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Blockade of glucocorticoid receptors with ORG 34116 does not normalize stress-induced symptoms in male tree shrews

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Abstract

Glucocorticoid receptors play an important role in the regulation of the activity of the hypothalamo–pituitary–adrenal axis, and are thought to be involved in the pathophysiology of depressive disorders. The present study investigated the effect of the specific glucocorticoid receptor antagonist ORG 34116 (a substituted 11,21 bisarylsteroid compound) in the tree shrew (*Tupaia belangeri*) chronic psychosocial stress model, an established animal model for depressive disorders. Animals were stressed for 10 days before treatment with ORG 34116 started (25 mg/kg p.o. for 28 days). Stress induced a decrease in body weight, which just failed significance, whereas ORG 34116 did not affect body weight in stress and control animals. ORG 34116 enhanced the stress-induced increase in the concentration of urinary-free cortisol, although no differences between the different experimental groups existed during the last week of treatment. In stressed animals, ORG 34116 did not affect marking behavior, but decreased locomotor activity. *Post mortem* analysis of 5-HT_{1A} receptors revealed a decreased affinity of ^{3}[H]-8-OH-DPAT (^{3}[H]-8-hydroxy-2-[di-*n*-propylamino]tetralin) binding sites in the hippocampus of animals treated with the glucocorticoid receptor antagonist. In conclusion, under our experimental conditions, the glucocorticoid receptor antagonist ORG 34116 did not normalize the depressive-like symptoms in the psychosocial stress model of male tree shrews. This finding, however, does not exclude that specific central, neuroendocrine and behavioral features are affected by the compound.

Keywords: Tree shrew; Stress; Behavior; Corticosteroid; Glucocorticoid receptor; 5-HT_{1A} receptor

1. Introduction

A dysregulation of the hypothalamo–pituitary–adrenal axis is one of the most consistently measured neuroendocrine changes in major depression. The activity of the hypothalamo–pituitary–adrenal axis is centrally regulated by corticosteroid hormones such as cortisol via two receptor types, i.e. the mineralocorticoid receptor and the glucocorticoid receptor. Within the brain, mineralocorticoid receptors are mainly located in the hippocampus, whereas glucocorticoid receptors show a wide distribution in the brain and pituitary (Reul and De Kloet, 1985, 1986; Van Eekelen et al., 1987; Herman et al., 1989; Meyer et al., 1998). Corticosteroids exert a negative feedback regulation via the glucocorticoid receptor during stress or the diurnal peak (Reul et al., 1987; De Kloet and Reul, 1987). Depressed patients seem to be resistant to this negative feedback regulation. This is demonstrated by increased cortisol levels in plasma and urine, increased corticotropin-releasing hormone (CRH) concentrations in the cerebrospinal fluid, an escape from the dexamethasone suppression test and an increased adrenocorticotropic hormone (ACTH) response to a CRH challenge in dexamethasone pretreated patients (see for review Holsboer, 2000). In particular, psychotic delusions may develop during hypercorticism in the context of depressive episodes (Schatzberg and Rothschild, 1988).

Clinical improvement during antidepressant treatment is associated with a normalization of the hypothalamo–pituitary–adrenal axis (Heuser et al., 1996), possibly due to antidepressant-induced changes in brain corticosteroid capacity (Barden et al., 1995). Rats treated with antidepres-

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sants showed increased levels of glucocorticoid receptor and mineralocorticoid receptor in the hippocampus, a region implicated in neuroendocrine and cognitive symptoms of depression (Reul et al., 1993, 1994). Transgenic mice with a partial glucocorticoid receptor gene expression knockout show defective glucocorticoid feedback together with characteristics of human depression. Treating these animals with antidepressants induces a reduction in hypothalamo–pituitary–adrenal axis activity and increased glucocorticoid receptor mRNA (Barden et al., 1995). These findings lead to the suggestion that corticosteroid receptors may be a primary site of action for antidepressants (Barden et al., 1995). Results from studies performed with the glucocorticoid receptor antagonist RU486 (mifepristone) revealed antidepressant effects in patients (Murphy et al., 1993). More specifically, the anti-glucocorticoid RU486 caused a rapid reversal of acute psychotic symptoms in Cushing patients (Van der Lely et al., 1991) and in patients suffering from psychotic major depression (Rothschild and Belanoff, 2000; Belanoff et al., 2001a). However, RU486 is not a pure glucocorticoid receptor antagonist, but also has a high affinity for the progesterone receptor (Gagne et al., 1985). The newly developed glucocorticoid receptor antagonist ORG 34116 (a substituted 11,21 bisarylsteroid compound) investigated in the present study does not possess intrinsic agonist activity in a glucocorticoid activity assay and has little affinity for the progesterone receptor (Gebhard et al., 1995).

In the present study, we tested if the glucocorticoid receptor antagonist ORG 34116 affects the depressive-like symptoms observed in chronically stressed tree shrews. Chronic psychosocial stress in male tree shrews induces hyperactivity of the hypothalamo–pituitary–adrenal axis, which is also a cardinal feature of major depression. In the tree shrew brain, chronic stress down regulates both glucocorticoid receptor and mineralocorticoid receptor mRNA in the hippocampus (Meyer et al., 2001), while a glucocorticoid receptor antagonist is thought to increase receptor expression. The stress-induced endocrine and behavioral effects seen in male tree shrews, like an increase in urinary-free cortisol and a decrease in marking behavior, show resemblance with depressive symptoms in humans (Van Kampen et al., 2002; Fuchs et al., 1996). The behavioral and endocrine changes induced by stress have been shown to be normalized by treating the animals with the tricyclic antidepressant clomipramine (Fuchs et al., 1996). Here, we report the effect of ORG 34116 on cortisol and noradrenaline excretion as well as marking behavior and locomotor activity of chronically stressed male tree shrews. We also measured the binding properties of 5-HT_{1A} receptors in distinct brain regions in stressed and control animals treated with ORG 34116 or vehicle since a variety of observations suggest a link between this index for serotonergic activity, stress and depression (Flügge, 1995; Maes and Meltzer, 1995; McQuade and Young, 2000).

2. Materials and methods

2.1. Animals and housing

Experimentally naive adult male tree shrews (*Tupaia belangeri*; $n=19$) were obtained from the breeding colony at the German Primate Center (Göttingen, Germany). The animals were housed individually on a regular day/night cycle (lights on from 8:00 am to 8:00 pm) with free access to food and water (for details see Fuchs, 1999). All animal experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/EEC) and had been approved by the Government of Lower Saxony, Germany.

2.2. Drug administration

To minimize uncontrollable stress and to mimic the situation in humans, the drug was applied orally. The glucocorticoid receptor antagonist ORG 34116 (Organon, Oss, the Netherlands) was suspended in 5% Mulgofen/0.9% NaCl. The suspension was given to the animals in the morning (at 8:00 am) in a dose of approximately 25 mg/kg.

2.3. Experimental procedure

Animals were divided into four groups, namely the *Control* ($n=4$), the *Control + ORG* ($n=4$), the *Stress* ($n=4$) and the *Stress + ORG* ($n=7$) group. During the first 10 days, the activity of the hypothalamo–pituitary–adrenal axis and sympathoadrenal system was determined daily by measuring cortisol and noradrenaline in the morning urine. Daily, body weight was monitored and the animals were videotaped in the evening for analysis of behavior (marking behavior and locomotor activity; see below). The second phase of the experiment was a 10 day long period, during which the animals of the *Stress* and *Stress + ORG* group were submitted to daily psychosocial stress. Psychosocial stress was induced by introducing a naive male animal into the cage of a socially experienced male. This confrontation resulted in active competition for control over the territory (the cage). After establishment of a stable dominant/subordinate relationship, a wire mesh barrier separated the two males so that they could still see each other. The third experimental phase consisted of the ORG treatment lasting 28 days. During this time, the subordinate animals remained in a psychosocial conflict situation and were treated daily with the glucocorticoid receptor antagonist ORG 34116 (25 mg/kg/day p.o.; *Stress + ORG* group) or received vehicle (*Stress* group). Animals of the *Control + ORG* group remained singly housed in their cages and received ORG 34116 (25 mg/kg) daily for 28 days. Animals of the C*ontrol* group also remained singly housed in their cages and received vehicle. Throughout the whole experiment, morning urine samples were collected from all animals, body weight was monitored and behavior was recorded daily.

2.4. Monitoring and analysis of behavior

The behavior of each experimental animal was videotaped daily between 6:45 and 7:15 pm. During 15 min, the locomotor activity of the animals was measured automatically with the Insight software (Octec, Bracknell, UK). For this purpose, the cage had been divided on the monitor in six rectangles (w: 25 cm × h: 40 cm; head to tail length of the animals: approximately 30 cm), and movements were counted when the animal moved from one rectangle to another. Marking behavior was quantified by measuring the total time the animal spent during these 15 min with different forms of scent marking: marking with the abdominal gland, the sternal gland and urinary marking (Aue, 1988). This behavior was analyzed using the Hindsight 1.3 Behavioral Observer software (S. Weiss, Leeds, UK).

2.5. Analysis of urine samples

Urinary-free cortisol was measured by a scintillation proximity radioimmunoassay and urinary noradrenaline was quantified by high-pressure liquid chromatography (see Fuchs et al., 1996). To correct for physiological dilution of the urine, the resulting concentrations were related to creatinine concentrations measured with the Beckman Creatinine Analyzer 2.

2.6. Tissue preparation and autoradiography

At the end of the experiment, animals were decapitated between 8:00 and 9:00 am while one subordinate and one control animal were killed simultaneously. Brains were quickly removed and frozen in liquid nitrogen. Brain sections (10 μm) were cut at −18 °C on a cryostat, thaw-mounted onto gelatin-coated glass slides and stored at −80 °C. Corresponding brain sections from one *Stress + vehicle*, one *Control + vehicle*, one *Stress + ORG* and one *Control + ORG* animal were cut on the same day and processed in the same receptor binding experiment.

To determine 5-HT_{1A} receptor binding, saturation experiments were performed with ^{3}[H]-8-OH-DPAT (^{3}[H]-8-hydroxy-2-[di-*n*-propylamino]tetralin; specific activity 143.8 Ci/mmol; DuPont—NEN, Köln, Germany) according to Vergé et al. (1986) with minor modifications (Flügge, 1995; Flügge et al., 1998). Non-specific binding was determined in the presence of a 1000-fold excess of serotonin (Sigma). Before the saturation experiment started, the sections were placed under vacuum at 4 °C overnight. Briefly, consecutive sections (at least three sections per brain area, radioligand concentration and animal) were preincubated for 30 min at room temperature in buffer (170 mM of Tris–HCl, pH 7.6, 4 mM of $CaCl_2$, 0.01% ascorbic acid, 10 μM of pargyline; 10 μM of fluoxetine hydrochloride), incubated with ^{3}[H]-8-OH-DPAT in concentrations ranging from 0.05 to 6 nM (120 min), washed in buffer (twice for 5 min on ice), in distilled water (30 s on ice) and dried under a stream of cold air (pargyline from Sigma; fluoxetine hydrochloride from Biotrend, Köln, Germany). After washing and drying, the sections were apposed to tritium-sensitive Hyperfilm-^{3}H together with ^{3}H-microscale standards for 5 weeks (Amersham, Braunschweig, Germany).

2.7. Analysis of autoradiographic data

The films were densitometrically analyzed with a computerized image analysis system (MCID AIS, Imaging, St. Catherines, Canada) while gray values of the standards were used to determine the amount of radioactivity bound to tissue sections. The anatomical localization of radioligand binding was performed with the aid of Nissl-stained sections adjacent to the sections that had been processed for autoradiography and with a tree shrew brain atlas (Tigges and Shanta, 1969). In the 5-HT_{1A} receptor binding studies, the maximal number of binding sites (B_{max}) and the equilibrium constants (K_d) were derived from saturation experiments and were calculated using a non-linear regression analysis. Saturation curves were generated with a curve-fitting program (Graph Pad Prism, Graph Pad Software, San Diego, USA).

2.8. Statistical analysis

2.8.1. Body weight, urinary cortisol and noradrenaline, behavioral parameters

The statistical analysis was performed with the SPSS 7.5 software (SPSS, Chicago, USA). To avoid influences of interindividual pretest differences, values were transformed to the percentage of the mean value from the pre-stress period (days 1–10). Data of every group were divided into five data blocks: pre-stress period without any treatment (days 1–10), mere stress period (days 11–20) and stress period while treating the animals with ORG 34116 or vehicle (days 21–30, 31–40 and 41–52). Data were subjected to a non-parametric analysis of variance (ANOVA) for repeated measures (factor time) using the Friedman test followed by the Wilcoxon signed ranks test to detect significant differences among the time blocks while significance was set at $P < 0.05$. To compare for differences in the last time block (days 41–48) between the four groups of non-parametric ANOVA, the Kruskall–Wallis test was used, followed by the Mann–Whitney *U*-test as a post hoc test when appropriate. Statistical significance was set at $P < 0.05$. Data are expressed as mean ± S.E.M.

2.9. 5-HT_{1A} receptor binding

Binding data (B_{max} and K_d values) were analyzed by one-way ANOVA and comparisons between animal groups were performed by Tukey honestly significantly difference post hoc test. Statistical significance was set at $P < 0.05$. Data are expressed as mean ± S.E.M.

3. Results

3.1. Stress-induced changes in body weight, adrenocortical and sympathetic activity are not normalized by ORG 34116 treatment

The effect of stress and ORG 34116 treatment on body weight is summarized in Fig. 1. Psychosocial stress induced a non-significant decrease in body weight in the *Stress + ORG* group (Fig. 1A). The *Stress* group showed a decrease in body weight during the 38 days of stress (Fig. 1B), although the effect ($P = 0.052$) was not as pronounced as in former experiments. No changes in body weight were observed in control animals either treated with ORG 34116 (*Control + ORG*) or with vehicle (*Control*; Fig. 1C,D).

As indicated by the analysis of urinary cortisol excretion, psychosocial stress induced a significant activation of the hypothalamo–pituitary–adrenal axis in animals in the *Stress* group ($P = 0.011$). Differences to the pre-stress period were shown for days 11–20, 21–30 and 41–48 in the stressed animals ($P = 0.068$; Fig. 2B). A sustained activation was also seen in the *Stress + ORG* group ($P = 0.004$; Fig. 2A). ORG 34116 treatment in control animals (*Control + ORG* group) did not significantly affect urinary-free cortisol, neither did the application of the vehicle (*Control* group; Fig. 2C,D). No differences were found between the groups, comparing the last time block of the experiment (days 41–48).

The activity of the sympathetic system was determined by measuring concentrations of noradrenaline in urine (Fig. 3). Urinary noradrenaline was significantly increased during stress in the *Stress + ORG* group ($P < 0.0001$), and the ORG 34116 treatment further increased noradrenaline concentrations (days 31–40 vs. days 11–20, $P < 0.05$; days 41–48 vs. days 11–20, $P < 0.05$, Fig. 3A). Stressed animals receiving the vehicle (*Stress* group) showed a significant increase in urinary noradrenaline concentrations ($P = 0.027$; Fig. 3B). Differences were observed for days 11–20, 21–30, 31–40 and 41–48 ($P = 0.068$). For the control animals, no effect of either ORG 34116 treatment (*Control + ORG* group) or vehicle (*Control* group) was observed (Fig. 3C,D). Comparing the last time block between the four different groups revealed significant differences between the groups. Animals from the *Stress + ORG* group showed higher noradrenaline concentrations compared to the animals from the *Control + ORG* group ($P < 0.05$), similarly for the *Stress* group compared to the *Control* group ($P < 0.05$). The *Stress + ORG* group and the *Stress* group did not differ from each other and also comparison between the *Control + ORG* and *Control* groups did not show significant differences, confirming that ORG 34116 itself has no influence on urinary noradrenaline concentrations.

3.2. Chronic treatment with ORG 34116 does not normalize stress-induced behavioral symptoms

Locomotor activity was significantly decreased in the *Stress + ORG* group ($P = 0.007$; Fig. 4A). Post hoc analysis revealed significant changes for days 21–30, 31–40 and 41–48 vs. pre-stress (days 1–10; $P < 0.05$). No changes in

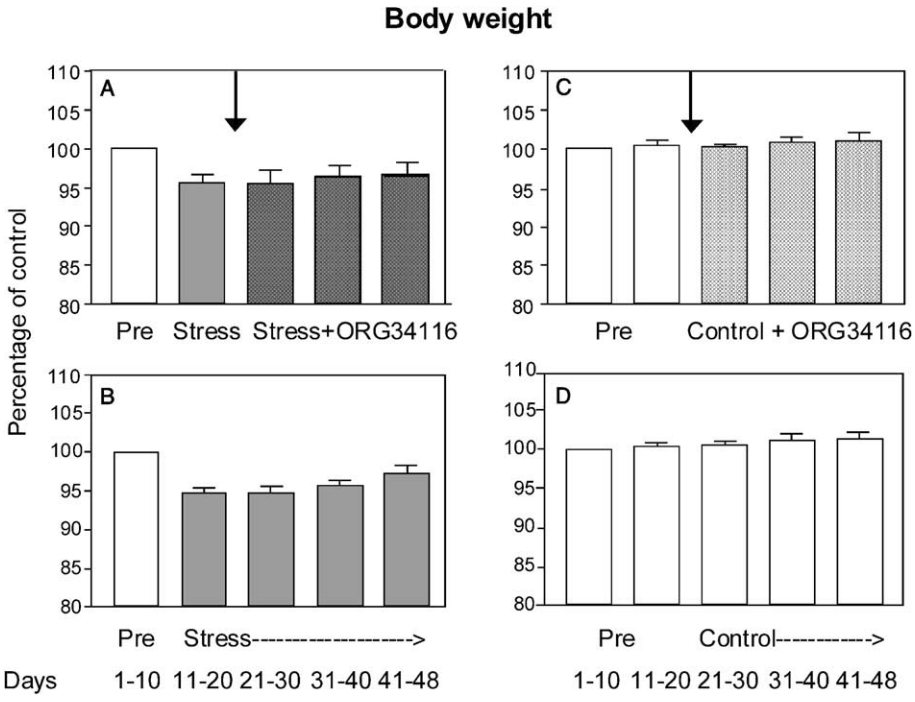

Fig. 1. Effect of chronic psychosocial stress and ORG 34116 on body weight. (A) *Stress + ORG* group; (B) *Stress* group; (C) *Control + ORG* group; (D) *Control* group. ORG 34116 was given orally in the morning, starting on day 20 (arrow) and continued until day 48. Data were expressed as a percentage of the mean individual body weight value during the pre-stress period (mean ± S.E.M.).

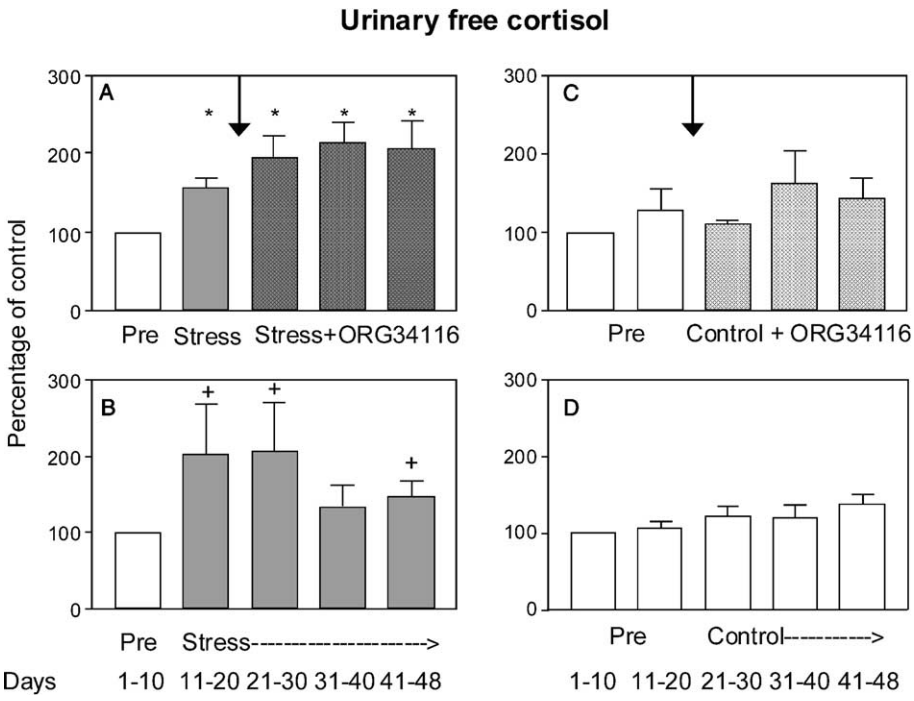

Fig. 2. Effect of chronic psychosocial stress and ORG 34116 treatment on urinary-free cortisol. (A) *Stress + ORG* group; (B) *Stress* group; (C) *Control + ORG* group; (D) *Control* group. ORG 34116 was given orally in the morning, starting on day 20 (arrow) and continued until day 48. Data were expressed as a percentage of the mean individual body weight value during the pre-stress period (mean ± S.E.M.). Significant differences: $*P < 0.05$ compared to the pre-stress period (days 1–10); $\#P < 0.05$ compared to the mere stress period (days 11–20); $+P = 0.068$ compared to the pre-stress period (days 1–10).

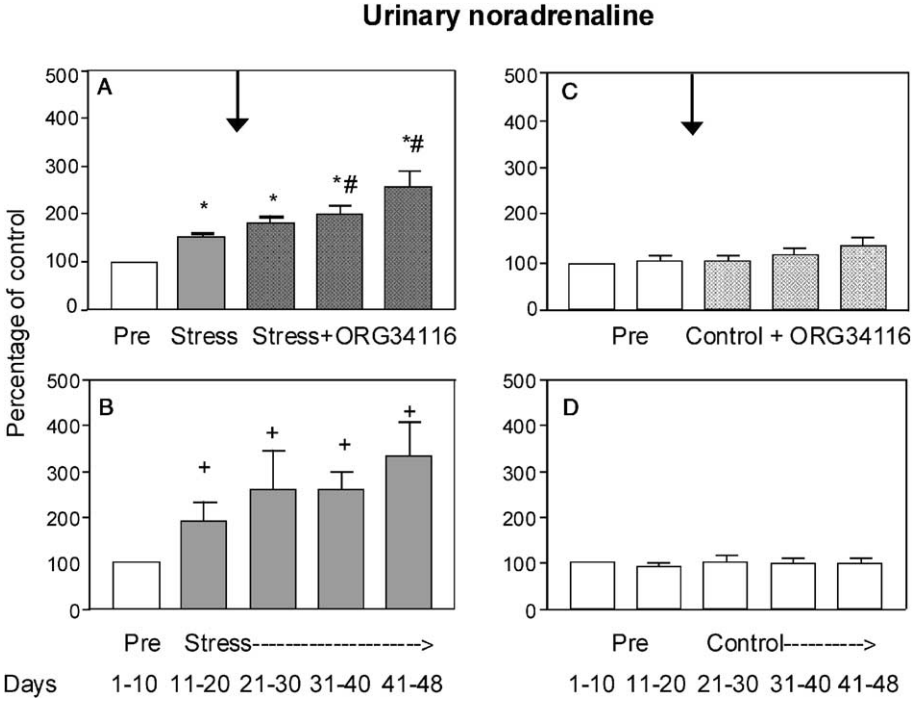

Fig. 3. Effect of chronic psychosocial stress and ORG 34116 treatment on urinary noradrenaline. (A) *Stress + ORG* group; (B) *Stress* group; (C) *Control + ORG* group; (D) *Control* group. ORG 34116 was given orally in the morning, starting on day 20 (arrow) and continued until day 48. Data were expressed as a percentage of the mean individual body weight value during the pre-stress period (mean ± S.E.M.). Significant differences: $*P < 0.05$ compared to the pre-stress period (days 1–10); $\#P < 0.05$ compared to the mere stress period (days 11–20); $+P = 0.068$ compared to the pre-stress period (days 1–10).

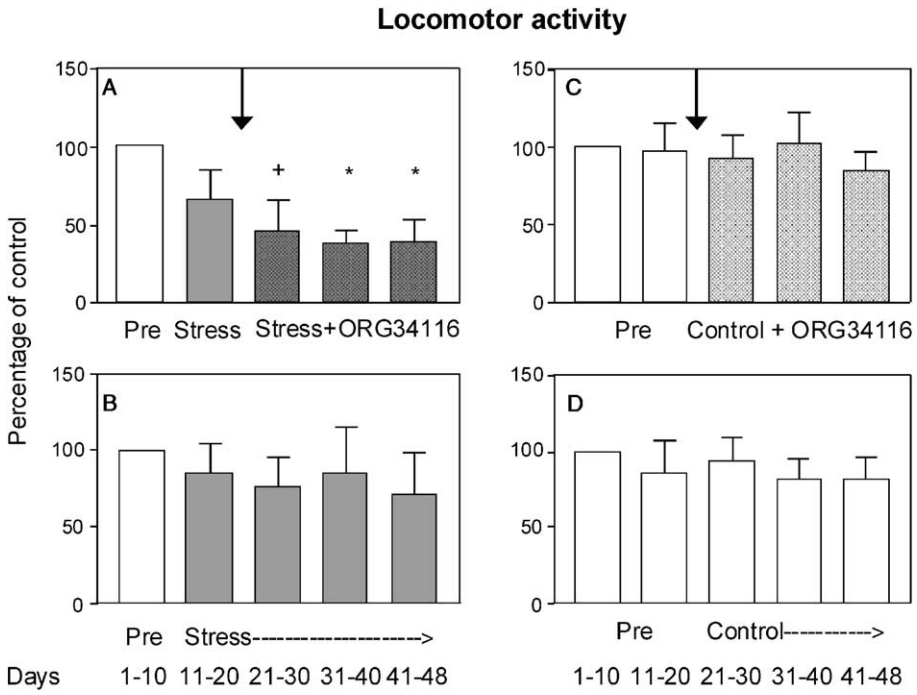

Fig. 4. Effect of chronic psychosocial stress and ORG 34116 treatment on locomotor activity. (A) *Stress + ORG* group; (B) *Stress* group; (C) *Control + ORG* group; (D) *Control* group. ORG 34116 was given orally in the morning, starting on day 20 (arrow) and continued until day 48. Data were expressed as a percentage of the mean individual body weight value during the pre-stress period (mean ± S.E.M.). Significant differences: $*P<0.05$ compared to the pre-stress period (days 1–10); $+P=0.068$ compared to the pre-stress period (days 1–10).

locomotor activity were observed in the *Stress* group, probably due to the large interindividual differences, and control animals treated with ORG 34116 (*Control + ORG* group) or vehicle (*Control* group; Fig. 4B,C,D). No differences between the groups were observed, comparing the last time blocks of all groups.

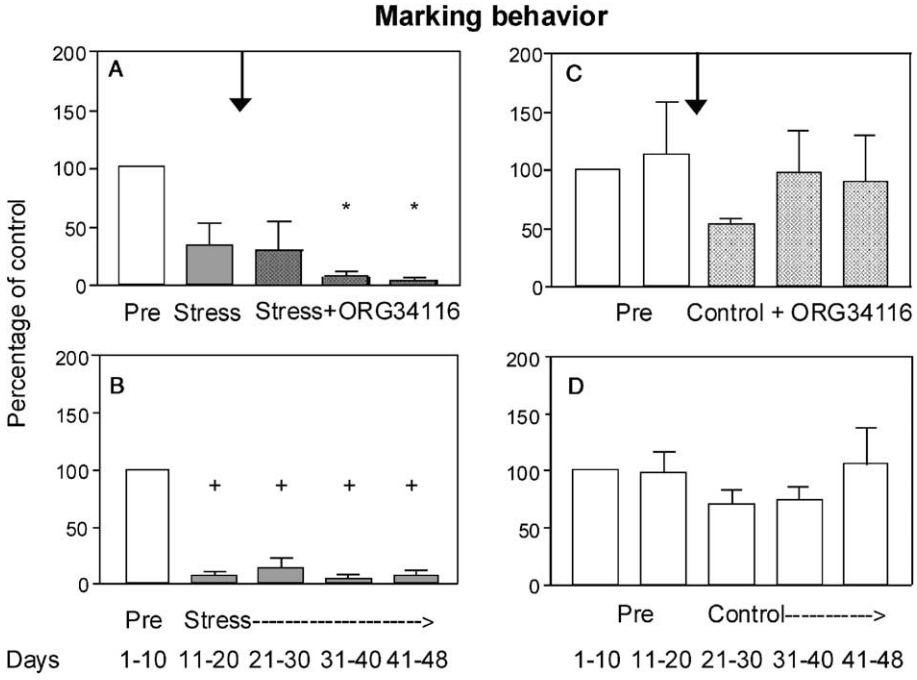

Fig. 5. Effect of chronic psychosocial stress and ORG 34116 treatment on marking behavior. (A) *Stress + ORG* group; (B) *Stress* group; (C) *Control + ORG* group; (D) *Control* group. ORG 34116 was given orally in the morning, starting on day 20 (arrow) and continued until day 48. Data were expressed as a percentage of the mean individual body weight value during the pre-stress period (mean ± S.E.M.). Significant differences: $*P<0.05$ compared to the pre-stress period (days 1–10); $+P=0.068$ compared to the pre-stress period (days 1–10).

Psychosocial stress decreases marking behavior (Fig. 5) in both stress groups (*Stress* group: $P=0.041$; *Stress + ORG* group: $P=0.013$). Stress and treatment with ORG 34116 decreased marking behavior further as compared to just stress (days 11–20) in the *Stress + ORG* group (days 31–40 vs. pre-stress, days 41–48 vs. pre-stress days 1–10: $P<0.05$; Fig. 4A). Marking behavior was not changed in the control groups either treated with ORG 34116 (*Control + ORG* group) or with vehicle (*Control* group; Fig. 4C,D). Comparing the last time block (days 41–48) from the different groups revealed a trend for decreased marking behavior in the *Stress + ORG* group compared to the *Control + ORG* group ($P=0.052$) and a significant decrease in marking behavior in the *Stress* group compared to the *Control* group ($P<0.05$). No changes were observed when comparing the last time blocks of the *Stress* and *Stress + ORG* groups with each other, indicating that the further decrease in marking behavior in the stressed animals treated with ORG 34116 may not be due to the treatment, but may be a consequence of the prolonged stress. Stressed animals treated with ORG 34116 (*Stress + ORG* group) or with vehicle (*Stress* group) showed a significant decrease in marking behavior compared to their control groups (*Control + ORG* group and *Control* group, respectively) at the end of the experiment (days 41–48; $P<0.05$).

3.3. Effects of stress and ORG 34116 treatment on 5-HT$_{1A}$ receptor binding properties

To determine whether ORG 34116 affects 5-HT$_{1A}$ postsynaptic receptors, we determined ^{3}[H]-8-O*H*-DPAT binding in the hippocampus (dentate gyrus and CA1) and the retrobulbar region. Complete saturation binding experiments were performed and the maximal number of binding sites (B_{max}) and K_d values were calculated (Tables 1 and 2).

Psychosocial stress did not induce changes in the maximal number of binding sites of 5-HT$_{1A}$ receptors in the dentate gyrus, CA1 and retrobulbar region in both the *Stress* group and the *Stress + ORG* group, neither did ORG 34116 in the *Control + ORG* and the S*tress + ORG* groups (Table 1).

Psychosocial stress did not induce changes in the K_d of the ^{3}[H]-8-OH-DPAT binding sites in the *Stress* group. Treatment with ORG 34116 increased K_d values in the CA1 region of the hippocampus of the animals in the *Control + ORG* and *Stress + ORG* groups compared to the *Control* and *Stress* groups, respectively (Table 2). In the dentate gyrus, ORG 34116 treatment showed an increase in K_d in the *Stress + ORG* group compared to the *Stress* group ($P<0.05$) and a trend was observed for an increase in K_d in the *Control + ORG* group compared to the *Control* group ($P=0.074$), indicating that ORG 34116 induced a decrease in 5-HT$_{1A}$ receptor affinity in the dentate gyrus and CA1 region of the hippocampus. In the retrobulbar region, no changes in affinity have been found (Table 2).

Table 1
Effects of stress and ORG 34116 on numbers of ^{3}[H]-8-OH-DPAT binding sites (B_{max}: fmol/mg)

	Control ($n=4$)	Stress ($n=3$)	Control + ORG ($n=4$)	Stress + ORG ($n=3$)
Retrobulbar region	120.6 ± 2.9	124.0 ± 4.7	133.4 ± 3.9	132.2 ± 3.3
Hippocampal region CA1	142.7 ± 2.7	136.7 ± 3.2	141.4 ± 2.1	143.5 ± 3.3
Dentate gyrus	83.6 ± 2.5	85.3 ± 2.5	84.5 ± 2.6	70.4 ± 2.5

Data are expressed as mean ± S.E.M. No significant differences were observed.

Table 2
Effect of stress and fluvoxamine on affinity of ^{3}[H]-8-OH-DPAT binding sites (K_d values: nM)

	Control ($n=4$)	Stress ($n=3$)	Control + ORG ($n=4$)	Stress + ORG ($n=3$)
Retrobulbar region	0.28 ± 0.03	0.294 ± 0.05	0.28 ± 0.03	0.28 ± 0.03
Hippocampal region CA1	0.11 ± 0.01	0.13 ± 0.02	0.20 ± 0.01*ab*	0.20 ± 0.02*ab*
Dentate gyrus	0.26 ± 0.03	0.27 ± 0.026	0.34 ± 0.044	0.364 ± 0.05*ab*

Data are expressed as mean ± S.E.M. *a*: significantly different from *Control*, $P<0.05$; *b*: significantly different from *Stress*, $P<0.05$.

4. Discussion

Hyperactivity of the hypothalamo–pituitary–adrenal axis may be involved in inducing the depressive-like behavioral and central nervous changes observed in psychosocially stressed tree shrews. In the present study, we tested the effect of the anti-glucocorticoid ORG 34116 on endocrinological and behavioral parameters and 5-HT$_{1A}$ receptor-binding properties in stressed male tree shrews.

ORG 34116 showed antagonistic activity on human glucocorticoid receptors and, contrary to RU486, no transactivation agonistic activity, which implies that ORG 34116 is a potent and selective glucocorticoid receptor antagonist (Gebhard et al., 1995). In vivo studies showed antagonistic effects of ORG 34116 on the hypothalamo–pituitary–adrenal axis, when given to rats ± 3.5 h before a mild stress (Karst et al., 1997). In addition, also chronic treatment with ORG 34116 increased stress-induced corticosterone levels in rats (Bachmann et al., 1999). Furthermore, ORG 34116 shows antagonism on corticosteroid-mediated effects on $Ca2^+$ currents in the hippocampus (Karst et al., 1997).

4.1. Stress, treatment with ORG 34116 and effects on body weight and adrenocortical activity

In the present study, behavioral and neuroendocrine effects during chronic treatment with ORG 34116 were investigated. We mimicked a realistic situation of antidepressant intervention with daily oral ORG 34116 application, which started after establishment of the stress-induced neurobiological, physiological and behavioral alterations.

The action of the drug was followed across a clinically relevant time period of 4 weeks while the psychosocial stress continued during the whole treatment period.

Measurements of urinary-free cortisol revealed antagonistic effects of ORG 34116 on hypothalamo–pituitary–adrenal axis activity, as reflected by an enhanced urinary-free cortisol in the stressed animals treated with ORG 34116. Similar effects have been found during the diurnal peak in non-stressed rats after i.c.v. administration of the partial glucocorticoid receptor antagonist RU486, whereas during the diurnal through no effect of RU486 was found (Van Haarst et al., 1996). This increased amplitude in the diurnal rhythm of plasma corticosterone is opposite from the flattened rhythm seen in depressed patients (Carroll et al., 1976; Linkowski et al., 1985). This may suggest that anti-glucocorticoids may have antidepressant properties. Murphy et al. (1993) treated depressed patients with RU486 and found an improvement of depressive symptoms in three patients. One possible mechanism behind the antidepressant effects of glucocorticoid receptor antagonists may be based on its ability to up-regulate the glucocorticoid receptor (McQuade and Young, 2000). This up-regulation was observed when treating unchallenged rats with tricyclic antidepressants (Reul et al., 1993; Seckl and Fink, 1992). However, Bachmann et al. (1999) showed that chronic treatment of ORG 34116 and other glucocorticoid receptor antagonists decreased glucocorticoid receptor binding in the anterior pituitary, amygdala and hippocampus of the rat. In addition to this down regulation, a reduced hypothalamic drive was observed, indicated by an attenuated stress-induced ACTH response. Also, an increase in adrenal weight has been found (Bachmann et al., 1999).

4.2. Stress, treatment with ORG 34116 and effects on behavior

Decreased marking behavior has been reported in subordinate tree shrews in response to chronic psychosocial stress (Fuchs et al., 1996; Van Kampen et al., 2000). In the present study, marking behavior also decreased during stress, but different from the antidepressants clomipramine and fluvoxamine, treatment with ORG 34116 did not normalize this behavior.

Animals in the *Stress + ORG* group showed a significant decrease in locomotor activity during treatment with the glucocorticoid receptor antagonist. This is consistent with the findings of the group of Piazza that dopamine-dependent behaviors are dependent on glucocorticoid receptors (Piazza et al., 1996). Psychotic depressed patients show increased cortisol and dopamine activity (Belanoff et al., 2001b), and it was suggested that glucocorticoids increase dopaminergic activity and hereby induce psychotic symptoms (Schatzberg and Rothschild, 1988). Treatment with anti-glucocorticoids reverses psychotic symptoms in Cushing patients who have increased cortisol levels (Van der Lely et al., 1991) and in patients with psychotic major depression (Belanoff et al., 2001a). The decrease in locomotor activity observed in stressed male tree shrews treated with ORG 34116 may be linked to the dopaminergic system, which activity is supposed to be decreased during anti-glucocorticoid treatment. Also Marinelli et al. (1998) found a decrease in dopamine release and in dopamine-dependent locomotor activity after administration of RU486.

4.3. Stress, treatment with ORG 34116 and effects on 5-HT_{1A} receptors

Stress and hyperactivity of the hypothalamo–pituitary–adrenal axis are often associated with changes in central 5-HT_{1A} receptors. The maximal binding capacity of 5-HT_{1A} receptors is often found to be decreased during chronic psychosocial stress (Flügge, 1995; McKittrick et al., 1995) and serotonin-mediated neuronal responses are shown to be attenuated after prolonged high corticosteroid levels (Karten et al., 1999). Corticosterone is known to increase serotonin synthesis and release and due to the homologous regulation of 5-HT_{1A} receptors by their agonist (serotonin), the amount of receptors may be down regulated and/or their affinity may be decreased. In the present study, a decrease in affinity of the 5-HT_{1A} receptor was found in the dentate gyrus and the CA1 region of the hippocampus in both stressed and control tree shrews treated with the glucocorticoid receptor antagonist ORG 34116. This finding may suggest that treatment with ORG 34116 is able to induce an increase in serotonin in these brain areas.

4.4. Conclusions

The present study was designed to evaluate potential antidepressant effects of the glucocorticoid antagonist ORG 34116. The treatment, however, does not normalize the stress-induced changes in male tree shrews. This may be a consequence of the dose we used. Also, the duration of the treatment and the long time span (11 h) between administration of the compound and recording the behavior of the animals may be a problem to determine the effects exerted by ORG 34116; although this time period is shown to be the time point on which the stress-induced changes are most prominent (Kramer et al., 1999). To achieve a longer exposure to the glucocorticoid receptor antagonist, it may be useful to give the compound twice a day or via the drinking water. Alternatively, as is shown that glucocorticoid receptor antagonists are effective in reducing psychotic symptoms, our animal model may not be suitable to demonstrate effects of glucocorticoid receptor antagonists, since no psychotic symptoms have been analyzed. In the case of psychosis, the mesolimbic dopamine system is involved which can be driven in a hyperactive state during hypercorticism (Piazza et al., 1996; De Kloet et al., 1998), whereas acute anti-glucocorticoid treatment decreased hyperactivity of the dopamine system (Marinelli et al., 1998).

In conclusion, the glucocorticoid receptor antagonist ORG 34116 does show antagonistic effects on the hypothalamo–pituitary–adrenal axis regulation, leads to a decrease in 5-HT_{1A} receptor affinity and suppresses locomotor activity in stressed animals. The data show that the anti-glucocorticoid is not able to counteract the stress-induced symptoms in this model, but do not exclude that specific behavioral features are affected.

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