sensitive Line rats that received vehicle. In contrast, there were no significant effects in the Flinders Resistant Line rats. Note also that the immobility is much less in the vehicle-treated Flinders Resistant Line rats compared to the vehicle-treated Flinders Sensitive Line rats, confirming previous studies (see Overstreet, 2002, for review).

Analyses with one-way analyses of variance indicated significant treatment effects in the Flinders Sensitive Line rats [$F(4,38)=8.78$, $P<0.001$] but not in the Flinders

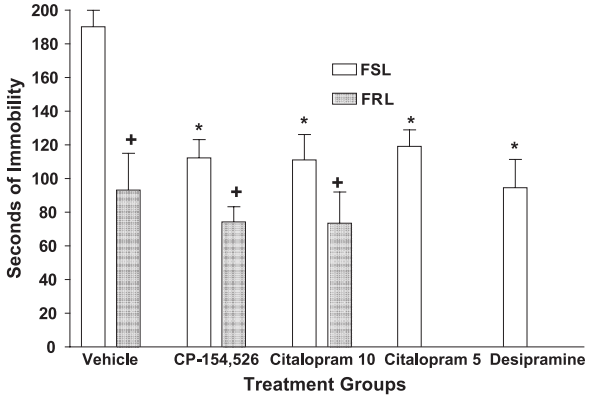

Fig. 1. Effects of Citalopram, CRF_1 Receptor Antagonist CP154,526, and Desipramine on Time Spent Immobile in the Forced Swim Test. Rats were treated for 14 consecutive days with the respective treatments and then tested in the 5-min forced swim test approximately 24 h after the last treatment. Data represent the mean sec ± S.E.M. immobile for 8–11 rats. *Indicates significant difference from Flinders Sensitive Line rat treated with vehicle, $P<0.01$, Tukey's test. + Indicates that Flinders Resistant Line rats are significantly different from Flinders Sensitive Line rats receiving the same treatment.

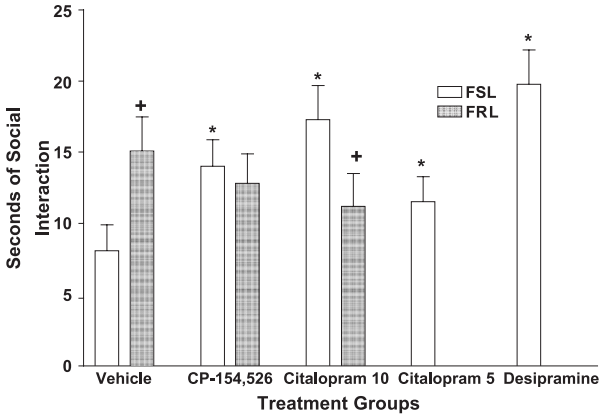

Fig. 2. Effects of Citalopram, CRF_1 Receptor Antagonist CP154,526, and Desipramine on Time Spent in Social Interaction in the Social Interaction Test. Rats were treated for 14 consecutive days with the respective treatments and then tested in the 5-min social interaction test approximately 22 h after the last treatment. Data represent the mean second ± S.E.M. spent in social interaction for 8–11 rats. *Indicates significant difference from Flinders Sensitive Line rat treated with vehicle, $P<0.01$, Tukey's test. + Indicates that Flinders Resistant Line rats are significantly different from Flinders Sensitive Line rats receiving the same treatment.

Resistant Line rats [$F(2,20)=0.41$, $P>0.10$]. All treated Flinders Sensitive Line rats were significantly different from the vehicle-treated Flinders Sensitive Line rats. A two-way analysis of variance revealed a highly significant strain effect [$F(1,44)=23.99$, $P<0.0001$] and a significant treatment effect [$F(2,44)=7.63$, $P<0.001$] and a nearly significant strain × treatment interaction [$F(2,44)=2.81$, $P=0.07$]. This analysis is consistent with the observation that the treatments significantly reduced swim test immobility in the Flinders Sensitive Line rats only. Note, however, that the Flinders Resistant Line rats treated with CP-154,526 and 10 mg/kg citalopram were still significantly less immobile than their Flinders Sensitive Line counterparts (see Fig. 1).

3.2. Social interaction test

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 [$F(4,38)=5.27$, $P<0.01$]. CP-154,426 increased social interaction. There was a dose-dependent effect of citalopram on social interaction, with the higher dose being effective. Finally, desipramine had the most significant effect on social interaction in the Flinders Sensitive Line rats. The higher social interaction exhibited by the Flinders Resistant Line rats was not altered by treatment with CP-154,525 or 10 mg/kg citalopram [$F(2,20)=0.49$, $P>0.05$]. Even though the Flinders Resistant Line rats spent almost twice as much time in social interaction than the Flinders Sensitive Line rats, the two-way analysis of variance revealed no significant strain effect [$F(1,44)=0.01$, $P>0.10$]. There was also no significant

treatment effect [$F(2,44) = 0.87$, $P > 0.10$]. Instead, there was a highly significant strain × treatment interaction effect [$F(2,44) = 5.72$, $P < 0.01$], because CP-154,526 and 10 mg/kg citalopram increased time spent in social interaction in the Flinders Sensitive Line rats but not in the Flinders Resistant Line rats.

Fig. 3 illustrates the effects on line crossings. Only citalopram at the 10 mg/kg dose increased activity in the Flinders Sensitive Line rats, but this increase was enough to result in a small but significant outcome in the one-way analysis of variance [$F(4,38) = 3.25$, $P < 0.05$]. No other treatment affected activity in the Flinders Sensitive Line rats and no treatment had a significant effect on activity in the Flinders Resistant Line rats [$F(2,20) = 0.49$, $P > 0.10$], which was higher than that the Flinders Sensitive Line rats. As for time spent in social interaction, the only significant effect in the two-way analysis of variance for line crosses was the significant interaction effect [$F(2,44) = 3.31$, $P < 0.05$]. This statistical outcome was most likely a reflection of the fact that 10 mg/kg citalopram significantly increased line crossings in the Flinders Sensitive Line rats but not in the Flinders Resistant Line rats.

Fig. 4 illustrates the effects on Center Entries. There is a fairly dramatic strain difference, with the Flinders Resistant Line rats entering the central area almost twice as much as the Flinders Sensitive Line rats. Again, no treatment affected the Flinders Resistant Line rats [$F(2,20) = 1.05$, $P > 0.10$], but several did increase the Center Entries in the Flinders Sensitive Line rats [$F(3,38) = 3.14$, $P < 0.05$]. Once again, CP-154,526 and 10 mg/kg citalopram were effective but 5 mg/kg citalopram was not. However, unlike with social interaction, desipramine had no significant effect on Center Entries. Consistent with the large difference between the untreated Flinders Sensitive and Resistant Line rats, the two-way analysis of variance revealed a significant strain effect [$F(1,44) = 4.18$, $P < 0.05$], but there was no overall significant treatment effect [$F(2,44) = 0.97$, $P > 0.10$]. As with the other measures, there was a significant strain × treatment interaction effect [$F(2,44) = 4.98$, $P < 0.01$], mainly because CP-154,526 and 10 mg/kg citalopram significantly increased Center Entries in the Flinders Sensitive Line rats but not in the Flinders Resistant Line rats.

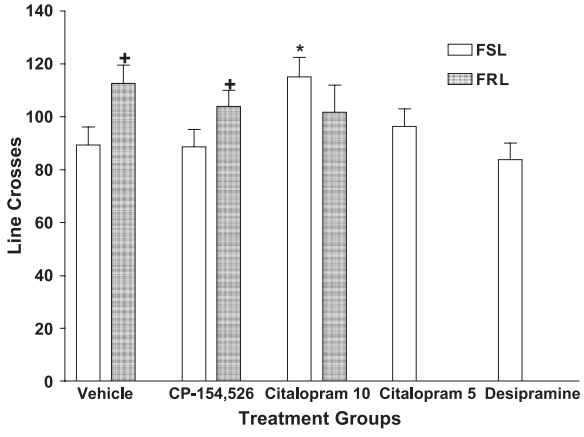

Fig. 3. Effects of Citalopram, CRF_1 Receptor Antagonist CP154,526, and Desipramine on Number of Line Crosses in the Social Interaction Test. Rats were treated for 14 consecutive days with the respective treatments and then tested in the 5-min social interaction test approximately 22 h after the last treatment. Data represent the mean ± S.E.M. lines crossed for 8–11 rats. *Indicates significant difference from Flinders Sensitive Line rat treated with vehicle, $P < 0.01$, Tukey's test. + Indicates that Flinders Resistant Line rats are significantly different from Flinders Sensitive Line rats receiving the same treatment.

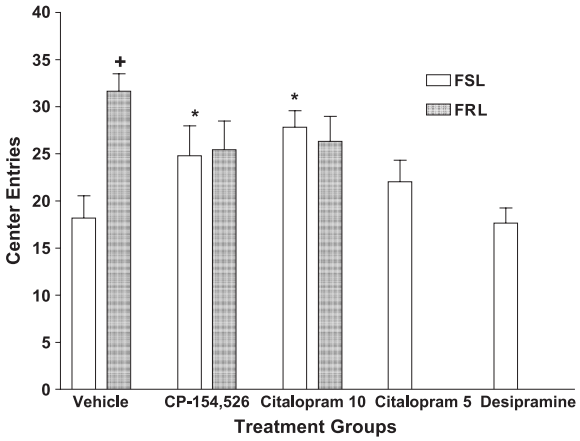

Fig. 4. Effects of Citalopram, CRF_1 Receptor Antagonist CP154,526, and Desipramine on Number of Center Entries in the Social Interaction Test. Rats were treated for 14 consecutive days with the respective treatments and then tested in the 5-min social interaction test approximately 22 h after the last treatment. Data represent the mean entries into the center ± S.E.M. for 8–11 rats. *Indicates significant difference from Flinders Sensitive Line rat treated with vehicle, $P < 0.01$, Tukey's test. + Indicates that Flinders Resistant Line rats are significantly different from Flinders Sensitive Line rats receiving the same treatment.

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 Flinders Sensitive Line rats, while the small reductions after CP-154,526 and 10 mg/kg citalopram in the Flinders Resistant Line rats were not significant. These findings are also consistent with previous literature indicating that antidepressants are effective in reversing the behavioral abnormalities of the Flinders Sensitive Line rats, but not in the Flinders Resistant Line rats (see Overstreet, 2002). The lack of change in the Flinders Resistant Line rats is unlikely to be the result of a floor effect, as the immobility score of the Flinders Resistant Line rats was about 90 s. Instead, it is proposed that the antidepressants, regardless of acute biochemical mechanism of action, are counteracting the exaggerated immobility of the Flinders Sensitive Line rats.

The present findings, therefore, support the predictive validity of the Flinders Sensitive Line rats in detecting antidepressants. The forced swim test by itself is little more than a bioassay for potential antidepressants and there have been a number of false positives and negatives (Borsini and Meli, 1988). However, when the swim test is combined with a chronic drug regimen and the Flinders Sensitive Line rats, the predictive validity is greatly enhanced. The psychomotor stimulants amphetamine and scopolamine do not alter swimming under these conditions (Overstreet et al., 1995). The original version of the forced swim test was unable to detect antidepressant-like effects of selective serotonin reuptake inhibitors (Porsolt et al., 1977), but these agents are very effective in the Flinders Sensitive Line rats (Overstreet, 2002; Pucilowski and Overstreet, 1993; Fig. 1). The predictive validity of the Flinders Sensitive Line rat model compares favorably with that of several environmentally induced models such as olfactory bulbectomy (Kelly et al., 1997) and chronic mild stress (Willner, 1997); however, fewer manipulations are required to produce the Flinders Sensitive Line rat.

Previous studies had shown that desipramine was quite effective when administered just once daily (see Overstreet, 2002). In a preliminary study the effects of twice daily injections of desipramine were examined to determine whether this treatment could be used as a comparison with citalopram and CP-154,526. However, Flinders Sensitive Line rats subjected to this treatment lost weight and it was considered prudent to maintain just once daily treatments for desipramine. There were no significant differences in weight gain ($P>0.10$).

The CRF_1 receptor antagonist CP154,526 was effective in counteracting the exaggerated immobility of the Flinders Sensitive Line rats. This outcome 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 receptor antagonists support this conclusion as well (see Griebel et al., 2002; Holsboer, 1999; Keck and Holsboer, 2001; Seymour et al., 2003; Zobel et al., 2000).

Whether the differential effects of CP-154,526 in the Flinders Sensitive and Resistant Line rats are related to the differences in basal CRF levels (Owens et al., 1991) cannot be resolved at this time. The fact that both desipramine, a tricyclic with primarily noradrenergic effects, and selective serotonin reuptake inhibitors both normalize the serotonergic and noradrenergic abnormalities in the limbic regions of the Flinders Sensitive Line rats (Zangen et al., 1997, 1999) suggests that there might not be a tight relationship between the antidepressant-like effects of drugs and their neurochemical consequences. It would be of interest to determine whether CRF_1 receptor antagonists also normalize the neurochemical abnormalities in the Flinders Sensitive Line rats.

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. For locomotor activity, the Flinders Sensitive Line rats were slightly, but significantly, less active than the Flinders Resistant Line rats. This supports previous findings on these strains in novel environments (see Overstreet, 2002). Only citalopram at the 10 mg/kg dose significantly increased locomotor activity in the Flinders Sensitive Line rats; as with immobility, 10 mg/kg citalopram did not significantly affect activity in the Flinders Resistant Line rats.

As indicated above, because the social interaction test is carried out in a large square open field arena, the locomotor activity can be divided into total line crossings and center entries. These center entries have been regarded by some investigators as a measure of anxiety-like behavior or, rather, the absence of anxiety-like behavior (e.g. Ramos et al., 1997). The fact that the Flinders Resistant Line rats went into the center much more often than the Flinders Sensitive Line rats suggests that there might be a difference in the anxiety-like behavior in the two strains. This is a novel finding, because we have previously reported that the Flinders Sensitive and Resistant Line rats do not differ in the elevated plus maze test of anxiety (Schiller et al., 1991; Overstreet et al., 1995). Assuming that the low center entries of the Flinders Sensitive Line rats does reflect an anxiety-like behavior, then it can be proposed that CP-154,526, the CRF_1 antagonist, and the high dose of citalopram had anxiolytic effects. However, the low dose of citalopram and desipramine (the tricyclic) did not. The difference between the 5 and 10 mg/kg doses of citalopram might be related to dose. Because center entries could include a general activity component, an Analysis of Covariance was conducted, with line crossings as the covariate. There was still a significant treatment effect on center entries in the Flinders Sensitive Line rats.

The time spent in social interaction is another highly regarded index of anxiety-like behavior (e.g., File and Seth, 2003). Although not as dramatic as for center entries, Flinders Sensitive Line rats spent less time in social interaction than the Flinders Resistant Line rats, suggesting again that they may be more anxious than the Flinders Resistant Line rats. An Analysis of Covariance with line crossings as the covariate confirmed the significant treatment effects on time spent in social interaction in the Flinders Sensitive Line rats. How can these results be reconciled with the earlier reports of no differences in the elevated plus maze (e.g., Overstreet et al., 1995)? Perhaps the parameters in the social interaction test are more sensitive to differences in serotonergic function than the elevated plus maze, as suggested by File et al. (File et al., 1993, 1996; Gonzalez et al., 1998). Indeed, the High DPAT Sensitive line of rats, which is more sensitive to $5\text{-}HT_{1A}$-induced hypothermia like the Flinders Sensitive Line rats (Overstreet et al., 1994, 1996, 1998), is more immobile in the swim test and engages in less social

interaction than the Low DPAT Sensitive line of rats, but they do not differ in the elevated plus maze (File et al., 1999; Gonzalez et al., 1998; Overstreet, 2002; Overstreet et al., 1996).

Because CP-154,526, 10 mg/kg citalopram and desipramine increase time spent in social interaction in the Flinders Sensitive Line rats, they have putative anxiolytic effects. In contrast, 5 mg/kg citalopram did not increase time spent in social interaction. These negative effects could be dose-related, as suggested above. In any case, both CP-154,526 and 10 mg/kg citalopram had anxiolytic profiles for both time spent in social interaction and center entries. Yet neither of these drugs altered the time spent in social interaction or center entries in the Flinders Resistant Line rats. Once again, the conclusion seems to be that citalopram, a selective serotonin reuptake inhibitor, and CP-154,526, a CRF_1 receptor antagonist, normalized the pathologically low behavior of the Flinders Sensitive Line rats but did not affect the "normal" behavior of the Flinders Resistant Line rats. Further evidence for this principle has been afforded by recent findings showing that citalopram (Papp et al., 2002) or a CRF_1 receptor antagonist (Griebel et al., 2002) can counteract the behavioral abnormalities associated with chronic mild stress.

The profile outlined above, showing that citalopram possesses both an antidepressant and anxiolytic profile, whereas desipramine was devoid of anxiolytic effects, is supported by clinical studies that demonstrate citalopram to be an effective antidepressant, which also possesses clinical efficacy in a number of anxiety disorders, whereas desipramine has only shown efficacy as an antidepressant drug (Bezchlibnyk-Butler et al., 2000; McLeod et al., 2000; Perna et al., 2001; Pollock, 2001; Sasson et al., 1999; Varia and Rauscher, 2002; Varia et al., 2002). These clinical findings suggest that CP154,526 may have both antidepressant and anxiolytic effects in humans because of its favorable profile in Flinders Sensitive Line rats.

The fact that both CP154,526 and 10 mg/kg citalopram increased both swimming and social interaction in the Flinders Sensitive Line rats suggests that these agents did not have any adverse effects in the rats. Indeed, all rats looked very healthy at the time of each of their injections. Furthermore, body weights taken at weekly intervals were similar in the various treatment groups.

In conclusion, the CRF_1 receptor antagonist CP154,526 has antidepressant-like effects in the Flinders Sensitive Line rats as reflected by the decreases in the exaggerated swim test immobility. In addition, CP154,526 has anxiolytic effects as reflected by an increase in the abnormally low social interaction behavior and center entries of the Flinders Sensitive Line rats. These data demonstrate that the CRF_1 receptor antagonist has a profile comparable to citalopram and that clinical testing of CRF_1 receptor antagonists as antidepressants or anxiolytics could be warranted.

Acknowledgements

We thank Mili Senapati for technical assistance.

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.

Bezchlibnyk-Butler, K., Aleksic, I., Kennedy, S.H., 2000. Citalopram—a review of pharmacological and clinical effects. J. Psychiatry Neurosci. 25, 241–254.

Borsini, F., Meli, A., 1988. Is the forced swim test a suitable model for revealing antidepressant activity? Psychopharmacology 94, 147–160.

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. , Zangrossi Jr., H., Andrews, N. 1993. Social interaction and elevated plus maze tests: changes in release and uptake of 5-HT and GABA. Neuropharmacology 32, 217–221.

File, S.E., Gonzalez, L.E., Andrews, N., 1996. Comparative study of pre- and postsynaptic $5\text{-}HT_{1A}$ receptor modulation of anxiety in two ethological animal tests. J. Neurosci. 16, 4810–4815.

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.

Gonzalez, L.E., File, S.E., Overstreet, D.H., 1998. Selectively bred lines of rat differ in social interaction and hippocampal $5\text{-}HT_{1A}$ receptor function: a link between anxiety and depression? Pharmacol. Biochem. Behav. 59, 787–792.

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*-[(1S)-2-cyclopropyl-1-(3-fluror-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.

Guy, A.P., Gardner, C.R., 1985. Pharmacological characterisation of a modified social interaction model of anxiety in the rat. Neuropsychobiology 13, 194–200.

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

Irvine, E.E., Bagnalasta, M., Marcon, C., Motta, C., Tessari, M., File, S.E., Chiamulera, C., 2001. Nicotine self-administration and withdrawal: modulation of anxiety in the social interaction test in rats. Psychopharmacology 153, 315–320.

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

Kelly, J.P., Wrynn, A.S., Leonard, B.E., 1997. The olfactory bulbectomized rat as a model of depression: an update. Pharmacol. Ther. 74, 299–316.

Lightowler, S., Kennett, G.A., Williamson, I.J.R., Blackbourn, T.P., Tulloch, I.F., 1994. Anxiolytic-like effect of paroxetine in a rat social interaction test. Pharmacol. Biochem. Behav. 49, 281–285.

McLeod, D.R., Hoehn-Saric, R., Porges, S.W., Kowalski, P.A., Clarke, C.M., 2000. Therapeutic effects of imipramine are counteracted by the metab-

olite, desipramine, in patients with generalized anxiety disorder. J. Clin. Psychopharmacol. 20, 615–621.

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., Rezvani, A.H., Pucilowski, O., Gause, L., Janowsky, D.S., 1994. Rapid selection for serotonin-1A Sensitivity in rats. Psychiatr. Genet. 4, 57–62.

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., Rezvani, A.H., Knapp, D.J., Crews, F.T., Janowsky, D.S., 1996. Further selection of rat lines differing in 5-HT-1A receptor sensitivity: behavioral and functional correlates. Psychiatr. Genet. 6, 107–117.

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.

Owens, M., Overstreet, D.H., Knight, D.L., Rezvani, A.H., Ritchie, J.C., Bissette, G., Janowsky, D.S., Nemeroff, C.B., 1991. Alterations in the hypothalamic–pituitary–adrenal axis in a proposed animal model of depression with genetic muscarinic supersensitivity. Neuropsychopharmacology 4, 87–94.

Papp, M., Nalepa, I., Antkiewicz-Michaluk, C., Sanchez, C., 2002. Behavioural and biochemical studies of citalopram and WAY 100635 in rat chronic mild stress model. Pharmacol. Biochem. Behav. 72, 465–474.

Perna, G., Bertani, A., Caldirola, D., Smeraldi, E., Bellodi, L., 2001. A comparison of citalopram and paroxetine in the treatment of panic disorder: a randomized, single-blind study. Pharmacopsychiatry 34, 85–90.

Pollock, B.G., 2001. Citalopram: a comprehensive review. Expert Opin. Pharmacother. 2, 681–698.

Porsolt, R.D., Anton, G., Blavet, N., Jalfre, M., 1977. Behavioral despair in rats: a new model sensitive to antidepressant treatment. Eur. J. Pharmacol. 47, 379–391.

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.

Ramos, A., Berton, O., Mormede, P., Chaouloff, F., 1997. A multiple-test study of anxiety-related behaviours in six inbred rat strains. Behav. Brain Res. 85, 57–69.

Sanchez, C., Bergqvist, P.B.F., Brennum, L.T., Gupta, S., Hogg, S., Larsen, S., Wilborg, O., 2003. Escitalopram. The *S*-(+)-enantiomer of citalopram, is a selective serotonin reuptake inhibitor with potent effects in animal models predictive of antidepressant and anxiolytic activities. Psychopharmacology 167, 353–362.

Sasson, Y., Iancu, I., Fax, M., Traub, M., Dannon, P.N., Zohar, J., 1999. A double-blind crossover comparison of clomipramine and desipramine in the treatment of panic disorder. Eur. Neuropsychopharmacol. 9, 191–196.

Schiller, G.D., Daws, L.C., Overstreet, D.H., Orbach, J., 1991. Absence of anxiety in an animal model of depression with cholinergic supersensitivity. Brain Res. Bull. 26, 443–447.

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.,J.C., J.C., Gillin, J.C., 1988. Increased REM sleep in rats selectively bred for cholinergic hyperactivity. Neuropsychopharmacology 1, 127–133.

Varia, I., Rauscher, F., 2002. Treatment of generalized anxiety disorder with citalopram. Int. Clin. Psychopharmacol. 17, 103–107.

Varia, I.M., Cloutier, C.A., Doraiswamy, P.M., 2002. Treatment of social anxiety disorder with citalopram. Prog. Neuro-psychopharmacol. Biol. Psychiatry 26, 205–208.

Willner, P., 1997. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology 134, 129–139.

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.

Zangen, A., Overstreet, D.H., Yadid, Y., 1999. Increased catecholamine levels in specific brain regions of a rat model of depression: normalization by chronic antidepressant treatment. Brain Res. 824, 243–250.

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.