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Antidepressant-like activity of corticotropin-releasing factor type-1 receptor antagonists in mice

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Abstract

The development of selective corticotropin-releasing factor type-1 (CRF_1) receptor antagonists represents a potential novel treatment for depression. These studies evaluated CRF_1 receptor antagonists for antidepressant-like activity in mice. Subchronic dosing of both R 121919 (3-[6-(dimethylamino)-4-methyl-pyrid-3-yl]-2,5-dimethyl-*N*,*N*-dipropyl-pyrazolo[2,3-*a*]pyrimidin-7-amine) and DMP 696 (4-(1,3-dimethoxyprop-2-ylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)-pyrazolo[1,5-*a*]-1,3,5-triazine) significantly decreased immobility time in the tail suspension test (at 30 and at 3 and 10 mg/kg, i.p., respectively). These antidepressant-like effects were observed at doses that did not impair general locomotor activity. Neither antalarmin (*N*-butyl-*N*-ethyl-[2,5,6-trimethyl-7-(2,4,6)trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]amine) nor DMP 904 (4-(3-pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)-pyrazolo-[1,5-*a*]-pyrimidine) had an effect indicative of antidepressant-like activity. These results suggest that the tail suspension assay may have utility to identify CRF_1 receptor antagonists with antidepressant-like activity. Moreover, the results lend support to the theory that some nonpeptidic CRF_1 receptor antagonists may possess antidepressant-like activity and therefore represent a promising novel pharmacotherapeutic strategy in the treatment of depression.

Keywords: Corticotropin-releasing factor (CRF); Depression; Tail suspension test; Forced swim test; R 121919; DMP 696; DMP 904; Antalarmin; (Mouse)

1. Introduction

Depression is often thought of as a stress-related disorder as initial signs of depression often occur following some form of stressful life event. However, the majority of people who experience a stressful event do not become clinically depressed, whereas people who do become depressed do so after what may ultimately be considered a relatively mild stressor. Hypersensitivity in the hypothalamic–pituitary–adrenal system, the central regulator of the stress response, is now considered to be key in the pathogenesis of depression. In response to a stressor, the paraventricular nucleus of the hypothalamus releases corticotropin-releasing factor (CRF), which stimulates the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, which in turn stimulates the release of glucocorticoids (primarily cortisol in humans and corticosterone in rodents) from the adrenal gland. In healthy controls, glucocorticoids provide negative feedback to decrease the synthesis and release of CRF, ACTH, and eventually glucocorticoids themselves. However, when given a synthetic glucocorticoid (dexamethasone), many depressed people fail to suppress plasma ACTH and cortisol levels, suggesting an abnormality in the normal hypothalamic–pituitary–adrenal negative feedback loop (Galard et al., 2002). People with depression also exhibit elevated basal levels of both cortisol (Catalan et al., 1998; Galard et al., 2002) and

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CRF (Catalan et al., 1998; Galard et al., 2002; Nemeroff et al., 1984). Furthermore, following a CRF challenge, people with depression exhibit decreased ACTH, but normal cortisol responses (Gold et al., 1986; Holsboer et al., 1986; Holsboer, 1999) supporting the hypothesis that CRF hypersecretion causes down-regulation and desensitization of pituitary CRF receptors. Overall, these data suggest that dysregulated central CRF systems play an essential role in the etiology of depression. Indeed, decreased CRF binding was observed in the frontal cortex of suicide victims known to have been depressed (Nemeroff et al., 1988). Other postmortem studies have noted increased levels of CRF positive cells and CRF mitochondrial ribonucleic acid in the paraventricular nucleus of the hypothalamus in individuals known to have been depressed (Raadsheer et al., 1994). Furthermore, treatment of depression with various antidepressants (De Bellis et al., 1993) or electroconvulsive shock therapy (Kling et al., 1994; Nemeroff et al., 1991) reduced cerebrospinal fluid CRF levels, suggesting that a dysregulated CRF level is normalized by successful antidepressant treatment. However, the most direct evidence supporting the involvement of CRF in depression comes from an open-label clinical trial demonstrating that a nonpeptide corticotropin-releasing factor type-1 (CRF_1) receptor antagonist, R 121919 (3-[6-(dimethylamino)-4-methyl-pyrid-3-yl]-2,5-dimethyl-*N*,*N*-dipropyl-pyrazolo[2,3-*a*]pyrimidin-7-amine), reduced measures of anxiety and depression in patients with major depression, which then relapsed when drug administration was discontinued (Zobel et al., 2000).

Studies in animal models also support a role of CRF in depression and anxiety. Central administration of CRF has been shown to increase behavioral measures indicative of depression (Dunn and Berridge, 1990; Hammack et al., 2002; Owens and Nemeroff, 1991) and anxiety (Britton et al., 1982; Dunn and Berridge, 1990; Dunn and File, 1987; Koob et al., 1994; Momose et al., 1999; Sutton et al., 1982; Swerdlow et al., 1986), whereas central administration of CRF receptor antagonists (Berridge and Dunn, 1987; Heinrichs et al., 1992; Korte et al., 1994; Menzaghi et al., 1994; Radulovic et al., 1999; Swerdlow et al., 1989; Takahashi et al., 1989, 2001) decrease anxiety-like behaviors. Similarly, genetically engineered mice that overproduce CRF exhibit behaviors indicative of increased anxiety and depression (Stenzel-Poore et al., 1994; van Gaalen et al., 2002). Furthermore, analogous to people with depression, transgenic mice that overexpress CRF postnatally also exhibit increased basal plasma corticosterone, dexamethasone nonsuppression, and hypertrophy of the adrenal glands (Groenink et al., 2002).

Thus far, two major CRF receptor subtypes have been identified: CRF_1 and CRF_2. Most studies suggest that increased CRF_1 receptor function is primarily involved in anxiety and depression. In animal models, central administration of antisense oligonucleotides directed against CRF_1 but not CRF_2 receptors decreases anxiety-like behaviors (Heinrichs et al., 1997; Liebsch et al., 1995, 1999; Skutella et al., 1998; Takahashi et al., 2001). Furthermore, CRF_1-receptor-deficient mice exhibit less anxiety-like behavior (Contarino et al., 1999; Smith et al., 1998; Timpl et al., 1998), whereas CRF_2-receptor-deficient mice exhibit no behavioral differences (Coste et al., 2000) or increased behavioral measures indicative of anxiety (Bale et al., 2000; Kishimoto et al., 2000) and depression (Bale and Vale, 2003).

Recently, nonpeptide CRF_1 receptor antagonists have been synthesized and shown to have anxiolytic-like properties in various animal models (Gilligan et al., 2000; Griebel et al., 1998, 2002; Habib et al., 2000; He et al., 2000; Keck et al., 2001; Lundkvist et al., 1996; Maciag et al., 2002; Millan et al., 2001; Zorrilla et al., 2002). However, relatively few nonpeptide CRF_1 receptor antagonists have been tested in animal models used to screen for antidepressant-like activity. Since, as stated above, alterations in CRF function are suggested to contribute to the pathogenesis of depression, this study sought to evaluate the effectiveness of several CRF_1 receptor antagonists in animal models commonly used to screen for antidepressant-like efficacy. The forced swim and tail suspension tests are well-established paradigms that have been shown to have utility as screens to identify potential clinically active antidepressants (Borsini and Meli, 1988; Bourin et al., 2001; Porsolt et al., 1977; Porsolt, 2000; Steru et al., 1985) and were used in the present studies to assess the potential efficacy of the CRF_1 receptor antagonists DMP 904 (4-(3-pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)-pyrazolo-[1,5-*a*]-pyrimidine), DMP 696 (4-(1,3-dimethoxyprop-2-ylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)-pyrazolo[1,5-*a*]-1,3,5-triazine), R 121919, and antalarmin (*N*-butyl-*N*-ethyl-[2,5,6-trimethyl-7-(2,4,6)trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]amine).

2. Materials and methods

2.1. Subjects

Naive male CF-1 mice (18–20 g, Harlan) were group-housed (either 5–6 mice in small plastic shoebox cages or 9–11 in large plastic cages), at room temperature, with a 12:12 h light/dark cycle (lights on 06:00 h) with ad libitum access to food and water. Animals were housed in a vivarium for at least 1 week prior to use. Separate cohorts of mice were utilized in each test, and each mouse was tested only once (n=8–11 mice per dose group). All mice in a given cohort came from similar housing conditions, either from small or large cages. For all tests, mice were randomly assigned to the dose groups and were dosed with compounds either acutely (30 min prior to testing for all

compounds except DMP 696 which was administered 60 min prior to testing) or subchronically (once in the morning and once in the evening the day before and once 30 or 60 min prior to testing). All compounds were dissolved or suspended in 2.5% methylcellulose and administered intraperitoneally (i.p.) at a volume of 10 ml/kg body weight, and drug doses were calculated as free base equivalents. All studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act and guidelines established by the Pfizer Animal Care and Use Committee, at Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facilities.

2.2. Tail suspension test

The procedure used was similar to that described by Steru et al. (1985). Mice were suspended by their tail using adhesive tape placed approximately 1 cm from the tip of the tail attached to a wood applicator stick and hung approximately 30 cm above a table. The duration of immobility was scored manually during the last 4 min of a 6-min test. Mice were considered immobile only when they hung passively. Comparisons of the mean duration of immobility (in s) were performed using one-way analysis of variance (ANOVA) followed by Dunnett's test. All results are expressed as mean±S.E.M.

2.3. Forced swim test

Mice were placed into an 800-ml glass beaker containing 450–500 ml of room temperature water (approximately 22 °C). The water was changed between subjects. The duration of immobility was manually scored during the last 4 min of a 6-min test. Mice were considered immobile when floating and only making movements necessary to keep their head above water. Comparisons of the mean duration of immobility (in s) were performed using one-way ANOVA followed by

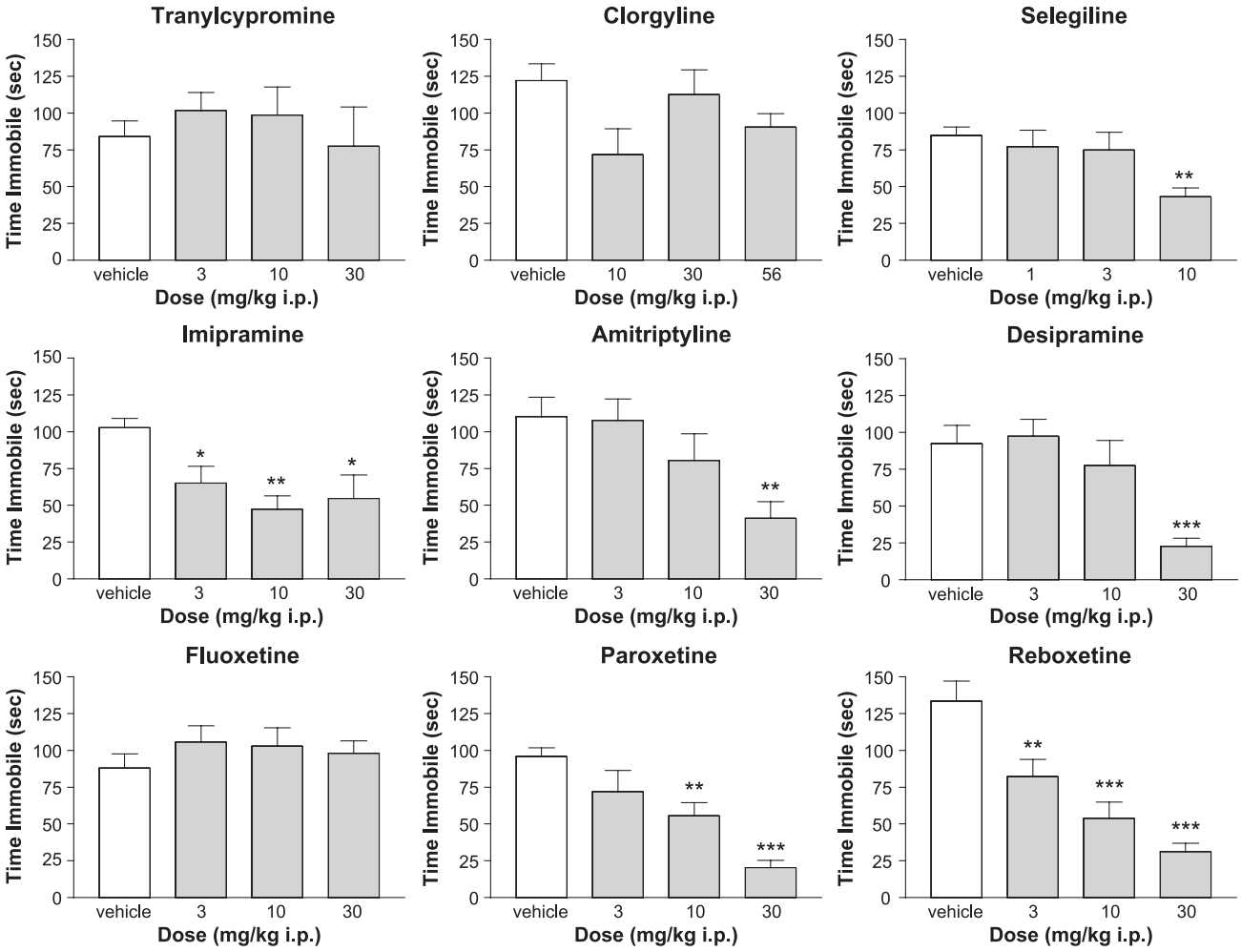

Fig. 1. Tail suspension test: known antidepressants, acute dosing. Male CF-1 mice were acutely treated with an antidepressant 30 min prior to testing. Top row: monoamine oxidase inhibitors. Middle row: tricyclic antidepressants. Bottom row: selective serotonin or norepinephrine reuptake inhibitors. Data are expressed as mean±S.E.M. for n=9–10 mice per treatment (*P<0.05, **P<0.01, ***P<0.001; one-way ANOVA, Dunnett post hoc).

Dunnett's test. All results are expressed as mean±S.E.M. An experimenter blinded to treatment groups conducted scoring for all of the tail suspension and forced swim tests.

2.4. Locomotor activity

Mice were placed in an open-field chamber (Med Associates, St. Albans, VT) made of clear Plexiglas equipped with infrared beams and sensors located in sound-attenuating boxes. Horizontal locomotor activity was recorded for 10 min. Comparisons of the mean horizontal locomotor counts were performed using one-way ANOVA followed by Dunnett's test.

3. Results

3.1. Tail suspension test: acute dosing

The tail suspension test was validated with different classes of antidepressants (Fig. 1). Acute treatment with tranylcypromine (a monoamine oxidase mixed A/B inhibitor) did not significantly change immobility time in the tail suspension test ($F_{3,36}$=0.40, P=0.76). There was a non-significant trend for an acute treatment of clorgyline (a monoamine oxidase A inhibitor) to decrease immobility time in the tail suspension test ($F_{3,36}$=2.53, P=0.07). Acute treatment with selegiline (a monoamine oxidase B inhibitor) significantly decreased immobility time in the tail suspension test ($F_{3,36}$=4.00, P<0.01) specifically at 10 mg/kg (P<0.01). Acute treatment with the tricyclic antidepressants imipramine, amitriptyline, and desipramine significantly affected immobility time ($F_{3,36}$=4.13, P<0.01; $F_{3,36}$=4.92, P<0.01; $F_{3,35}$=7.13, P<0.001; respectively). Specifically imipramine at 3, 10, and 30 mg/kg, amitriptyline at 30 mg/kg, and desipramine at 30 mg/kg significantly decreased immobility time in the tail suspension test compared to vehicle-treated mice. Acute treatments of both paroxetine (a selective serotonin reuptake inhibitor) and reboxetine (racemate R,R+S,S, a selective norepinephrine reuptake inhibitor) significantly reduced immobility in the tail suspension test ($F_{3,36}$=11.75, P<0.001; $F_{3,35}$=16.88, P<0.001; respectively). Specifically, paroxetine at 10 and 30 mg/kg and reboxetine at 3, 10, and 30 mg/kg significantly decreased immobility time in the tail suspension test. An acute treatment with fluoxetine did not significantly change immobility time in the tail suspension test ($F_{3,36}$=0.55, P=0.65).

The effects of acute treatments with CRF_1 receptor antagonists on immobility time in the tail suspension test are illustrated in Fig. 2. Immobility time in the tail suspension test was not significantly affected by acute administration of 3, 10, or 30 mg/kg of antalarmin ($F_{3,35}$=1.12, P=0.36), DMP 904 ($F_{3,36}$=0.38, P=0.77), or DMP 696 ($F_{3,35}$=0.85, P=0.48). There was a nonsignificant trend for R 121919 to acutely decrease immobility time ($F_{3,35}$=2.18, P=0.11).

3.2. Tail suspension test: subchronic dosing

Since antidepressant-like effects with selective serotonin or norepinephrine reuptake inhibitors are most often observed after multiple doses, a subchronic dosing regime was also evaluated in the tail suspension test. Following

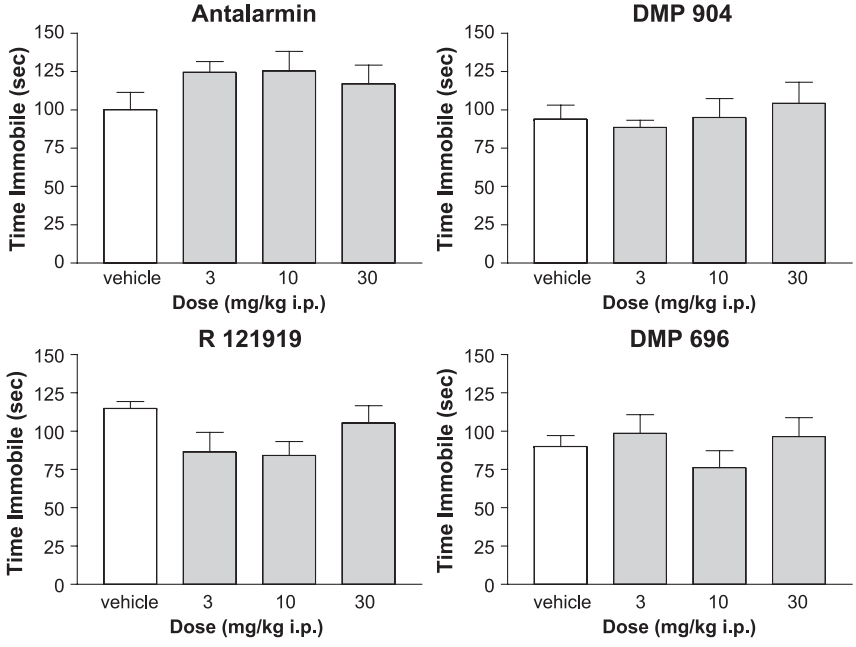

Fig. 2. Tail suspension test: CRF_1 receptor antagonists, acute dosing. Male CF-1 mice were acutely treated with a CRF_1 receptor antagonist 30 min prior to testing (60 min for DMP 696). Data are expressed as mean±S.E.M. for n=9–10 mice per treatment.

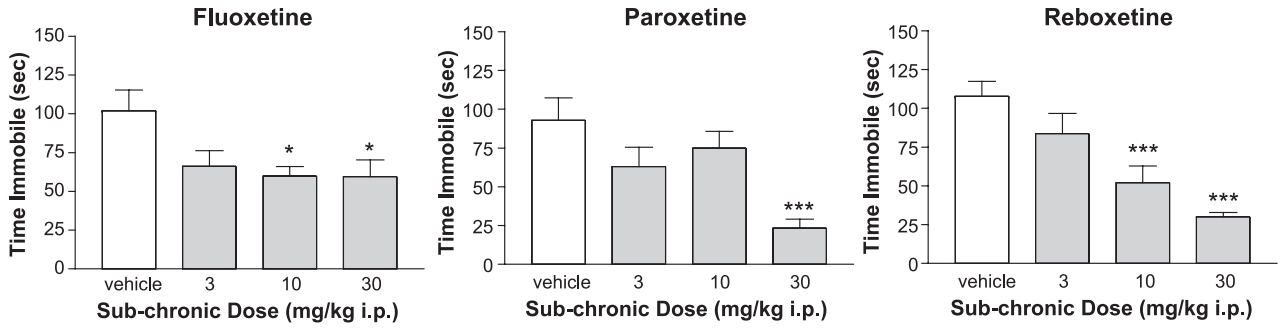

Fig. 3. Tail suspension test: known antidepressants, subchronic dosing. Male CF-1 mice were subchronically treated (once in the morning and in the evening the day before and once 30 min prior to testing) with selective serotonin or norepinephrine reuptake inhibitors. Data are expressed as mean±S.E.M. for n=8–11 mice per treatment (*P<0.05, ***P<0.001; one-way ANOVA, Dunnett post hoc).

subchronic dosing, significant effects were seen with fluoxetine, paroxetine, and reboxetine in the tail suspension test ($F_{3,36}$=3.51, P<0.03; $F_{3,42}$=6.73, P<0.001; $F_{3,38}$=11.60, P<0.001, respectively; Fig. 3). Specifically, immobility time was significantly reduced by subchronic fluoxetine at 10 and 30 mg/kg, paroxetine at 30 mg/kg, and reboxetine at 10 and 30 mg/kg. Subchronic treatment with R 121919 and DMP 696 significantly reduced immobility in the tail suspension test ($F_{3,39}$=4.434, P<0.01; $F_{3,37}$=5.40, P<0.003; respectively, Fig. 4). Specifically, subchronic R 121919 at 30 mg/kg and subchronic DMP 696 at 3 and 10 mg/kg significantly reduced immobility in the tail suspension test. Neither subchronic antalarmin ($F_{3,36}$=0.704, P=0.56) nor subchronic DMP 904 ($F_{3,35}$=1.655, P=0.19) significantly changed immobility time in the tail suspension test.

3.3. Forced swim test: acute dosing

The forced swim test was validated with different classes of antidepressants (Fig. 5). Acute treatment with tranylcypromine (a monoamine oxidase mixed A/B inhibitor), clorgyline (a monoamine oxidase A inhibitor), and selegiline (a monoamine oxidase B inhibitor) significantly changed immobility time in the forced swim test ($F_{3,34}$=6.53, P<0.001; $F_{3,36}$=3.17, P<0.04; $F_{3,36}$=3.08, P<0.04, respectively). Specifically, tranylcypromine at 10 and 30 mg/kg, clorgyline at 56 mg/kg, and selegiline at 10 mg/kg significantly decreased immobility time in the forced swim test. There was a nonsignificant trend for an acute treatment of amitriptyline to change immobility in the forced swim test ($F_{3,34}$=2.42, P=0.08). Acute treatment with the tricyclic

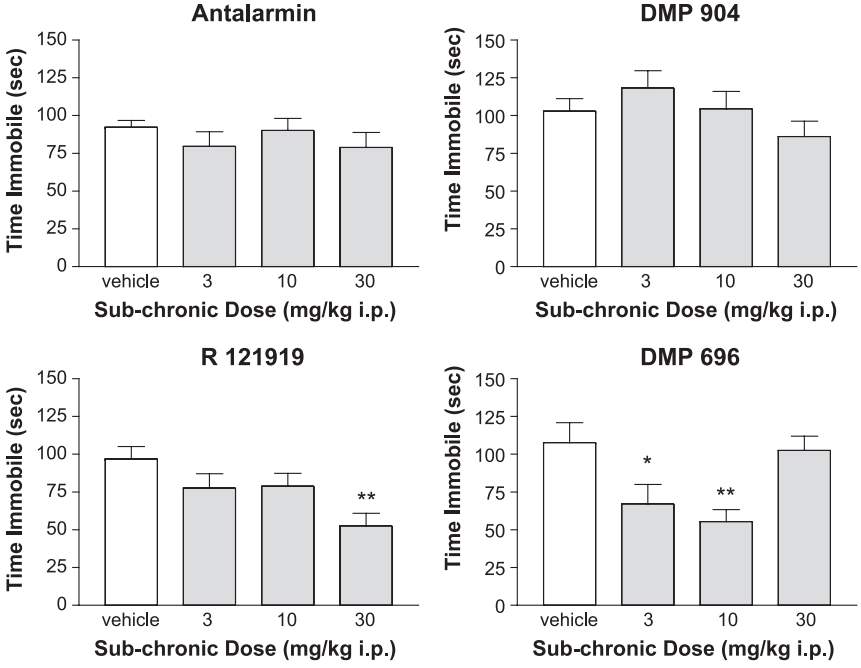

Fig. 4. Tail suspension test: CRF_1 receptor antagonists, subchronic dosing. Male CF-1 mice were subchronically treated (once in the morning and in the evening the day before and once 30 min prior to testing; 60 min for DMP 696) with a CRF_1 receptor antagonist. Data are expressed as mean±S.E.M. for n=8–11 mice per treatment (*P<0.05, **P<0.01; one-way ANOVA, Dunnett post hoc).

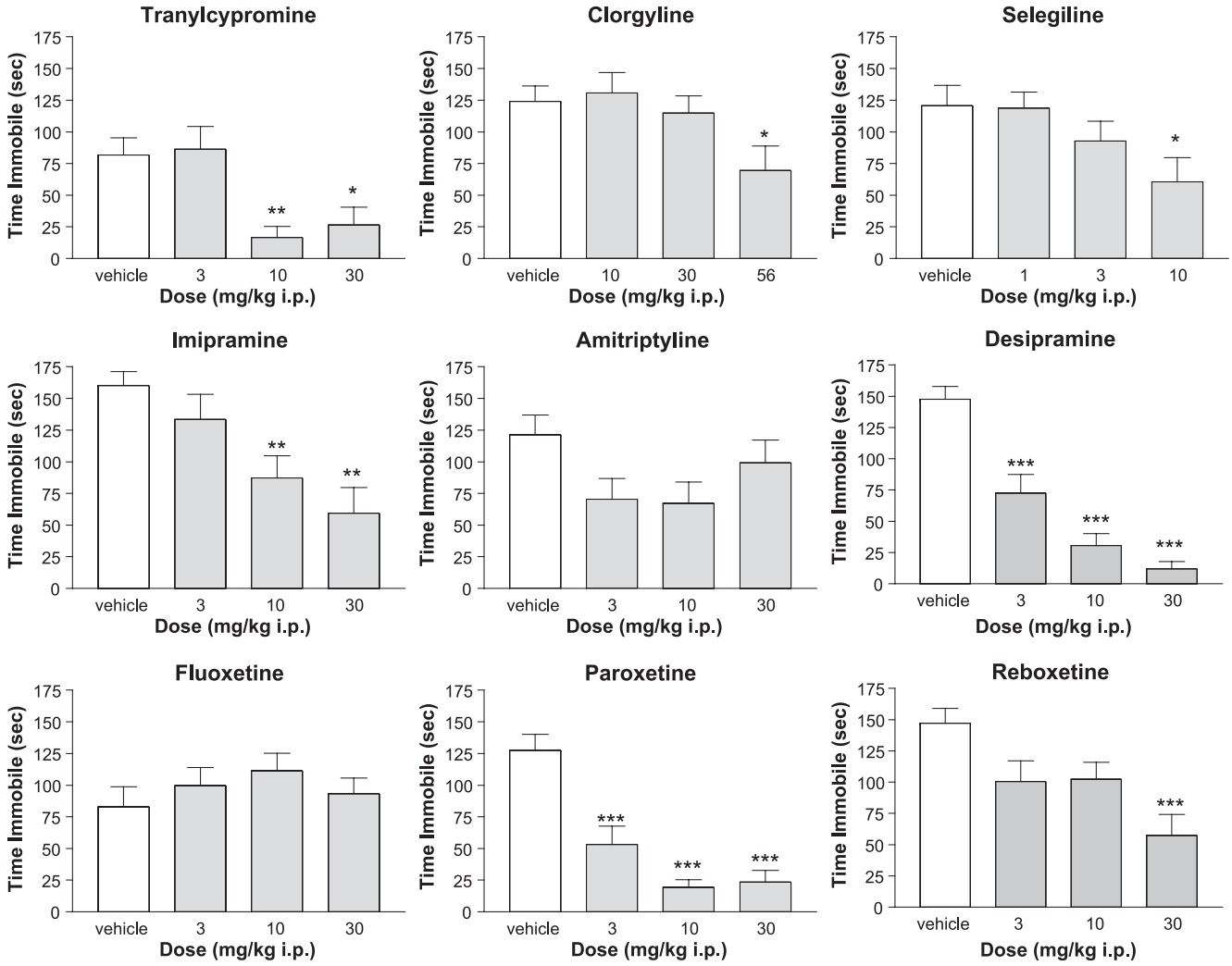

Fig. 5. Forced swim test: known antidepressants, acute dosing. Male CF-1 mice were acutely treated with an antidepressant 30 min prior to testing. Top row: monoamine oxidase inhibitors. Middle row: tricyclic antidepressants. Bottom row: selective serotonin or norepinephrine reuptake inhibitors. Data are expressed as mean±S.E.M. for n=9–11 mice per treatment (*P<0.05, **P<0.01, ***P<0.001; one-way ANOVA, Dunnett post hoc).

antidepressants imipramine and desipramine significantly affected immobility time ($F_{3,34}$=6.83, P<0.001; $F_{3,38}$=29.47, P<0.001, respectively). Specifically, imipramine at 10 and 30 mg/kg and desipramine at 3, 10, and 30 mg/kg significantly decreased immobility time in the forced swim test. Acute treatments with the selective serotonin reuptake inhibitor paroxetine, and the selective norepinephrine reuptake inhibitor reboxetine significantly affected immobility time ($F_{3,36}$=19.99, P<0.001; $F_{3,36}$=6.12, P<0.002; respectively). Specifically, paroxetine at 3, 10, and 30 mg/kg and reboxetine at 30 mg/kg significantly reduced immobility time in the forced swim test. An acute treatment with the selective serotonin reuptake inhibitor fluoxetine did not significantly change immobility time in the forced swim test ($F_{3,36}$=0.72, P=0.55).

Acute treatment with the CRF_1 receptor antagonists antalarmin, DMP 904, R 121919, or DMP 696 did not significantly change immobility time in the forced swim test ($F_{3,36}$=0.31, P=0.82; $F_{3,35}$=2.29, P=0.10; $F_{3,36}$=2.27, P=0.10; $F_{3,34}$=2.12, P=0.10; respectively; Fig. 6).

3.4. Forced swim test: subchronic dosing

With subchronic dosing, significant effects were observed with paroxetine and reboxetine in the forced swim test ($F_{3,36}$=5.57, P<0.01; $F_{3,34}$=21.10, P<0.001; respectively, Fig. 7). Specifically, subchronic reboxetine at 10 and 30 mg/kg and subchronic paroxetine at 30 mg/kg significantly decreased immobility in the forced swim test. Subchronic doses of fluoxetine (3, 10, or 30 mg/kg) did not significantly change immobility in the forced swim test ($F_{3,37}$=0.341, P=0.80). Subchronic dosing with the CRF_1 receptor antagonists DMP 904 and DMP 696 did not significantly affect immobility time in the forced swim test ($F_{3,34}$=1.60, P=0.21; $F_{3,36}$=1.71, P=0.18, respectively; Fig. 8). There was a trend for subchronic dosing of R 121919 or antalarmin to effect immobility in the forced

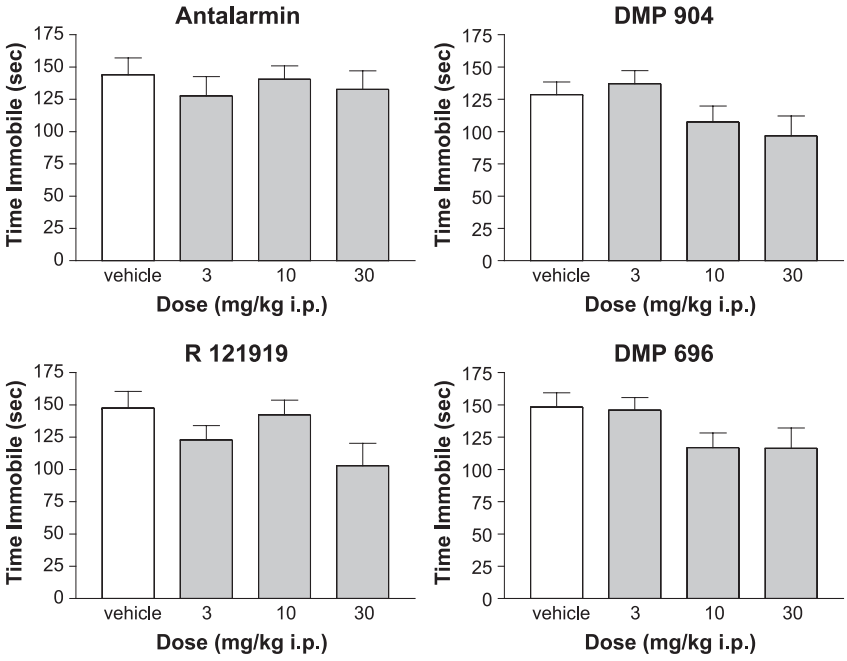

Fig. 6. Forced swim test: CRF_1 receptor antagonists, acute dosing. Male CF-1 mice were acutely treated with a CRF_1 receptor antagonist 30 min prior to testing (60 min for DMP 696). Data are expressed as mean±S.E.M. for n=8–11 mice per treatment.

swim test ($F_{3,30}$=2.49, P=0.08; $F_{3,35}$=2.53, P=0.07; respectively).

3.5. Locomotor activity: acute dosing

Acute treatment with clorgyline, imipramine, amitriptyline, paroxetine, and reboxetine significantly affected horizontal locomotor activity (Table 1, $F_{3,39}$=8.66, P<0.001; $F_{3,36}$=4.27, P<0.01; $F_{3,36}$=11.53, P<0.001; $F_{3,36}$=12.22, P<0.001; $F_{3,36}$=7.35, P<0.001; respectively). Clorgyline significantly decreased locomotor activity at 56 mg/kg (P<0.01) but not at 10 or 30 mg/kg. Imipramine and amitriptyline both significantly decreased locomotor activity at 30 mg/kg (P<0.01 and P<0.001, respectively) but not at 3 or 10 mg/kg. Paroxetine significantly increased horizontal locomotor activity at both 3 and 10 mg/kg (P<0.01 and P<0.05, respectively). Reboxetine significantly decreased horizontal locomotor activity at both 3 and 30 mg/kg (P<0.05 and P<0.001, respectively).

Acute administration of R 121919, DMP 696, DMP 904, or antalarmin did not significantly affect locomotor activity at any of the doses tested (3, 10, and 30 mg/kg i.p. Table 1).

3.6. Locomotor activity: subchronic dosing

Subchronic administration of paroxetine resulted in significant changes in horizontal locomotor activity (Table 1, $F_{3,35}$=35.75, P<0.001). Specifically, subchronic paroxetine resulted in significantly decreased horizontal locomotor activity at 30 mg/kg (P<0.001), however, at 3 mg/kg, significantly increased activity (P<0.01). Subchronic

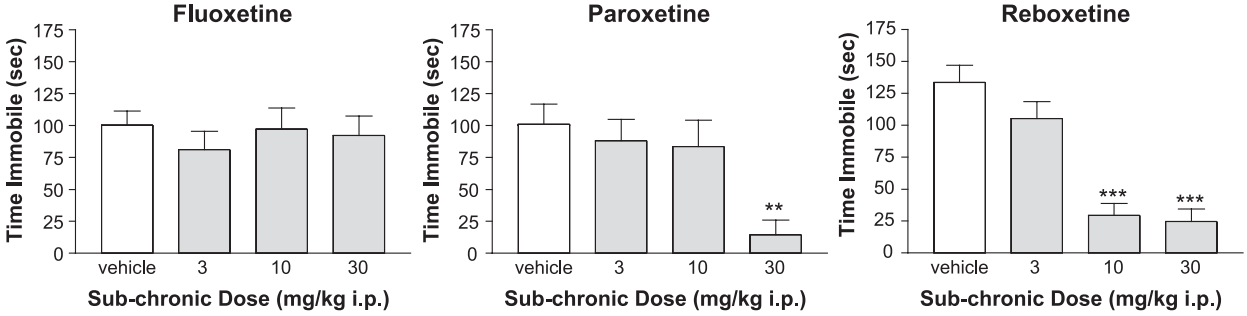

Fig. 7. Forced swim test: known antidepressants, subchronic dosing. Male CF-1 mice were subchronically treated (once in the morning and in the evening the day before and once 30 min prior to testing) with selective serotonin or norepinephrine reuptake inhibitors. Data are expressed as mean±S.E.M. for n=9–10 mice per treatment (**P<0.01, ***P<0.001; one-way ANOVA, Dunnett post hoc).

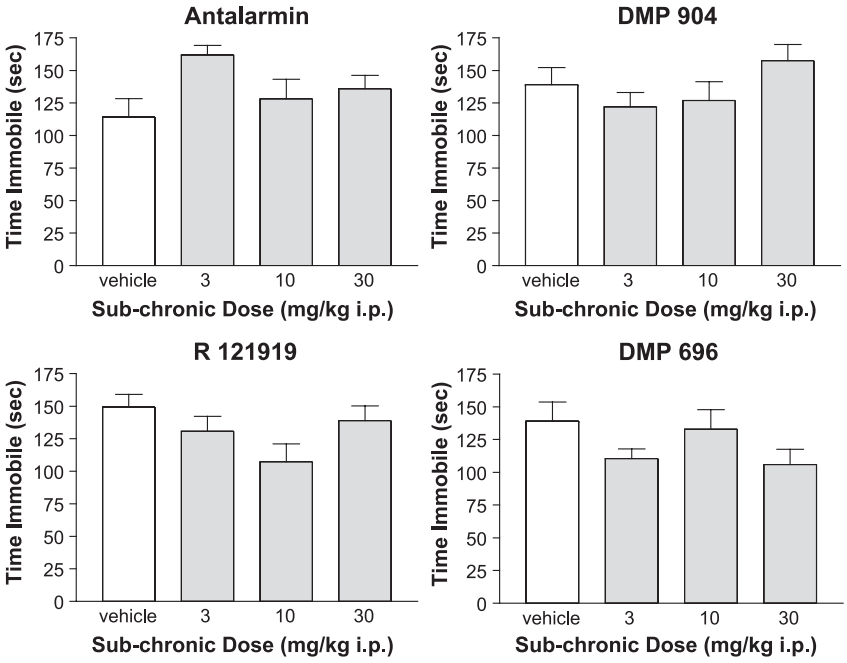

Fig. 8. Forced swim test: CRF_1 receptor antagonists, subchronic dosing. Male CF-1 mice were subchronically treated (once in the morning and in the evening the day before and once 30 min prior to testing; 60 min for DMP 696) with a CRF_1 receptor antagonist. Data are expressed as mean±S.E.M. for n=8–10 mice per treatment.

administration of R 121919, DMP 696, DMP 904, or antalarmin did not significantly affect locomotor activity at any of the doses tested (3, 10, and 30 mg/kg i.p., Table 1).

4. Discussion

The present study demonstrated that the forced swim and tail suspension tests, two animal assays predictive of antidepressant activity, can display similar sensitivity with various known antidepressant compounds. Additionally, the effect of four CRF_1 receptor antagonists was investigated in these tests with two demonstrating antidepressant-like activity.

Since differences in activity may confound the interpretation of the results in the forced swim and tail suspension tests, the effects of the various compounds on locomotor activity were assessed. The compounds that exhibited sedative effects in locomotor activity nevertheless significantly decreased immobility with at least one dose in one of or both the forced swim and tail suspension assays. However, the decrease in activity seen with amitriptyline at 30 mg/kg may have masked an antidepressant-like effect in the forced swim test at this dose, although this dose of amitriptyline exhibited a significant decrease in immobility in the tail suspension test. Overall, the changes observed in activity were not likely to confound the results seen in the forced swim and tail suspension tests.

The current results from the forced swim and tail suspension tests were relatively consistent and are analogous to previous reports of intrastrain drug-induced differences observed between these two tests (Bai et al., 2001; David et al., 2001). In the present study, known clinically active antidepressants exhibited similar effects with acute dosing in the forced swim and tail suspension tests in that at least one dose was active in either one or, frequently, in both tests. The exception to this was fluoxetine, which was inactive in both tests following acute treatment. It has been suggested that the antidepressant-like activity of the selective serotonin reuptake inhibitors is more consistently observed with the tail suspension test than with the forced swim test (Borsini, 1995; Detke et al., 1995; Lucki et al., 2001; Perrault et al., 1992). However, in the current study, an acute treatment of fluoxetine was inactive in the tail suspension test and this may have been influenced by the mouse strain utilized. Numerous studies suggest that the sensitivity for detecting antidepressants of various pharmacological mechanisms may be influenced by the background mouse strain used in the tail suspension test (van der Heyden et al., 1987; Vaugeois et al., 1997) as well as in the forced swim test (David et al., 2003; Liu and Gershenfeld, 2001; Lucki et al., 2001; Porsolt et al., 1978). Indeed, there are other reports of the ineffectiveness of an acute treatment of fluoxetine in the tail suspension test with mice of the same strain as the present studies (Wong et al., 2000) as well as other strains (Naudon et al., 2002; Takeuchi et al., 1997), although Naudon et al. (2002) demonstrated that while fluoxetine was ineffective after an acute treatment in the tail suspension test, efficacy was seen after chronic treatment. Similarly, in the present studies, after subchronic dosing, fluoxetine exhibited antidepressant-like activity in the tail suspension test. Thus, the sensitivity of screens such as the

Table 1
Horizontal locomotor activity counts

Compound	Dose (mg/kg)	Acute dose	Subchronic dosing
Clorgyline	0	1083±123	
	10	815±107	
	30	796±101	
	56	303±110***	
Imipramine	0	1052±114	
	3	884±101	
	10	993±138	
	30	535±89**	
Amitriptyline	0	1220±149	
	3	1176±117	
	10	847±174	
	30	192±110***	
Paroxetine	0	1032±95	1058±68
	3	1762±213**	1417±64***
	10	1624±175*	1297±98
	30	549±133	414±50***
Reboxetine	0	1030±124	
(racemate R,R+S,S)	3	672±84*	
	10	809±91	
	30	413±74***	
Antalarmin	0	1015±109	1210±110
	3	924±100	1236±130
	10	1007±103	1332±127
	30	896±108	927±104
DMP 904	0	862±118	1128±90
	3	1006±99	980±42
	10	1200±143	1140±117
	30	928±118	976±78
DMP 696	0	1118±117	1148±64
	3	962±82	1291±112
	10	1264±119	1024±153
	30	1207±108	940±133
R 121919	0	736±43	1106±146
	3	805±75	1077±73
	10	709±77	1252±70
	30	863±91	1040±108

Data are shown for known antidepressants that significantly changed locomotor activity and for all CRF_1 receptor antagonists tested. Compounds were administered i.p. to male CF-1 mice either acutely (30 min prior to testing; 60 min for DMP 696) or subchronically (in the morning and in the evening the day before and once 30 min prior to testing; 60 min for DMP 696). Data are expressed as mean±S.E.M. for n=9–10 mice per treatment.
* $P<0.05$ vehicle-treated mice (one-way ANOVA with Dunnett post hoc).
** $P<0.01$ vs. vehicle-treated mice (one-way ANOVA with Dunnett post hoc).
*** $P<0.001$ vs. vehicle-treated mice (one-way ANOVA with Dunnett post hoc).

tail suspension and forced swim tests may be enhanced by using chronic or subchronic dosing, consequently increasing the possibility of detecting efficacy that may not be evident acutely with some compounds.

In the present studies, four CRF_1 receptor antagonists were evaluated in both the forced swim and tail suspension tests after both acute and subchronic dosing. Neither antalarmin nor DMP 904 had a significant effect indicative of antidepressant activity. However, both R 121919 and DMP 696 significantly decreased immobility time, suggesting possible clinical efficacy in the treatment of depression.

The antidepressant-like effects of R 121919 and DMP 696, seen only after subchronic dosing and only in the tail suspension test, were observed at doses that did not impair motor activity. We are not aware of any previous reports of antidepressant-like activity with R 121919 in animals. Nevertheless, R 121919 did reduce symptoms of major depression in an open-label clinical study (Zobel et al., 2000). The present results demonstrate that certain selective CRF_1 receptor antagonists display efficacy in an animal model used to screen compounds for antidepressant-like activity.

This study is among the first to report of antidepressant-like activity with DMP 696. Previous reports demonstrate anxiolytic-like activity with DMP 696 (He et al., 2000; Maciag et al., 2002) and suggest that DMP 696 is most effective in rodents that are hyperresponsive to stress and have increased basal CRF levels (Maciag et al., 2002). Similarly, R 121919 has been shown to reduce anxiety only in rats bred for high levels of anxiety (Keck et al., 2001) or previously stressed rats (Heinrichs et al., 2002). However, R 121919 has been shown to decrease CRF binding equivalently in both high- and low-anxiety rats (Keck et al., 2001). These studies support the idea that the anxiolytic-like activity of CRF_1 receptor antagonists, such as DMP 696 and R 121919, is best observed in animals in which the hypothalamic–pituitary–adrenal axis has been activated either by a stressor or in animals that are more susceptible to the effects of stress (Griebel et al., 2002; Maciag et al., 2002; Menzaghi et al., 1994; Okuyama et al., 1999; Zorrilla et al., 2002). Thus, DMP 696 and R 121919 appear to have anxiolytic- and antidepressant-like efficacy in animal models.

While DMP 904 has been shown to exhibit anxiolytic-like properties (Gilligan et al., 2000), we are not aware of any previous reports of DMP 904 in assays predictive of antidepressant activity. In the present studies, DMP 904 did not exhibit any signs of antidepressant-like efficacy. Likewise, antalarmin did not exhibit antidepressant-like activity. The inactivity of antalarmin in the current studies was not surprising since it was recently demonstrated that CP-154,526 (butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]-amine), an unmethylated form of antalarmin, lacked antidepressant-like effects in the tail suspension test (Yamano et al., 2000). However, antidepressant-like activity has been shown with CP-154,526 in learned helplessness assays (Mansbach et al., 1997; Takamori et al., 2001). Additionally, antalarmin exhibited antidepressant-like activity in the rat forced swim test (Griebel et al., 2002) and in a forced swim test in CRF_2-receptor-deficient mice (Bale and Vale, 2003). Both the learned helplessness and rat forced swim paradigms involve a pretest exposure to an inescapable stressor. In the learned helplessness paradigm, rats are exposed to a form of inescapable shock prior to the actual

test. Similarly, when rats are utilized in the forced swim paradigm, as was the case in the study by Griebel et al. (2002), there are two exposures to the water: a pretest exposure followed 24 h later by the actual test session. Interestingly, a similar preswim was utilized 24 h prior to the forced swim test to evaluate CRF_2-receptor-deficient mice in the study by Bale and Vale (2003). Analogous to the suggestion made for the efficacy of some CRF_1 receptor antagonists in certain models of anxiety, presumably some CRF_1 receptor antagonists, such as antalarmin, may only exhibit antidepressant-like efficacy in animals that are hypersensitive to stress (e.g. the CRF_2-receptor-deficient mice) or in which the hypothalamic–pituitary–adrenal axis has been primed prior to the actual test situation. In support of this hypothesis, Yamano et al. (2000) demonstrated that CP-154,526 does demonstrate antidepressant-like activity in the tail suspension test in mice but only in those in which the hypothalamic–pituitary–adrenal axis was previously activated by administration of interferon-alpha. Furthermore, it has recently been shown that compared to naive subjects, mice pre-exposed to an inescapable swim session exhibit increased immobility in the forced swim test (Alcaro et al., 2002). While the current study detected antidepressant-like effects with CRF_1 receptor antagonists in relatively nonstressed mice (except for the stress of injections), the anxiolytic and antidepressant-like efficacy of CRF_1 receptor antagonists may be best measured in animals in which the hypothalamic–pituitary–adrenal axis has been experimentally activated, although the critical level of activation has yet to be defined. In conclusion, the present results demonstrate that the tail suspension assay may have utility to identify novel CRF_1 receptor antagonists with antidepressant-like activity. Furthermore, these results provide additional support that the nonpeptidic CRF_1 receptor antagonists represent a promising novel pharmacotherapeutic strategy in the treatment of depression.

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