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Corticosterone Is Permissive to the Anxiolytic Effect That Results From the Blockade of Hippocampal Mineralocorticoid Receptors

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BITRAN, D., M. SHIEKH, J. A. DOWD, M. M. DUGAN AND P. RENDA. *Corticosterone is permissive to the anxiolytic effect that results from the blockade of hippocampal mineralocorticoid receptors.* PHARMACOL BIOCHEM BEHAV **60**(4) 879–887, 1998.—The effects of RU 28318, a mineralocorticoid receptor antagonist (A-MR), and RU 38486, a glucocorticoid receptor antagonist (A-GR) on behavior in three animal models of anxiety were assessed after microinjection into the dorsal hippocampus. Significant anxiolytic effects were observed after intrahippocampal injection of 0.5, and 1 ng of A-MR in thigmotaxic behavior in the open field, in the elevated plus-maze, and in the defensive burying test. Lower (0.2 ng) or higher (5 ng) doses of A-MR were ineffective, as were comparable injections of A-GR or microinjections of combined A-MR and A-GR. The anxiolytic effect of intrahippocampal A-MR administration observed in the elevated plus-maze and in the open field was not observed in adrenalectomized animals or in animals pretreated with a systemic injection of dexamethasone (80 mg/kg). Intrahippocampal injection of 1 ng of A-MR or A-GR prevented the return to basal corticosterone levels observed 90 min after restraint stress. This effect was reversed in dexamethasone-pretreated animals. The results are discussed in light of recent findings implicating the role of the MR in the hippocampus in adaptive behavioral responses to an aversive or threatening environment, and further implicate the permissive role of corticosterone in A-MR-induced behavioral responses.

RU 28318 RU 38486 Elevated plus-maze Defensive burying Thigmotaxis

THE hypothalamic–pituitary–adrenal (HPA) axis has long been suspected to play a role in affective and anxiety disorders. Basic research has been equivocal on the relationship of corticosteroids to behavioral responses in animal models of anxiety. Although intracranial injection of corticotropin releasing hormone (CRH) (12,31) and systemic administration of pituitary adrenocorticotropic hormone (ACTH) elicit anxiogenic effects (14), corticosterone (CORT) injection has the opposite effect (1,14). Recent work, however, has shown that CORT administration produced anxiogenic effects (39).

A source of variance in CORT-induced effects may be due to differential activation of two receptor subtypes. Corticosteroids bind with high affinity to mineralocorticoid receptors (MR) and with low affinity to glucocorticoid receptors (GR) (37). Occupation of MR and GR in the hippocampus, where they are found in high concentrations (45), contributes to regulation of the HPA response to stress (8,13,38). The modulation of neural excitability by CORT acting on this dual receptor system has led to the suggestion that CORT at low concentrations act in a permissive fashion to increase excitability via the MR, and at high concentrations suppress excitability via the GR (10,24,27).

The involvement of the MR in behavior in animal models of anxiety was implicated in findings that intracranial injection of MR antagonist (A-MR) decreased shock-conditioned immobility, whereas treatment with an A-MR or a GR antagonist (A-GR) prevented the anxiogenic effect in the elevated plus-maze elicited by shock conditioning; similar treatment with a combination of A-MR and A-GR was without effect (20). More recently, an anxiolytic effect of A-MR was confirmed after intrahippocampal microinjection (39). In contrast, another study found that ICV infusion of A-MR or A-GR had

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no effect in the defensive burying test, but an injection combining both drugs increased immobility in the defensive burying test and increased fear-potentiated startle response (21).

Our experiments were designed to further clarify the role of hippocampal CORT receptors in animal models of anxiety. The defensive burying test and elevated plus-maze were chosen as tests of anxiety because they represent active and passive avoidance of aversive stimuli, respectively. Thigmotaxis in a novel open field was also used to measure reactivity to novelty. Blood serum CORT response to restraint stress after intrahippocampal administration of A-MR or A-GR was measured to provide a physiological validation of the pharmacological manipulation used in the behavioral studies (36). Finally, the role of the HPA axis in the behavioral effects after CORT receptor blockade was determined in adrenalectomized or dexamethasone-pretreated animals. The rationale for these experiments was to determine whether the occupation of MR or GR with CORT was a necessary condition for the putative effects of A-MR or A-GR microinjection into the hippocampus on behavior in animal models of anxiety (9,17,28,46). Thus, adrenalectomy would presumably eliminate the presence of CORT at both receptor subtypes, whereas dexamethosone pretreatment would occupy only the GR, as it is a specific GR agonist (40), while at the same time, it would decrease endogenous levels of CORT, thereby reducing their presence at the MR.

METHOD

Animals

Adult male Long–Evans rats (Harlan Sprague–Dawley, Indianapolis, IN), weighing between 300–400 g at the time of the experiments, were maintained in a temperature- and humidity-controlled vivarium on a reversed 12 L:12 D cycle (lights on at 2300 h). Males were housed singly in standard polycarbonate cages containing wood chip bedding with free access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee of the College of the Holy Cross and were in compliance with guidelines established by the National Institutes of Health.

Surgery

Adrenalectomy (ADX) or sham surgery was performed through a dorsal approach using ketamine HCl (60 mg/kg, IP) and xylazine HCl (12 mg/kg, IP) anesthesia. Sham-operated animals were subjected to the same surgical procedure as ADX rats, but the adrenals were left intact. ADX rats received 0.9% saline as drinking solution. Standard stereotaxic procedures were used to implant bilateral cannulae to 2 mm above the dentate gyrus of the hippocampus. The coordinates for the cannulae implantations were 2.6 mm posterior to bregma, 1.7 mm lateral to the midline, and 3 mm ventral to the surface of the skull, with the incisor bar set at +3.3 mm (33). The outer guide cannulae were constructed from 23-ga. thin-wall stainless steel hypodermic tubing (Small Parts, Inc.). Removable obturators were made of 27 ga. and were cut flush with the guide cannula. The rats were allowed at least 1 week of postsurgical recovery.

Drugs

The A-MR, RU 28318 (spironolactone; 3-(3-oxo-7-propyl-17-hydroxy-androsta-4-one-17-yl)-propionic acid lactone), and the A-GR, RU 38486 (17-hydroxy-11-(4-dimethylaminophenyl)-17-(1-propynyl)-estra-4,9-diene-3-one), were generously donated by Roussel Uclaf Research Centre (Romainville, France). Dexamethasone was purchased from Sigma Chemical Co. (St. Louis, MO). All drugs were prepared daily in a 1% ethanol:0.9% saline vehicle.

Drug Infusions

An injection cannula made from 28-ga. hypodermic tubing was lowered into the permanently indwelling guide cannula. One end of a 1-m length of PE20 polyethylene tubing (Clay-Adams) was fitted over the end of the injection cannula, preventing extension beyond 2 mm below the outer cannula. The other end of the PE tubing was connected to a mechanized infusion pump (Harvard Instruments). A 0.5 μl aliquot was infused at a rate of 0.25 μl per minute. The injection cannula remained in place for an additional minute, and the procedure was repeated in the contralateral cannula. All injections were made in freely moving animals.

Behavioral Tests

Ten minutes after the completion of the intracranial microinjection, animals were tested. Indices of anxiolytic drug effects were assessed in the open-field, elevated plus-maze, and defensive burying test. All tests were conducted in the dark phase of the light cycle, beginning 2 h after lights off.

Thigmotaxis in the open field. The open field (100 × 100 × 30 cm) was built from Plexiglas with a line drawn on the floor 2 cm from the wall around its perimeter. Behavior was monitored for a 5-min period. Thigmotaxis was defined as the amount of time an animal spent in contact with the lines outlining the perimeter of the open field. An anxiolytic effect is noted as a reduction in thigmotaxic behavior (43).

Elevated plus-maze. The maze consisted of two "open" arms (50 × 10 cm) and two "closed" arms with walls (50 × 10 × 40 cm) and an open roof, arranged so that the two open arms were opposite to one another. The maze was elevated to a height of 50 cm. Transparent Plexiglas rails (50 × 1 cm) were fixed to both sides of the open arms to prevent subjects from falling. Two lamps (25 W) were mounted 50 cm above the open arms, providing a lighting intensity in the middle of the open arms of 100 lx. The number of open and closed arm entries and the time spent in the open and closed arms was recorded for a 10-min period via a closed-circuit television monitor by an observer who was naive to the treatment condition of the animal. Anxiolytic effects in the plus-maze are noted as an increase in the proportion of open arm entries and time spent on the open arms, relative to the total number of arm entries and time spent on open and closed arms; anxiogenic effects are noted as decreases in these measures (34).

Defensive burying. This test was conducted in a Plexiglas cage (44 × 30 × 44 cm) with a 5-cm layer of wood shavings. Animals were habituated to the testing chamber in 30-min periods on each of 3 consecutive days. On the fourth day, the animal was placed in the test cage where a 6.5 × 0.5 cm probe was inserted through a hole placed in the middle of one of the shorter walls, 2 cm above the wood shavings. Electric current was administered through two uninsulated wires wrapped around the probe. When the animal first touched the probe, it received a mild electric shock (2 mA). Immediately after the shock, burying behavior was recorded for a 10-min period once burying began, or for a 15-min period if no burying behavior was recorded. The latency and duration of each burying sequence was recorded via a closed-circuit television monitor housed in a separate room. Anxiolytic agents cause a reduction in the duration of burying behavior (44).

Histology. At the completion of the testing, rats were killed by CO_2 asphyxiation. Brains were removed and frozen to −20°C, and 40 μm coronal sections were cut on a freezing microtome (Zeiss, HM505N). Sections were mounted on slides, stained for cresyl violet, and coverslipped. Cannula placement was verified under a light microscope.

Experimental Procedures

Experiment 1: Effect of intrahippocampal microinjection of A-MR and A-GR on behavior in the elevated plus-maze and defensive burying test. Forty-nine animals were randomly assigned to one of seven groups. One week after bilateral cannulae implantation, animals received microinjections of one of the following: vehicle, RU 28318 at 1 or 5 ng, RU 38486 at 1 or 5 ng, or a combination of RU 28318 and RU 38486 at 1 or 5 ng. Behavior in the elevated plus-maze was conducted after the intrahippocampal injections as described above. One week later, all animals were randomly reassigned to receive one of the seven treatments, and behavior was examined in the defensive burying test.

Experiment 2: Effect of ADX on the anxiolytic effect of intrahippocampal RU 28318 in the elevated plus-maze. Thirty-six animals received bilateral cannulae aimed at the hippocampus, as described above. Eighteen of these animals were also adrenalectomized; the remaining animals received a sham operation. After a week of postsurgical recovery, all animals received a bilateral injection of vehicle or a single dose of RU 28318 (0.2 or 0.5 ng). Behavioral test in the elevated plus-maze was conducted as described above. One week later, animals that previously received a bilateral injection of RU 28318 now received an injection of vehicle, whereas animals that previously received an injection of vehicle now received a microinfusion of a single dose of RU 28318. Behavioral measures in the plus-maze were similarly collected. Thus, each sham and ADX animal was tested once a week over a 2-week period: once after an injection of vehicle, and once after an injection of RU 28318 at either 0.2 or 0.5 ng. Two days after the last intracranial injection, animals were killed by decapitation, trunk blood was collected, blood serum was separated and assayed for CORT content, and brains were removed and prepared for histological verification of cannula placement. Only animals with complete ADXs were included in the behavioral analyses.

Experiment 3: Effects of dexamethasone pretreatment and intrahippocampal microinjection of A-MR or A-GR on behavior in the open field and elevated plus-maze. One week after intracranial cannula implantation, 70 animals were randomly assigned to one of two pretreatment and five treatment groups (n = 7 per pretreatment/treatment combination). Animals received a pretreatment injection of either dexamethasone (80 g/kg, IP) or the ethanol/saline vehicle 3 h prior to the test. The five different treatment conditions consisted of the following bilateral intrahippocampal injections: RU 28318 at 0.2, 0.5, or 1 ng; RU 38486 at 0.2 or 0.5 ng. In addition, each animal served as its own control by receiving an intrahippocampal injection of the ethanol/saline vehicle. The order of intrahippocampal treatment conditions (vehicle or receptor antagonist) was counterbalanced, and the second series of tests was separated from the first by 1 week. After the intracranial microinjections, animals were placed in the open field test for 5 min, and then immediately placed in the elevated plus-maze for 10 min.

Experiment 4: Effects of dexamethasone pretreatment and intrahippocampal microinjection of A-MR or A-GR on blood serum CORT response to restraint stress. Ninety animals previously tested in Experiments 1 or 3 were used in this experiment. Half of the animals received a pretreatment injection of dexamethasone, the other half received an ethanol/saline vehicle pretreatment injection. Three hours later, animals received an intrahippocampal injection of 1 ng of RU 28318 or RU 38486, or the ethanol/saline vehicle. Thus, a total of six pretreatment/treatment groups were formed. Fifteen minutes after the intracranial injection, approximately one-third of each group was killed by decapitation (n = 5). The remaining animals were placed in acrylic restraint chambers for a 15-min period. After restraint stress, half of the remaining animals were killed, and the other half were returned to their home cages for a 90-min period, after which they were killed. Trunk blood was collected from all animals, and serum was separated by centrifugation and stored at −20°C until assay for CORT using a radioimmunoassay kit (Diagnostic Products Corp). Thus, CORT was assayed before, immediately after, and 90 min after restraint stress in animals pretreated with vehicle or dexamethasone, and treated with an intrahippocampal microinjection of vehicle or 1 ng of either RU 28318 or RU 38486.

For all experiments, only animals with accurate cannulae placements were included in the results. In experiments where the same animals were used for different tests or treatments, statistical analysis revealed no effect of prior treatment on the results of subsequent tests.

RESULTS

Experiment 1: Effects of Intrahippocampal Microinjection of A-MR and A-GR on Behavior in the Elevated Plus-Maze and Defensive-Burying Test

Forty-two of 49 animals had bilateral cannulae confirmed in the dentate gyrus of the hippocampus. The MR antagonist, RU 28318 produced a dose-dependent anxiolytic effect when injected into the hippocampus. As is shown in Table 1, the number of open-arm entries and time spent in the open arms were significantly increased by a 1 ng dose of RU 28318. The number of closed-arm entries remained unaffected by RU 28318. In contrast to the anxiolytic effect of RU 28318, the GR antagonist, RU 38486 did not affect any behavioral measure in the plus-maze. An injection combining GR and MR antagonists was also without effect.

A similar pattern of results was observed using the defensive-burying test (Fig. 1.) A 1 ng dose of RU 28318 increased the latency to burying behavior and decreased the amount of time animals spent burying the electrified probe. The total number of shocks received during the test was not affected by the A-MR (data not shown). A microinjection of RU 38486 alone or in combination with RU 28318 had no effect in the defensive burying test.

Experiment 2: Effects of ADX on the Anxiolytic Effect of Intrahippocampal RU 28318 in the Elevated Plus-Maze

The effect of adrenalectomy on circulating CORT levels is shown in Table 2. Accurate cannulae placement were found in 14 of the ADX animals, and in 12 of the sham control animals. Adrenal gland removal produced minor changes in the exploration of the elevated plus-maze (Table 2). The changes observed included a decrease in the number of closed arm entries, $t(24) = 2.71$, $p < 0.01$, and an increase in the amount of time spent in the closed arms, $t(24) = 2.17$, $p < 0.05$. Other measures reflecting anxiolytic indices, i.e., number of open-

TABLE 1

BEHAVIOR IN THE ELEVATED PLUS-MAZE AFTER MICROINJECTION OF GR AND/OR MR ANTAGONIST IN THE HIPPOCAMPUS

Drug	Dose (ng)	Number		Time	
		Open	Closed	Open	Closed
Vehicle		4.5 ± 0.4	10.5 ± 1.1	57.2 ± 5.6	161.0 ± 10.9
RU 486	1	8.2 ± 1.8	9.7 ± 0.2	90.7 ± 16.4	144.0 ± 12.2
	5	5.3 ± 1.4	11.1 ± 1.1	57.5 ± 18.8	165.0 ± 15.5
RU 318	1	12.3 ± 1.2*	10.8 ± 0.5	128.3 ± 10.4*	103.0 ± 13.5*
	5	6.2 ± 0.7	9.7 ± 0.8	67.5 ± 6.8	158.0 ± 5.9
486 and 318	1	4.5 ± 0.6	9.3 ± 1.1	57.0 ± 12.2	157.8 ± 10.6
	5	5.7 ± 1.1	11.5 ± 1.3	41.7 ± 9.6	178.3 ± 11.2

Data are mean ± SEM for $n = 6$ animals in each treatment condition. One-way analysis of variance revealed a significant effect of RU 318 on the number of open arm entries; $F(2, 15) = 24.8$, $p < 0.001$, and on the time spent on the open arms; $F(2, 15) = 23.8$, $p < 0.001$. Significant effects were subjected to pairwise comparisons with a Bonferroni adjustment.

*$p < 0.01$ against the vehicle response.

arm entries, proportion of time spent on the open arms, were not different in ADX animals.

In contrast to the absence of baseline effects seen in ADX animals, the anxiolytic effect of RU 28318 was eliminated by ADX (Fig. 2). Whereas the proportion of time spent on the arm was increased by an intrahippocampal injection of 0.5 ng RU 28318 in intact animals, $t(7) = 5.78$, $p < 0.001$, this effect was absent in ADX animals.

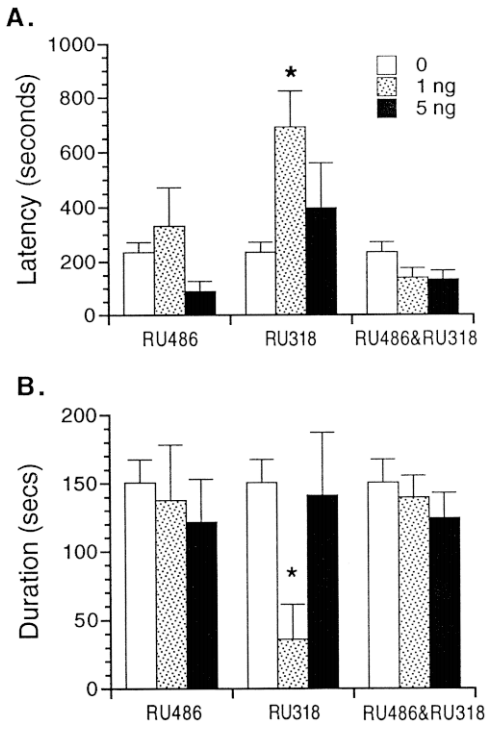

FIG. 1. Effect of RU 38486 and/or RU 28318 in the hippocampus on defensive burying behavior. Data are expressed as mean ± SEM. Main effect of RU 28318 on latency to burying behavior; $F(2, 15) = 3.62$, $p < 0.05$, and on the duration of burying behavior; $F(2, 15) = 4.02$, $p < 0.05$. Post hoc pairwise comparison with Bonferroni's adjustment, *$p < 0.05$.

Experiment 3: Effects of Dexamethasone Pretreatment and Intrahippocampal Microinjection of A-MR or A-GR on Behavior in the Open Field and Elevated Plus-Maze

Of the 60 animals used in this experiment, 50 were confirmed to have cannulae accurately placed within the hippocampus. Figure 3 summarizes the effects of dexamethasone pretreatment and intrahippocampal injection of either RU 38486 or RU 28318 on exploration of the open field and elevated plus-maze. Dexamethasone pretreatment alone had no effect on any behavioral measure in either test. Thigmotaxis in the open field was unaffected by blockade of the GR in the hippocampus. However, a decrease in thigmotaxis was observed after an intrahippocampal injection of 0.5 ng RU 28318; $t(4) = 2.80$, $p < 0.05$. This effect was not seen in animals pretreated with dexamethasone. Total locomotor activity in the open field was not affected by either A-GR or A-MR (data not shown).

Doses of RU 38486 lower than those tested in Experiment 1 were ineffective in altering behavior in the elevated plus-maze. Extension of the dose–response curve revealed that A-MR at low doses elicited an anxiolytic effect (Fig. 3). Thus, an increase in the proportion of time spent on the open arms was observed after 0.5 or 1 ng of RU 28318; $t(4) = 3.30$ and 5.97, $ps < 0.05$. Once again, the anxiolytic effect of RU 28318 was blocked by dexamethasone pretreatment. Indeed, a significant anxiogenic effect was observed in animals pretreated with dexamethasone receiving a microinjection of 0.5 ng RU 28318, $t(4) = 2.89$, $p < 0.05$.

Experiment 4: Effects of Dexamethasone Pretreatment and Intrahippocampal Microinjection of A-MR or A-GR on Blood Serum CORT Response to Restraint Stress

Histological analysis of cannulae placement resulted in the inclusion of data from 78 of 90 animals used in this experiment; 38 receiving dexamethasone pretreatment, 40 receiving a vehicle pretreatment injection. Serum CORT levels were analyzed using a $2 \times 3 \times 3$ analysis of variance, with systemic pretreatment (vehicle or dexamethasone), intrahippocampal microinjection treatment (vehicle, 1 ng RU 28318, or 1 ng RU

TABLE 2

BEHAVIOR IN THE ELEVATED PLUS-MAZE AFTER AN INTRAHIPPOCAMPAL INJECTION OF RU 28318 IN INTACT AND ADRENALECTOMIZED ANIMALS

Group	RU 318 (ng)	Number		Time				CORT (μg/dl)
		Open	Closed	%O:T	Open	Closed	%O:T	
Sham	Vehicle	9.5 ± 1.0	18.7 ± 1.6	33.5 ± 2.5	113 ± 13	271 ± 11	28.9 ± 2.7	15.6 ± 0.8
	0.2	8.7 ± 1.5	16.9 ± 1.3	33.5 ± 5.0	99 ± 20	262 ± 24	27.2 ± 5.3	
	0.5	14.4 ± 1.8*	15.8 ± 2.2	47.8 ± 3.1†	193 ± 18†	171 ± 24‡	52.5 ± 2.6‡	
ADX	Vehicle	7.0 ± 1.8	12.0 ± 3.3†	31.3 ± 5.5	87 ± 22	344 ± 36*	21.2 ± 5.3	0.84 ± 0.14‡
	0.2	6.3 ± 1.8	15.2 ± 4.1	27.6 ± 5.0	57 ± 19	347 ± 28	13.6 ± 4.5	
	0.5	6.4 ± 2.3	9.8 ± 4.7	31.1 ± 8.2	75 ± 33	342 ± 60	19.9 ± 9.0	

Significant difference relative to sham vehicle control: *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$.

38486), and stress condition (basal, stress, poststress) as main effects. Significant interactions were observed between pretreatment and treatment, $F(2, 61) = 10.49$, $p < 0.001$, pretreatment and condition, $F(2, 61) = 24.19$, $p < 0.001$, and treatment and condition, $F(4, 61) = 7.28$, $p < 0.001$. Simple effects contrasts of the data depicted in Fig. 4 revealed that RU 28318 in the hippocampus increased basal CORT levels, $F(1, 61) = 4.01$, $p < 0.05$, whereas an intrahippocampal injection of RU 38486 did not, $F(1, 61) = 0.02$. Although dexamethasone pretreatment did not, by itself, affect basal, stress, or poststress levels of serum CORT, the increase in basal CORT level induced by the MR blocker was not observed in dexamethasone-pretreated animals.

Restraint stress produced a marked elevation in serum CORT in all animals tested. An increase in serum CORT resulted from 15 min of restraint stress in animals that received a vehicle pretreatment and an intrahippocampal injection of vehicle, $F(1, 61) = 63.4$, $p < 0.0001$, RU 28318, $F(1, 161) = 34.9$, $p < .0001$, or RU 38486, $F(1, 61) = 41.8$, $p < 0.0001$. Dexamethasone pretreatment did not affect the stress response in any of these groups.

Ninety minutes after restraint stress, CORT levels had returned to baseline in vehicle and dexamethasone pretreated animals that had received an intrahippocampal vehicle injection, $F(1, 61) = 0.40$ and 1.80, respectively. This was not the case in animals receiving an injection of either A-MR or A-GR. CORT levels 90 min poststress in animals receiving RU 28318 or RU 38486 injection into the hippocampus were elevated relative to basal values, $F(1, 61) = 23.13$ and 22.72, ps < 0.0001. Dexamethasone pretreatment reinstated basal CORT 90 min poststress in animals treated with either A-MR or A-GR, $F(1, 61) = 0.21$ and 0.61, respectively.

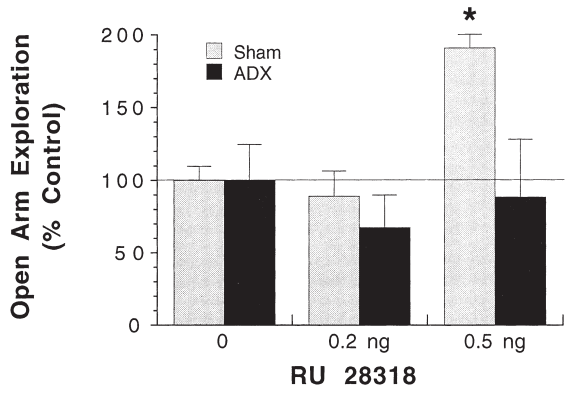

FIG. 2. Effects of intrahippocampal RU 28318 on the proportion of time spent on the open arms of the elevated plus-maze in intact and adrenalectomized rats. Data are standardized to percent of vehicle response. *$p < 0.01$ relative to sham vehicle control group.

DISCUSSION

The results clearly suggest a role for hippocampal MR in the regulation of affective behavior. In three different animal models of anxiety, blockade of hippocampal MR elicited dose-dependent anxiolytic effects. Thus, RU 28318 in the hippocampus decreased burying of the electrified probe in the defensive burying test, decreased thigmotaxis in the open field, and increased exploration of the open arms in the elevated plus-maze. These results are in partial agreement with previous research. Although an ICV injection of A-MR did not affect behavior in the defensive burying test (21), an anxiolytic effect in the elevated plus-maze was observed after ICV administration of A-MR or A-GR, but only in animals first exposed to a shock-conditioned stimulus (20). Several important methodological differences may have contributed to the different results. For example, Korte and colleagues (21) studied the effects of A-MR on burying behavior the day after the exposure to the electrified probe, whereas we studied it during the first exposure. Therefore, while our experiments assessed the impact of MR blockade on the immediate anxiogenic effects of being exposed to an electrified probe, studying the effects of A-MR on the reactivity to the probe a day after its initial exposure may have included a possible influence of glucocorticoids on learning and memory. It is interesting to note that Smythe and colleagues (39) also reported that intrahippocampal injection of RU 28318 elicited anxiolytic effects in a first (nonlearned) exposure to the black-white box test.

An interesting phenomenon that requires further analysis and experimentation is the nature of the inverted U-shaped dose–response curve we found for A-MR administration, an effect that has precedence in research on corticosteroid-mediated effects. For example, enhanced reactivity to a novel object was reported in ADX animals and in animals receiving an injection of a high dose of CORT (29). Other inverted U-shaped response characteristics of CORT-dependent events include an impairment of active and passive avoidance conditioning in ADX animals that was restored by low-dose CORT administration, but was reinstated by high doses of CORT (7,23,25),

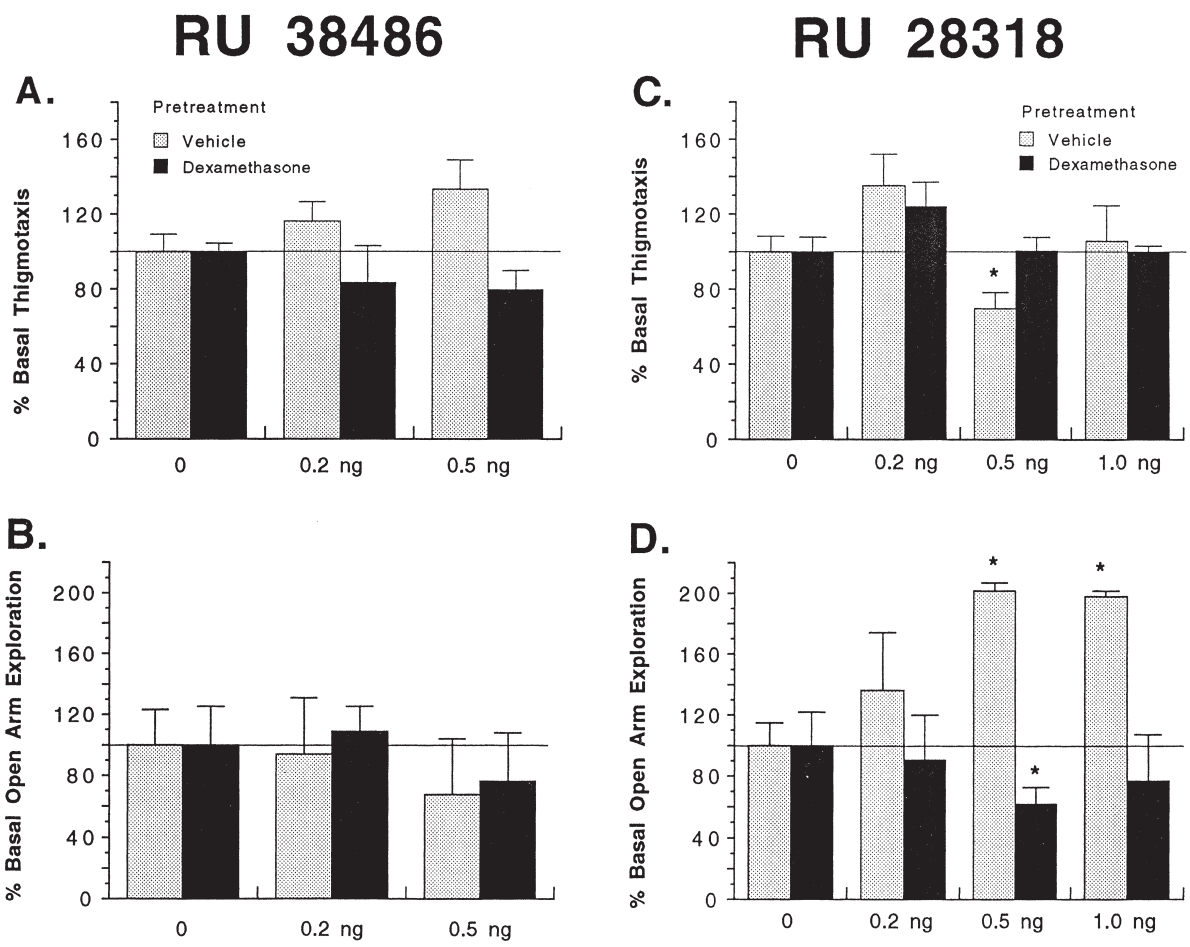

FIG. 3. Effect of dexamethasone pretreatment and intrahippocampal injection of RU 38486 or RU 28318 on thigmotaxis in the open field (A and C) and on behavior in the elevated plus-maze (B and D). Data are shown as mean percent ± SEM of the response observed after vehicle pretreatment and intrahippocampal vehicle injection. $*p < 0.05$ relative to vehicle control groups.

and suppression of long-term potentiation in ADX animals or ADX animals given high-dose CORT injections (32). The logic used for the explanation of these findings is that only low dose CORT administration results in a selective activation of MR, whereas high doses of the steroid act on both MR and GR. However, this explanation may not be appropriate for our results. RU 28318 has a 50-fold greater affinity for the MR than the GR, and therefore, the suggestion that there was spillover of the A-MR onto the GR does not seem probable because 0.5 and 1 ng were effective doses, but a 5 ng dose was not. Further experiments should be conducted to determine the pharmacological specificity of the A-MR-induced effect, for example, coadministration of a specific MR agonist, to rule out potential actions of the A-MR at sites other than corticosteroid receptors.

That the A-MR-induced anxiolytic effect was not a result of GR blockade was evident in that comparable intrahippocampal injections of RU 38486 had no effect on any of the behavioral measures we assessed. The lack of behavioral effects after A-GR administration alone or in combination with MR blockade is supported by previous reports (18,38), but stands in contrast to the anxiogenic effects observed in the defensive burying test and in fear-potentiated startle response after ICV injection of combined A-MR and A-GR (21). An important difference that may explain this discrepancy lies in the route of administration. Whereas significant effects were observed after ICV infusion of combined A-GR and A-MR (21), our experiments used injections into the hippocampus. It is, therefore, likely that other structures may have been involved in the effects observed after ICV injections. Although the MR and GR could have opposite effects within the hippocampus, their interactions may be more complex when considering the brain as a whole. Another apparent paradox is found in a report that ICV injection of A-MR increased mean arterial blood pressure and heart rate, while a similar injection of A-GR had no effect (19). These findings are contrary to changes that are expected to occur in these measures concomitantly with an anxiolytic effect. However, it is important to note that a stress-reducing effect was observed as A-MR treatment decreased the tachycardia induced by CRH administration (19). Whether injection of A-MR into the hippocampus elicits similar cardiovascular responses remains to be determined.

Blockade of MR in the hippocampus produced an increase in basal CORT levels, whereas the A-GR did not, a pattern of

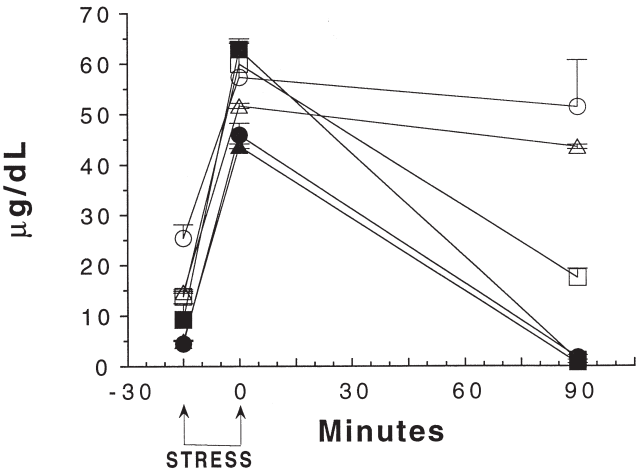

FIG. 4. Corticosterone in blood serum taken from animals prior to, immediately after, or 90 min after 15 min of restraint stress. Animals received a systemic injection of vehicle (clear symbols) or dexamethasone pretreatment (filled symbols) 3 h prior to an intrahippocampal injection of vehicle (squares), 1 ng of RU 28318 (circles), or 1 ng of RU 38486 (triangles). See text for detailed description of results from statistical analyses.

effects previously reported after ICV administration (36). We found that occupation of receptors in the hippocampus with A-MR or A-GR prevented the return of baseline CORT after restraint stress. These results confirm the role of the hippocampus in feedback regulation of the HPA axis (13,16), and corroborate earlier findings that MR and GR are responsible for normalizing CORT levels after a stressor (36). That the blockade of the MR produced an anxiolytic effect, whereas an A-GR did not, is especially noteworthy because we found that both MR and GR blockade in the hippocampus prevented the normalization of the CORT response to restraint stress. Thus, an increase in circulating CORT cannot be the proximal mechanism mediating A-MR-induced anxiolytic effects, as had been previously suggested (1,14).

The mechanism underlying the anxiolytic effect after hippocampal MR blockade remains undetermined. In experiments using ADX animals, the dose–response curve for A-MR treatment was expanded into the lower range to detect a leftward shift in the response curve, reflecting a potential hypersensitivity to MR blockade as a result of the upregulation of hippocampal CORT receptors following ADX. Unexpectedly, ADX produced a loss of sensitivity to the anxiolytic effect of intrahippocampal RU 28318 administration. This was surprising, especially because the resulting circulating levels of CORT after ADX were similar to basal diurnal levels in naive animals, and were probably sufficient to at least partly bind to the MR. It is important to note, however, that the behavioral experiments demonstrating anxiolytic effects after A-MR administration in intact animals were conducted during the dark phase of the dark/light schedule, a time when CORT levels are at their circadian peak. Perhaps complete occupation of the MR is necessary for the anxiolytic effects of A-MR to be detected, a state that was presumably compromised by ADX. Experiments testing this hypothesis should carefully evaluate the anxiolytic effects of A-MR administration as a function of varying levels of circulating CORT.

A possible explanation for the loss of anxiolytic efficacy of RU 28318 after ADX may be found in experiments using dexamethasone pretreatment. Systemic dexamethasone has been found to be a poor MR agonist but a good GR agonist in vivo (40). Thus, the principal action of dexamethasone pretreatment would have been to activate the inhibition of the HPA, thereby decreasing CORT secretion prior to the intrahippocampal injection of A-MR. In support of this assumption are our findings that dexamethasone administration decreased basal levels of CORT and reversed the prolonged CORT response to stress observed after A-MR or A-GR administration. Our studies reveal that CORT may play a permissive role in A-MR-induced effects. Dexamethasone pretreatment eliminated the anxiolytic effect of A-MR in both the open-field and plus-maze tests. Together with the results from the ADX experiment, these observations are consistent with hypothesis that occupation of the MR by CORT is a prerequisite for the anxiolytic effect of MR blockade. Our observations are also in agreement with the recent proposal that MR occupancy in the hippocampus may determine stress responsiveness (11).

These suggestions lead to the hypothesis that anxiolytic effects of A-MR may be more pronounced in animals whose hippocampal MRs are activated by CORT or by aldosterone, an MR agonist. Because the high-affinity MR is thought to be nearly saturated by basal CORT levels (37), a reduction in anxiety would be expected to occur when some neurochemical event produces a displacement of CORT from the almost always-occupied MR (15). The physiological significance of this putative mechanism is suggested in recent findings that progesterone binds to the MR with an affinity that is comparable to aldosterone (15). That progesterone is a potent anxiolytic steroid has been well documented (4,5,35), and although much research has attributed the anxiolytic effect of progesterone to its neuractive reduced metabolite, allopregnanolone, acting at the $GABA_A$ receptor (3,6), a role for progesterone in displacing CORT from the MR, and thus eliciting an anxiolytic effect, has not been examined.

The results from the dexamethasone experiment also exclude the possibility that the effects of the A-MR are mediated by an indirect A-MR–induced increase in CORT and subsequent activation of the GR, because GR stimulation by dexamethasone did not produced anxiolytic effects.

The celerity with which the anxiolytic response to RU 28318 was observed in these and other experiments (39) has prompted the speculation that a nongenomic mechanism may underlie the observed effects. The preponderance of evidence of corticosteroid effects on the electrical properties of hippocampal neurons points to a genomic mechanism [reviewed in (18)]. An indirect modulatory effect of CORT on ongoing signal transduction by other neurotransmitter systems has been hypothesized to account for the rapid depolarization of hippocampal neurons measured in response to the iontophoretic application of CORT (18). In this context, it is noteworthy that the anxiolytic property of A-MR in the hippocampus is shared by $5\text{-}HT_{1A}$ receptor agonists (22,41), benzodiazepines (42), and allopregnanolone (2). Presumed stimulation of MR by low-dose CORT has been reported to produce a rapid decrease in $5\text{-}HT_{1A}$ receptor expression (26). Similarly, low-dose CORT administration was observed to block the increased expression of various subunits of the $GABA_A$ receptor complex in the hippocampus after ADX (30). A hypothesis emerges from these findings that A-MR-induced anxiolytic effects may result from a magnification of the effector response observed as a result of stimulation of hippocampal $5\text{-}HT_{1A}$ or $GABA_A$ receptors. More definitive experiments should investigate the effects of A-MR administration into

the dorsal hippocampus on 5-HT_{1A} or $GABA_A$ receptor binding and effector responses.

In summary, the anxiolytic effect of blockade of hippocampal MR suggests that activity at this receptor site is important in the evaluation of an aversive or threatening environment, thereby promoting behavioral adaptations to environmental demands. Future research should be aimed at clarifying the mechanism(s) by which CORT elicits behavioral effects via its transduction of neural signals that may be principally mediated by neurotransmitter systems already implicated in the regulation of anxiety and the neuroendocrine response to stress.

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