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Effects of the selective nonpeptide corticotropin-releasing factor receptor 1 antagonist antalarmin in the chronic mild stress model of depression in mice

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Abstract

Several recent studies on corticotropin-releasing factor (CRF) have suggested that this neuropeptide may play a role in depression. Consequently, CRF receptor antagonists have been proposed as potential new agents for the treatment of this condition. This study investigated the effects of a 4-week treatment with the well-known CRF_1 receptor antagonist, antalarmin, and the prototypical selective serotonin reuptake inhibitor (SSRI), fluoxetine, in the chronic mild stress (CMS) model in BALB/c mice. Animals were exposed to 9 weeks of CMS which rapidly (within 2 weeks) produced decrease of physical state (PS), body weight gain and blunted emotional response in the light/dark test. Chronic treatment with antalarmin (10 mg/kg ip) and fluoxetine (10 mg/kg ip) led to an improvement of CMS-induced modifications. These results suggest that CRF_1 receptor antagonists may represent potential antidepressants.

Keywords: Antalarmin; BALB/c mice; Chronic mild stress (CMS); Corticotropin-releasing factor (CRF); Fluoxetine

1. Introduction

It has been convincingly demonstrated that corticotropin-releasing factor (CRF), a 41 amino acid-containing neuropeptide first characterized by Vale et al. (1981), represents the major physiological regulator of the hypothalamic–pituitary–adrenal axis, regulating basal and stress-induced release of adrenocorticotropic hormone (ACTH) and β-endorphin (Vale et al., 1983). The effects of CRF are mediated by two specific receptors called CRF_1 and CRF_2 (Chalmers et al., 1996). Tissue distribution analysis shows that CRF_1 receptor expression is most abundant in neocortical, cerebellar and limbic structures, whereas CRF_2 receptor expression is generally localized in subcortical structures (Chalmers et al., 1995). Those anatomical data suggested that CRF may contribute significantly both to behavioral responses to stress and emotional behavior itself. In patients with severe depressive illness, several studies have reported that CRF is elevated in the cerebrospinal fluid (CSF) and that after successful antidepressant treatment, CSF levels of CRF were decreased (Kasckow et al., 2001). In addition, some depressed patients display a blunted ACTH response to intravenously administered CRF, possibly due to desensitized CRF receptors at corticotropic cells (Holsboer et al., 1984). Taken together these findings suggest that CRF may be a key contributing factor in depression, and that CRF receptor antagonists may represent a novel class of agents for the treatment of this condition (Holsboer, 1999).

A few studies in animals, using small-molecule CRF receptor antagonists with selectivity for the CRF_1 subtype, have shown that these compounds are endowed with antidepressant-like properties. Mansbach et al. (1997) reported that the pyrrolopyridinine, CP-154,526, produced positive effects in the learned helplessness model of depression. Further, CP-154,526 was found to reduce IFN-α-induced

Abbreviations: ACTH, Adrenocorticotropic hormone; CMS, Chronic mild stress; CRF, Corticotropin-releasing factor; CRF_1, Corticotropin receptor type 1; CRF_2, Corticotropin receptor type 2; CSF, Cerebrospinal fluid; PS, Physical state; SSRI, Selective serotonin reuptake inhibitor.

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depression-like behavior in the forced-swimming test in mice (Yamano et al., 2000). Antalarmin, the 6-methyl homologue of CP-154,526, has been shown to impair both the induction and expression of conditioned fear following inescapable foot shock. In addition, the drug blocked the enhancement of fear conditioning produced by prior exposure to inescapable shock (Deak et al., 1999). Furthermore, a recent study revealed that antalarmin blocked the anxiogenic-like effect of CRF in the elevated plus maze, without affecting anxiety-like behavior in vehicle-treated animals and decreased spontaneous defensive withdrawal behavior in a novel, brightly illuminated open field (Zorrilla et al., 2002). Using the olfactory bulbectomized rat paradigm, which has been proposed as an animal model of depression, Okuyama et al. (1999) showed that the CRF_1 receptor antagonists, CRA1000 and CRA1001, reversed the behavioral changes (i.e. hyperemotionality) produced by the lesion. Finally, the CRF_1 antagonist SSR125543A as well as antalarmin, produced anxiolytic-like activity in models involving inescapable stress, including the conflict procedures, the social defeat-induced anxiety paradigm and the defense test battery, antagonized stress-induced hyperthermia, distress vocalization, and produced clear antidepressant-like effects in the forced-swimming test. . Furthermore, chronic SSR125543A improved the degradation of the physical state (PS), the reduction of body weight gain and anxiety produced by chronic mild stress (CMS), a rodent model of depressive symptoms (Griebel et al., 2002a). However, CRF_1 antagonists seem to have only weak behavioral effects in situations in which stress level is low (Keck et al., 2001; Griebel et al., 2002a), which suggests that this neuropeptide system may be active only in pathological conditions, such as depression and pathological anxiety, two closely related disorders exhibiting a very elevated comorbidity. On the clinical level, the first open-label study examining the effects of the CRF_1 antagonist R121910 (formerly NBI-30775) in 20 patients with major depression was recently completed (Zobel et al., 2000). The drug was well tolerated by patients and did not significantly affect ACTH or cortisol levels at baseline or following a CRF challenge. More importantly, significant reductions in depression scores were observed following 30-day treatment with the drug.

In this context, the aim of the present study was to compare the effects of repeated treatment with the CRF_1 receptor antagonist, antalarmin and the prototypical antidepressant, fluoxetine, in the CMS procedure in mice. The CMS has been proposed as a model of depression, in that it satisfies some criteria for face, predictive and construct validity (Willner, 1984). The test used in this study is based on the procedure originally designed by Willner et al. (1992) for rats, and was recently adapted for mice by Kopp et al. (1999). Briefly, rats or mice are exposed sequentially to a variety of mild and unpredictable stressors (e.g. restraint, overnight illumination). This procedure causes the occurrence of physical and behavioral abnormalities reminiscent of certain symptoms of human depression. CMS-induced deficits may be maintained for several months; however, normal behavior is restored, during continued application of CMS, by chronic treatment with tricyclic (Willner et al., 1987) or atypical antidepressants. It is important to note that the CMS has proved effective in identifying potential novel antidepressants (Griebel et al., 2002a,b; Munoz and Papp, 1999; Papp et al., 2000; Sanchez and Papp, 2000).

2. Methods

2.1. Animals

BALB/cByJIco male mice (Iffa Credo Lyon, France), aged 9 weeks at the beginning of CMS, were kept in the experimental room a week before the onset of the experiment in order to familiarize them with the testing environment. All animals were housed individually in small cages ($8 \times 13.5 \times 8.1$ cm) with food and water ad libitum and maintained under controlled conditions (22 ± 1 °C, 12/12 h light/dark cycle with lights on at 7 a.m.). The treatment of the animals was in accordance with the European Community Council directive 86/609/EEC and with French legislation from Ministère de l'Agriculture concerning research involving animal subjects.

2.2. Drugs

Fluoxetine and antalarmin (Webster et al., 1996) compounds were synthesized by Sanofi-Synthelabo Recherche.

2.3. Chronic mild stress

This procedure is a modified version of the one used by Kopp et al. (1999) and consists of restraint, forced bath, water and/or food deprivation, pairing with another stressed animal in wet sawdust, housing in wet sawdust, reversal of the light/dark cycle, housing in constant illumination or darkness each for a period ranging from 10 min to 24 h, in a schedule that lasts for 3 weeks, and is repeated thereafter at Week 1 (Table 1). Contrary to Kopp et al. (1999) who determined the effects of stress by measuring sucrose preference and emotional blunting, we measure the effects of the stress regime recording the PS, the body weight and the emotional blunting (light/dark test). Indeed, PS has been shown to be a particularly useful parameter to assess the effects of antidepressant activity, when compared with sucrose preference that seems not to be a reliable measure of the effects of chronic stress in this specie. Stress is applied daily for 9 weeks; the first 5 weeks being drug-free. The CMS procedure is the animal model of major depression having the highest face, predictive or construct validity when compared with other models of depression (Willner and Mitchell, 2002).

Table 1
CMS procedure

	Start of the stress	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Week 1	9:00 a.m.	Confinement in a small tube[a] (1 h)	Confinement in a small tube (1 h)	Food restriction (3 h)[b]	Confinement in a small tube (1 h)	Confinement in a small tube (1 h)	Access to an empty bottle (2 h)	Inversion of the light/dark cycle
	2:00 p.m.	Forced bath in water at 32 °C (30 min)	Confinement in a small tube (1 h)	Confinement in a small tube (1 h)	Paired housing in damp sawdust (10 min)[c]	Dark (2 h)	Housing in mild damp sawdust (24 h)	
	6:00 p.m.		Water and food deprivation (15 h)			Water deprivation (15 h)		
Week 2	9:00 a.m.	Confinement in a small tube (1 h)	Confinement in a small tube (1 h)	Confinement in a small tube (1 h)	Paired housing with food restriction (10 min)	Inversion of the light/dark cycle	Inversion of the light/dark cycle	Inversion of the light/dark cycle
	2:00 p.m.	Forced bath in water at 32 °C (30 min)	Paired housing in damp sawdust (18 h)[d]	Confinement in a small tube (1 h)	Inversion of the light/dark cycle			Housing in mild damp sawdust (19 h)
	6:00 p.m.			Water and food deprivation (15 h)				
Week 3	9:00 a.m.	Confinement in a small tube (1 h)	Food restriction (3 h)	Paired housing in damp sawdust (10 min)	Forced bath in water at 32 °C (30 min)	Access to an empty bottle (2 h)		Inversion of the light/dark cycle
	2:00 p.m.	Confinement in a small tube (1 h)	Confinement in a small tube (1 h)	Confinement in a small tube (1 h)	Confinement in a small tube (1 h)	Confinement in a small tube (1 h)	Inversion of the light/dark cycle	
	6:00 p.m.	Water and food deprivation (15 h)			Water deprivation (15 h)	Housing in mild damp sawdust (20 h)		

[a] Confinement tube: diameter = 4 cm, length = 5 cm.
[b] About 50 mg of food pellets.
[c] About 200 ml of water for 100 g of sawdust.
[d] In some cases, fighting occurred during this period but it never caused injuries to the mice.

2.4. Behavioral measures

Stress-induced modifications in mice were assessed using the measure of mice' PS variations, their body weight changes and an anxiety test: the light/dark test.

Schedule of stress-induced behavioral modifications recorded is presented in Fig. 1.

2.4.1. Physical state

It was controlled weekly using a scale from 1 to 3: a health state was noted 1 and a damaged state with piloerection and/or dirty fur was noted 3. Intermediate state was noted 2. The PS was not observed after the stressors of housing in damp sawdust or forced bath in order to avoid interaction of these stressors with this index.

2.4.2. Body weight

Mice were weighted weekly from Week 0 (initial week) to Week 9 of the procedure but the measure was never recorded after the food or water deprivation-stressor of the CMS procedure.

2.4.3. Light/dark choice paradigm

The apparatus was composed of two PVC cages ($20 \times 20 \times 14$ cm). One was darkened and the other one was lit by 300 lx illumination. The two boxes were linked with an opaque plastic tunnel ($5 \times 7 \times 10$ cm). The procedure is based on the natural avoidance of mice for lit places. Mice were placed individually in the lit box. The test started when they entered in the dark box. The time spent in the lit box and horizontal activity in the lit box were recorded automatically via light beams during a 4-min period. A mouse that has entered its four paws in the new box was considered as having changed boxes. This test was performed one day after the end of the CMS procedure (at Week 10).

2.5. Procedure

Treatment started at Week 5, while the stress regime was continuing. Mice were divided into three groups ($n = 20$ in each), each subjected to a 5-week period of CMS before being administered during a 4-week period with one of the following treatments: vehicle, fluoxetine (10 mg/kg) or antalarmin (10 mg/kg). Fluoxetine and antalarmin were dissolved in physiological saline with a drop of Tween 80. Mice were injected intraperitoneally in a volume of 20 ml/kg. Treatments were given each day between 9 and 10 a.m. A nonstressed and nontreated group was used as control in the light/dark box ($n = 15$).

2.6. Statistical analysis

As groups had short samples and nonensured normality, data were analysed using nonparametric exact analyses including the Monte Carlo correction. Data of PS were analysed with a Friedman test, followed by a Wilcoxon signed ranks test comparing initial week with CMS weeks and treatment weeks. Data of body weight were treated with a Kruskal–Wallis test for each week followed by a permutation test for two independent samples (Siegel and Castellan, 1988) as post hoc test. The light/dark test was submitted to a Kruskal–Wallis test followed by an a posteriori permutation test. In order to avoid multiple comparison errors, an ε risk was used. In fact, we used an ε risk in which $\varepsilon = \alpha/k - 1$, k being the number of groups and α the risk that was not corrected for multiple comparisons as suggested by Siegel and Castellan (1988) in the case of planned multiple comparisons.

3. Results

3.1. Body weight

From the second week of chronic treatment (i.e. Week 7), a significant difference in body weight appeared between the three stressed groups ($H = 10.42$, $P = .0038$). This effect persisted during the treatment, at Weeks 8 ($H = 11.97$, $P = .0023$) and 9 ($H = 12.20$, $P = .0014$). A weight increase is observed in the fluoxetine-injected group when compared with stressed-control animals during these 3 weeks (respectively: $P = .0032$, $P = .0007$ and $P = .0004$ with a ε value $= 0.025$ after correction). Antalarmin-injected mice failed to show a weight increase during this period ($P > .8$ during these weeks). These results are presented in Fig. 2.

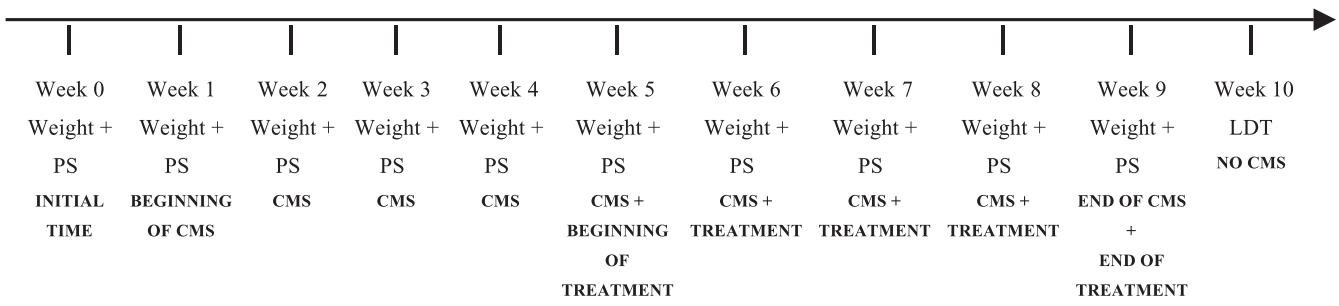

Fig. 1. Diagram of the sequence of the procedure and time of performed measures. PS means physical state and LDT means light/dark test.

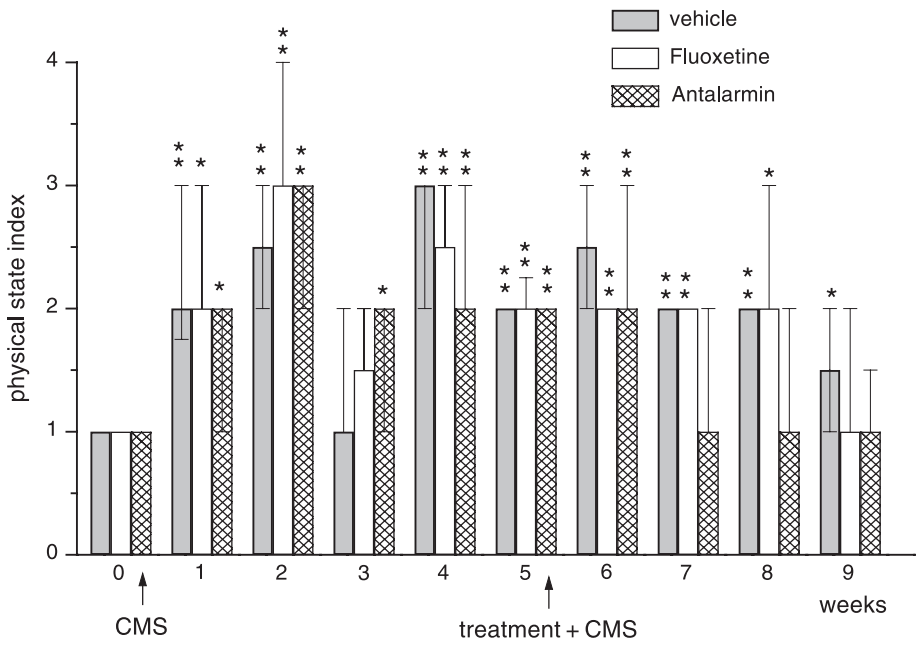

Fig. 2. Physical state index of mice in vehicle, fluoxetine (10 mg/kg) and antalarmin (10 mg/kg)-treated groups during the 9-week CMS (median and quartiles). * * Significantly different from Week 0 at 1% and * significantly different from Week 0 at 5% (P=.0055 after correction).

3.2. Physical state

A significant modification of mice PS appeared in the three groups (vehicle: F = 82.38, P < .001; fluoxetine: F = 70.54, P < .001; antalarmin: F = 95.67, P < .001). Indeed, after 2 weeks of CMS, this index increased (P < .0002 for each group, ε value being 0.0055 after correction) (Fig. 3). Both a 4-week fluoxetine and a 2-week antalarmin treatment induced a significant improvement of mice PS (fluoxetine, P=.0154 at Week 9; antalarmin, P=.0314 at Week 7; P=.0160 at Week 8 and P=.0627 at Week 9) while the vehicle-treated control group still displayed impaired PS (P=.006 at Week 9, ε value being 0.0056 after correction).

3.3. Light/dark test

Results are presented in Table 2. A significant difference among groups was found for time spent in the lit box (TLB) (H = 10.80, P=.0089) and activity in the lit box (H = 18.48, P < .001). TLB and activity are lower in nonstressed mice than in vehicle-treated stressed mice (respectively P=.0003, P=.0005 with ε values = 0.016 after correction). Adminis-

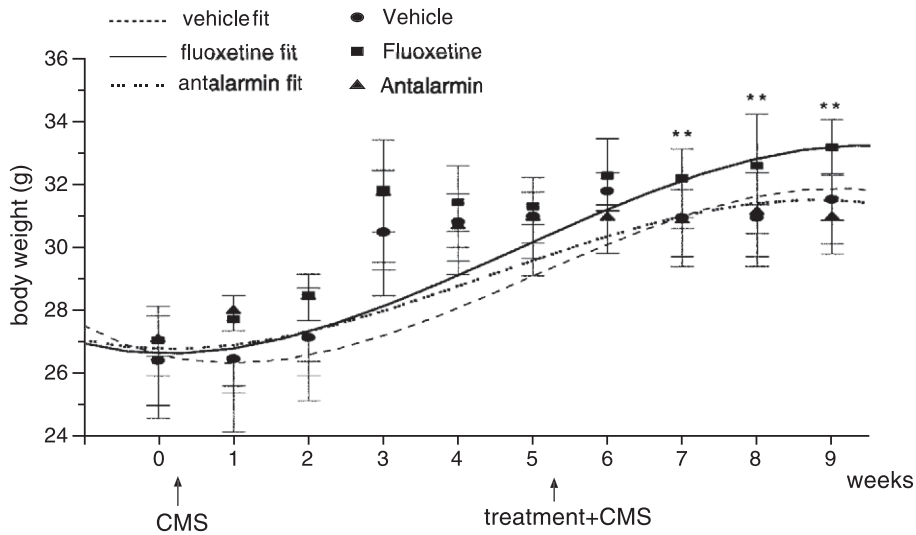

Fig. 3. Body weight changes in vehicle, fluoxetine (10 mg/kg) and antalarmin (10 mg/kg)-treated groups during the 9-week period of CMS (medians and quartiles). Polynomials fit curves are then plotted (r^2>.81, P < .0001 for all groups). * Significantly different from vehicle-treated group at 5% (P=.025 after correction).

Table 2
Activity and time spent in the lit box of the light/dark box procedure

Treatment	% of time in the lit box	Activity in the lit box
Stressed + vehicle	47.50 (− 9.7/ + 20.3)	51 (− 5/ + 22.5)
Stressed + fluoxetine (10 mg/kg)	44.6 (− 44.6/ + 26.5)	53.5 (− 1.5/ + 17.5)
Stressed + antalarmin (10 mg/kg)	30 (− 30/ + 15.1)	55.5 (− 12.5/ + 5.75)
Nonstressed	4.17 (− 4.2/ + 23.3)	94[a] (− 21/ + 30.5)

Data are medians and quartiles.
[a] Significantly different from the stressed vehicle-treated group at a 1% level (P=.016 after correction).

tration of fluoxetine and antalarmin induced a nonsignificant decrease of TLB and activity when compared to controls (respectively P=.2712 and P=.0532 for TLB and P>.4 for activity with ε values = 0.016 after correction).

4. Discussion

This study showed that chronic treatment with the CRF_1 receptor antagonist antalarmin may counteract some of the behavioral modifications produced in mice by repeated exposure to mild stressors. On a behavioral level, data show that CMS induces a decrease of weight gain, a degradation of the PS of mice' coat and a decrease in anxiety-like behavior in the light/dark test.

One of the most important modification observed after CMS is the degradation of the mice PS. This may be explained by a decrease in animals' grooming behavior, related to a conservation of resources in favour of coping behaviors toward the stressing situation. Indeed, in another study, we showed that the stress-induced degradation of PS was associated with an increased latency to groom after a sucrose splash on the body; this increased latency was completely counterbalanced by fluoxetine (Ducottet; Surget, personal communication). This measure, which is easy to score, rapidly observed and reproducible, may be a good index of the response of mice to CMS. Furthermore, CMS induced an increase of the time spent in the lit box in the light/dark test. Similar results were obtained by Kopp et al. (1999) in the light/dark box test and by D'Aquila et al. (1994) in the elevated plus-maze procedure; this effect can be interpreted as a blunting of the emotional response after CMS. However, this result contrasts with other data showing an anxiogenic effect of the CMS (Griebel et al., 2002a,b). Differences in stress procedures, strains and light/dark apparatus may account for this discrepancy.

A chronic treatment with antalarmin and fluoxetine improved the PS of the coat. This is in line with recent data showing that antidepressants such as fluoxetine, V1b antagonist and SSR125543A (another CRF1 antagonist), efficiently act on this parameter. This therefore strengthens the proposal that this PS may be a useful variable to model some depressive-like symptoms in mice. Moreover, administration of fluoxetine induced a weight gain, which is rather unexpected since the selective serotonin reuptake inhibitor (SSRI) is known for its anorectic effect (Berton et al., 1999; Heisler et al., 1997, 1999). This fluoxetine-induced weight gain could be explained by the restoration of eating behavior which was decreased during CMS, suggesting that the antidepressant effects of fluoxetine overrode its anorectic properties. Unfortunately, we did not record food and water intake, so that these remarks are only speculative. The reason for the inability of antalarmin to produce a weight gain in stressed animals is unclear, but cannot be explained by an anorexic effect of the drug since antalarmin has no such properties (Bornstein et al., 1998). Finally, both compounds were rather ineffective in counteracting the effects of stress in the light/dark procedure. This contrasts with data from other studies using fluoxetine-treated mice in the light/dark test and showing that fluoxetine was able to normalize either stress-induced emotional blunting (Kopp et al., 1999) or stress-induced anxiogenesis (Griebel et al., 2002a,b). Differences in the basal activity level could account for this difference. For example, in the Kopp et al. (1999) study, the activity level of nonstressed controls was 5 transitions: this level was increased to about 10 in stressed mice and normalized to 7 in stressed-fluoxetine mice. In the study by Griebel et al. (2002a), the activity level in nonstressed controls was around 20, it decreased in stressed mice to a level of approximately 5 and was counteracted by fluoxetine to about 18. In our study, the basal level of nonstressed controls was 94.

Except for the measures from the light/dark test, our mice subjected to the CMS regime were not compared to nonstressed mice. In fact, the most important measure of the effects of the stress regime is the one related to PS. As this parameter is optimal in nonstressed mice (see first measure on Week 1), it will not be possible to observe an improvement of this parameter with antidepressant treatments because of ceiling effects. Data concerning body weight in nonstressed mice would certainly provide some additional explanations on the effects of antalarmin and fluoxetine per se on body weight gain; this is one of the limits of the present study.

In conclusion, our data suggest that antalarmin may have antidepressant-like properties. However, these effects seem to have a weaker magnitude than the ones of fluoxetine, which is not surprising since fluoxetine is generally described as the most efficient pharmacological treatment of depression. The idea that nonpeptide small-molecule CRF_1 receptor antagonists may represent novel treatment for depressive disorders is substantiated by a large body of animal and clinical data (Heisler et al., 1999; Holsboer, 1999; O'Brien et al., 2001). Altogether, the present results with antalarmin in the CMS model further strengthen the idea that the blockade of central CRF_1 receptors may be an innovative target for future antidepressants.

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