ANXIOLYTIC-LIKE EFFECTS OF SELECTIVE MINERALOCORTICOID AND GLUCOCORTICOID ANTAGONISTS ON FEAR-ENHANCED BEHAVIOR IN THE ELEVATED PLUS-MAZE

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SUMMARY

The effects of intracerebroventricular (ICV) administration of the mineralocorticoid receptor (MR) antagonist, RU28318, and the glucocorticoid receptor (GR) antagonist, RU38486, were studied on behavior of rats exposed to a compartment previously associated with a stressor, and placed subsequently in an elevated plus-maze test. Fear-motivated immobility behavior was attenuated by the MR antagonist in a dose of 50 or 100 ng ICV, whereas the GR antagonist alone or simultaneous administration of both antagonists had no significant effect. In the elevated plus-maze, immediately after the exposure to the conditioned stressor, both the GR antagonist (50 ng) and MR antagonist (50 ng) increased the percentage of time the rats spent on open arms, and increased the amount of entries into these open arms. These data are interpreted in terms of the involvement of the GR and MR in fear and anxiety.

Keywords—Mineralocorticoid; Glucocorticoid; Receptor; Immobility; Plus-maze: Anxiety.

INTRODUCTION

ADRENAL GLUCOCORTICOIDs APPEAR to have a dual and often opposing action on a variety of homeostatic processes including the behavioral expressions of fear and anxiety (see review: Bohus, 1994; De Kloet, 1991). Removal of endogenous glucocorticoids by adrenalectomy (ADX) produces anxiogenic-like effects (File et al., 1979; Weiss et al., 1970), whereas administration of corticosterone caused anxiolytic-like effects (File et al., 1979). In contrast, it was reported that fear-motivated immobility was abolished by ADX and was restored after corticosterone replacement (Bohus, 1987). We propose that these different actions of corticosteroids are mediated by different corticosteroid receptor types located in diverse brain structures.
The existence of two intracellular receptor systems for corticosteroids has been demonstrated in the rat brain by radioligand binding, immunocytochemistry and in-situ hybridization (Chao et al., 1989; Fuxe et al., 1985; Herman et al., 1989; Reul & De Kloet, 1985; Van Eekelen et al., 1988). These receptors are designated as mineralocorticoid receptors (MRs; type I) and glucocorticoid receptors (GRs; type II). The two receptor types differ in their neuroanatomical distribution, their affinity for corticosterone, their regulation, and their function. The MRs are aldosterone selective in the periventricular brain and bind both corticosterone and aldosterone with high affinity in the limbic brain regions, for example, hippocampus, septum, and amygdala (De Kloet et al., 1975; McEwen et al., 1968, 1986, 1992; Reul & De Kloet, 1985). GRs bind corticosterone with a 10-fold lower affinity than the MRs. The GRs are widely distributed in the brain with highest concentration in regions involved in regulation of the stress response (Fuxe et al., 1985; Ratka et al., 1989; Reul & De Kloet, 1985; Sawchenko & Bohn, 1989; Van Eekelen et al., 1987). Low circulating levels of corticosterone predominantly occupy MRs, whereas after stress and during the circadian peak both receptor types are activated (Reul et al., 1987). MRs and GRs coordinate and differentially mediate the corticosteroid control of ion regulation and transmitter responsiveness (Bradbury et al., 1991; De Kloet & Reul, 1987; Evans & Arriza, 1989; Joëls & De Kloet, 1992; Munck & Náray-Fejes-Tóth, 1992; Pearce & Yamamoto, 1993).

The infusion of selective MR antagonists (RU28318), GR antagonists (RU38486), and selective agonists intracerebroventricularly (ICV) or locally in hippocampus allows to define receptor mediated effects in intact rats (De Kloet, 1991). Behavioral effects of the GR antagonist or the MR antagonist have been described in tests designed to examine learning and memory, and learned helplessness (De Kloet et al., 1988; Jeffereys et al., 1985, 1987; Oitzl et al., 1992a, 1992b, 1993; Papolos et al., 1993; Peeters et al., 1992). It was proposed that GRs are involved in consolidation processes, whereas MRs are needed for spatial and inhibitory avoidance learning. However, less attention was given to the constructs of fear and anxiety. Earlier, we observed that MR and GR antagonist treatment reduced the initial phase of the centrally CRF-induced heart rate stress response (Korte et al., 1993), suggesting stress reducing properties of both antagonists.

The purpose of the present study was to investigate the role of central MRs and GRs in behavioral tests shown to have predictive validity as model of fear and anxiety by using these specific corticosteroid antagonists to selectively block the function of each corticosteroid receptor type. There is a growing body of evidence that different behavioral tests measure different aspects of fear and anxiety, and that these hypothetical constructs may have different representations in the brain, both in terms of neuroanatomy and neurochemistry (Bohus et al., 1989; Korte et al., 1992b; LeDoux, 1993; Silveira et al., 1993). The fear-motivated immobility test was used, in which animals show passive behavior, that is, immobility-freezing behavior to a situation previously associated with an uncontrollable stressor. This type of fear-motivated behavior is attenuated by classical and novel anxiolytics (Conti et al., 1990; Korte et al., 1990). Directly after the fear-motivated immobility test, rats were exposed to the elevated plus-maze test, in which they usually show a preference for the closed arms instead of open arms. The percentage of time spent on the open arms and the number of open arms entries are validated measures of anxiolytic-like effects (Pellow et al., 1985).
METHODS

Subjects
For the fear-motivated immobility test and the elevated plus-maze, male Wistar rats ($n = 96$) (originally derived from Cpb, TNO, Zeist, The Netherlands and bred in our laboratory) weighing 300–340 g at the beginning of the experiments were used. They were housed individually in clear Plexiglas cages ($25 \times 25 \times 30$ cm) on a 12-h light-dark regime (lights on between 0700 and 1900h). All animals had free access to standard rat chow and tapwater. The experiments were carried out between 1000 and 1400h.

Surgery
The rats were secured in a stereotaxic frame for implantation of unilateral intracerebroventricular (ICV) cannulae, under complete ether anaesthesia. The guide cannulae were positioned 1.0 mm above the right lateral ventricle with the tooth bar set at +5.0 mm at AP 0.6 mm and L ± 2.0 from bregma and DV −3.2 mm from point of entry (Heinrichs et al., 1992; Pellegrino & Cushman, 1976). The rats were allowed at least 1 week for postsurgery recovery.

Treatment
The steroid receptor antagonists were injected ICV at a dose range of 50–150 ng/2 μl per rat 30–35 min before the exposure of the animals to the test situation. The glucocorticoid antagonist (anti-GR; RU38486: 17β-hydroxy-11β-(4-dimethylamino-phenyl)17α-(1-propynyl)estro-4,9-diene-3-one) (Gaillard et al., 1984; Moguilewski & Philibert, 1984) and the mineralocorticoid antagonist (anti-MR; RU28318; 3,3-oxo-7-propyl-17-hydroxy-androstan-4-en-17yl-propionic acid-lactone) (Perroteau et al., 1984) were provided by Roussel-UCLAF, Romainville, France. Both steroids were dissolved in ethanol; subsequently, a 0.9% NaCl solution was added to reach the appropriate concentration. The final concentration of ethanol was 2%. The vehicle control contained the same ethanol concentration. Each animal received only one ICV injection. The observations were made by trained observers who were blind to the treatment order.

Statistics
Comparisons between behavioral data of vehicle treated stressed and control animals were made by Student’s $t$-tests. One-way analysis of variance (ANOVA) was performed on the data of vehicle- and drug-treated stressed animals. The Dunnett’s posthoc $t$-test was used to compare the drug-treated stressed animals to the vehicle-treated stressed group. A probability level of $p \leq .05$ was taken as significant.

FEAR-MOTIVATED IMMOBILITY

Procedure
The rats were trained in a dark compartment in which the electric foot shock would be applied (Korte et al., 1990). Briefly, the rats were placed in a waiting Plexiglas ($25 \times 25 \times 30$ cm) cage next to the apparatus for 1 min, after which they were trained to enter from a platform to a dark compartment where they were allowed to stay for 5 min. This procedure was repeated three times. During the final training trial an inescapable scrambled foot shock (0.6 mA, AC for 3 s) was given immediately upon entering the dark from the lit platform compartment. The rats were removed from the dark compartment 40 s after termination of the foot shock. On the next day, the animals were placed in the
waiting cage for 1 min, whereafter they were directly transferred into the dark compart-
ment but without any further foot shock. This procedure has been shown to induce
immobility behavior accompanied by elevations in plasma corticosterone and plasma
catecholamine levels in the rat (Korte et al., 1992a). Each rat received an ICV injection of
RU38486, RU28318, both antagonists together, or vehicle 30 min before reexposure to
the former shock compartment. The effect of drug treatment on the duration of immobility
(defined by the total lack of motion) was measured through a mirror (tilted with an angle
of 45°) above the dark compartment during the second, third, and fourth minute of
exposure to the dark former shock compartment (5-min period).

ELEVATED PLUS-MAZE

Procedure

Directly after the 5-min period exposure to the compartment in which they had pre-
viously been shocked, rats were tested in the elevated plus-maze located in another
soundproof room. The animals were already treated with vehicle, anti-MR (50, 150 ng)
or anti-GR (50, 150 ng) or both anti-MR + anti-GR (50 ng + 50 ng) 30 min before
reexposure to the former shock compartment. The elevated plus-maze consisted of two
open, 50 x 10 cm, and two enclosed arms, 50 x 10 x 40 cm, with an open roof, arranged
such that the two arms of each type were opposite to each other (Pellow et al., 1985).
Light intensity in the open arms and closed arms was 200–350 lx and < 1 lx, respectively.
The maze was elevated to a height of 100 cm. Animals were placed in the center of the
maze facing an enclosed arm at the start of the experiment. Each rat was tested for 5
min on the elevated plus-maze. The maze was carefully wiped with a damp cloth after
each animal. The number of entries into closed arms, and into open arms and the time
spent in the open arms and in the closed arms in the elevated plus-maze were measured.
Time spent on open arms relative to open + closed arms is used as an index for the
anxiolytic or anxiogenic effects (Pellow et al., 1985; Wahlestedt et al., 1993).

RESULTS

Behavioral Effects of RU38486 and RU28318 on Fear-Motivated Immobility

Figure 1 shows that during reexposure to the former shock compartment duration of
immobility was increased in stressed animals vs. nonshocked controls after vehicle
treatment (p = .0013). ANOVA of data of stressed animals revealed a significant drug
treatment effect, $F(7, 80) = 3.85; p = .0012$. This was because two doses of the anti-
MR RU28318 (50 and 100 ng, ICV) reduced the fear-motivated immobility in stressed
rats compared to vehicle treated stressed rats ($p <= .05$). The anti-GR RU38486 failed
to show significant effects at these doses. No significant effects were found with the
highest dose (150 ng) of either antagonist or with simultaneous treatment of MR and GR
antagonists (both 50 ng).

Behavioral Effects of RU38486 and RU28318 in the Elevated Plus-Maze Test

Figure 2 shows that exposure to the former shock compartment prior to the plus-
maze test decreased the percentage of time on the open arms in stressed vs. nonshocked
vehicle treated animals ($p = .0283$). Analysis of variance revealed a significant drug
treatment effect, $F(5, 47) = 6.616; p < .0001$. Furthermore, both RU28318 (50 ng) and
RU38486 (50 ng) increased the percentage of time spent on the open platform (both $p <$
Corticosteroid Receptor Antagonists, Fear, and Anxiety

Fear-motivated Immobility

FIG. 1: Time spent on immobility behavior of rats during exposure to the former shock compartment, 30 min after ICV-administered vehicle (controls, n = 8; stressed, n = 19), antimineralocorticoid (RU28318; 50 ng, n = 8; 100 ng, n = 13; 150 ng, n = 7), antiglucocorticoid (RU38486; 50 ng, n = 8; 100 ng, n = 15; 150 ng, n = 7) or RU28318 + RU38486 (both 50 ng, n = 11). *p < .05; **p < .01, significantly different from control.

.01) in stressed animals. Neither the higher doses of RU28318 and RU38486 nor the combination of the drugs altered the behavior in the plus-maze.

Figure 3 shows that neither prior exposure to a situation previously associated with a stressor nor drug treatment produced a significant effect in the number of closed arm entries in the plus-maze test.

Elevated plus-maze

FIG. 2: Percent of time spent in the open arms relative to cumulative time on all four arms in the elevated plus-maze directly after the rats' exposure to the conditioned emotional stressor. Directly after the 5-min period exposure to the former shock compartment, animals treated with vehicle (controls, n = 8 stressed, n = 12), antimineralocorticoid (RU28318; 50 ng, n = 8; 150 ng, n = 7), antiglucocorticoid (RU38486; 50 ng, n = 8; 150 ng, n = 7), or RU28318 + RU38486 (both 50 ng, n = 11) were placed in the elevated plus-maze. **p < .01, significantly different from control.
Figure 3: Closed arm entries (mean ± SEM) in rats given a 5-min test in the elevated plus-maze, directly after the rats’ exposure to the emotional stressor. For further explanations see Fig. 2.

Figure 4 shows that prior exposure to a situation previously associated with a stressor significantly decreased the number of open arm entries in the plus-maze test ($p = .001$). However, drug treatment in the stressed animals showed a significant effect, $F(5, 47) = 7.811; p < .0001$, due to an increase in the number of open entries in the RU28318 (50 ng) ($p < .01$) and RU38486 (50 ng) ($p < .01$) treated animals.

**DISCUSSION**

The present study shows that if rats are reexposed to a compartment previously associated with a stressor, the MR is implicated in the control of fear-motivated immobility exerted by corticosterone. The MR antagonist, RU28318 (50 and 100 ng, ICV), reduced this fear-motivated immobility. In contrast, the GR antagonist, RU38486, did
not significantly affect the fear-motivated immobility. Fear-motivated immobility is also abolished following ADX and reinstated following corticosterone substitution. The synthetic glucocorticoid dexamethasone that activates GR, does not restore the immobility response (Bohus, 1987). Threat-induced freezing in pups may be of the same nature as the immobility response in adult rats. It is supposed that threat-induced behavioral inhibition reduces the risk of attack from predators. Recently, Takahashi and Rubin (1993) have demonstrated that removal of adrenal hormones effectively reduced the occurrence of threat-induced freezing in pups. Daily injections of corticosterone were effective in restoring the developmental appearance of freezing in ADX pups. Collectively, the data suggest that MRs may be involved in behavioral passivity during stressful conditions in which the animal has no active control. In contrast, consolidation of acquired immobility in the Porsolt-swimming test was disrupted after posttrial (poststress) administration of the GR antagonist, whereas the MR antagonist was not effective (De Kloet et al., 1988). However, this immobility response is probably not similar to the threat-induced immobility. Indeed, there exists evidence to indicate that rats have learned to inhibit rapid swimming movements as an energy-conserving strategy (Hawkins et al., 1978).

In the elevated plus-maze, the "anxious" behavior was enhanced by prior exposure to a conditioned stressor, that is, reexposure to a compartment previously associated with an inescapable uncontrollable stressor. The behavior in the plus-maze seems more easily to be altered by the emotional consequences of the stressor (present study; Heinrichs et al., 1992) than the stressor itself (Falter et al., 1992; Steenbergen et al., 1991). Furthermore, our recent findings suggest that absence of controllability of the stressor—i.e., inescapable foot shock—leads to the enhancement of "anxious" behavior (Bohus et al., in preparation). Accordingly, prior exposure to an emotional stressor as induced by uncontrollable aversive stimuli enhances the anxiety state (generalized anxiety?) as indicated by the reduced exploration of the open arms of the plus-maze as compared to nonstressed controls. Both the MR antagonist (RU28318) and the GR antagonist (RU38486) treatment led to an increase in time spent on these open arms and an increase of number of entries into the open arms. Such a preference for the open arms may be interpreted as an anxiolytic-like effect. The effect of the MR antagonist on the rat's performance in the plus-maze is not necessarily a direct one but may be a consequence of its action in the former test situation. Interestingly, the MR and GR antagonists showed no effects on plus-maze behavior in case fear was not potentiated by prior exposure to an emotional stressor (Oitzl & De Kloet, unpublished results). Therefore, it is concluded that the MR and GR antagonists do not have a general anxiolytic action, but may have anxiolytic-like actions when the state of anxiety is enhanced by prior stressor exposure. Accordingly, it has been reported that corticosteroids may play an important role in the processing of relevant stimuli (Fehm-Wolfsdorf et al., 1993). The ability to integrate incoming stimuli was decreased in patients lacking cortisol and was normalized by glucocorticoid replacement (Henkin, 1970). Such mechanisms may be involved in the present results.

In summary, the MR is implicated in the corticosterone control of behavioral passivity in stressful situations in which the animal has no active control. It is probably that steroid-serotonin interactions in the raphe-hippocampal system underly these MR-mediated behavioral effects (Biegon et al., 1985; Chalmers et al., 1993; De Kloet et al., 1986; Joëls & De Kloet, 1992; Joëls et al., 1991; Meijer & De Kloet, 1994; Mendelson & McEwen, 1990). The mechanism(s) by which GR-mediated effect on anxiety is organized is not known yet. The relation to the GABA\(_A\)/benzodiazepine receptor complex (Majewski
et al., 1985; Sutanto & De Kloet, 1993) may deserve attention. Accordingly, the GR antagonist may reduce the enhanced state of anxiety, probably via a different mechanism.

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REFERENCES


antiglucocorticoid activity that only disinhibits the human pituitary–adrenal system at a specific time of day. Proc Natl Acad Sci USA 81:3879–3882.


