Pergamon

Pharmacology Biochemistry and Behavior, Vol. 48, No. 2, pp. 497–503, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0003-Z

Mouse Strain Differences in the Behavioral Effects of Corticotropin-Releasing Factor (CRF) and the CRF Antagonist α-Helical CRF_{9-41}

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Received 4 June 1993

CONTI, L. H., D. G. COSTELLO, L. A. MARTIN, M. F. WHITE AND M. E. ABREU. *Mouse strain differences in the behavioral effects of corticotropin-releasing factor (CRF) and the CRF antagonist α-helical CRF_{9-41}.* PHARMACOL BIOCHEM BEHAV **48**(2) 497–503, 1994.—The effect of the corticotropin-releasing factor (CRF) antagonist α-helical CRF_{9-41} (αH CRF_{9-41}; 25 and 50 μg) was examined in four strains of mice (BALB/C, NIH Swiss, CF-1, and CD) in the elevated plus-maze anxiolytic test and found to significantly increase percent open arm activity in only the BALB/C mice. A marginal anxiolytic response was obtained in NIH Swiss, while no effect of the antagonist was noted in CF-1 or CD mice in this test. Diazepam (1–4 mg/kg IP) significantly increased percent open arm activity in all four mouse strains. Thus, all strains were sensitive to the effects of a known anxiolytic in this test. The locomotor-suppressing effect of the agonist CRF was assessed in the four strains of mice. While CRF suppressed locomotor activity in each of the strains, the peptide was more efficacious and more potent in the BALB/C strain than in any of the other three strains. The behavioral differences in responsiveness to CRF and the antagonist αH CRF_{9-41} could not be explained on the basis of differential binding of CRF to forebrain membranes in the four mouse strains. These data suggest that the BALB/C mouse is more sensitive to the behavioral effects of CRF and its antagonist than other strains and may be a useful strain for examining the effects of CRF and/or stress.

CRF α-Helical CRF_{9-41} Mouse strains Plus-maze BALB/C Locomotor activity Anxiolytic Anxiety model

AS a hypothalamic peptide, corticotropin-releasing factor (CRF) has a major role in stimulating the stress-induced release of adrenocorticotropin (ACTH) from the pituitary and, thus, glucocorticoids from the adrenal (16,20,21,26). Therefore, CRF contributes to an animal's hormonal response to stressful environmental demands.

Both CRF and CRF receptors are also localized in extrahypothalamic brain regions including the amygdala and the locus coeruleus (6–8,24). Such localization of the peptide indicates that CRF may act as a neurotransmitter in addition to acting as a hypothalamic hormone. Further evidence for this hypothesis comes from studies on the effects of CRF following CNS administration. ICV administration of CRF produces behavioral, physiological, and immunological responses similar to those induced by stress, independently of its pituitary/adrenal actions (4,10,13). For example, CRF ICV decreases locomotor and exploratory activity of rodents in a novel environment (3,23), is anxiogenic in animal models (1,9), stimulates sympathetic nervous system outflow (5,12), and decreases natural killer cell activity (14). These effects of CRF are attenuated by central but not peripheral administration of the CRF antagonist α-helical CRF_{9-41} (αH CRF_{9-41}), suggesting that these responses are due to CNS activity of the peptide. Additionally, central administration of CRF produces electrophysiological changes in the locus coeruleus, indicating that the peptide induces receptor-mediated events in the nucleus (27).

The CNS actions of CRF suggest that in stress- or anxiety-producing situations αH CRF_{9-41} would act as an anxiolytic

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by attenuating the effects of endogenously released CRF. In animal models of anxiety, behavior is assessed in a stressful environment under circumstances which presumably cause the release of CRF. Stress-induced behavioral effects which are attenuated by anxiolytics are also attenuated by αH CRF_{9-41}. In rats, αH CRF_{9-41} has been shown to decrease foot shock-induced freezing (15) and is anxiolytic in the fear-potentiated startle paradigm (25).

The elevated plus-maze test is widely used to assess the anxiolytic action of drugs from a number of classes in both rats and mice (17,19). In this test, behavior is dependent on rodents' natural tendency not to enter the open arms of an elevated maze. Thus, no discrete, unavoidable fear-eliciting stimulus is presented. In the present experiments, the plus-maze test was used to examine the behavioral effects of αH CRF_{9-41} in four strains of mice. In this way, the anxiolytic effect of the CRF antagonist was studied in strains which may be differentially sensitive to the stressfulness of the environ-

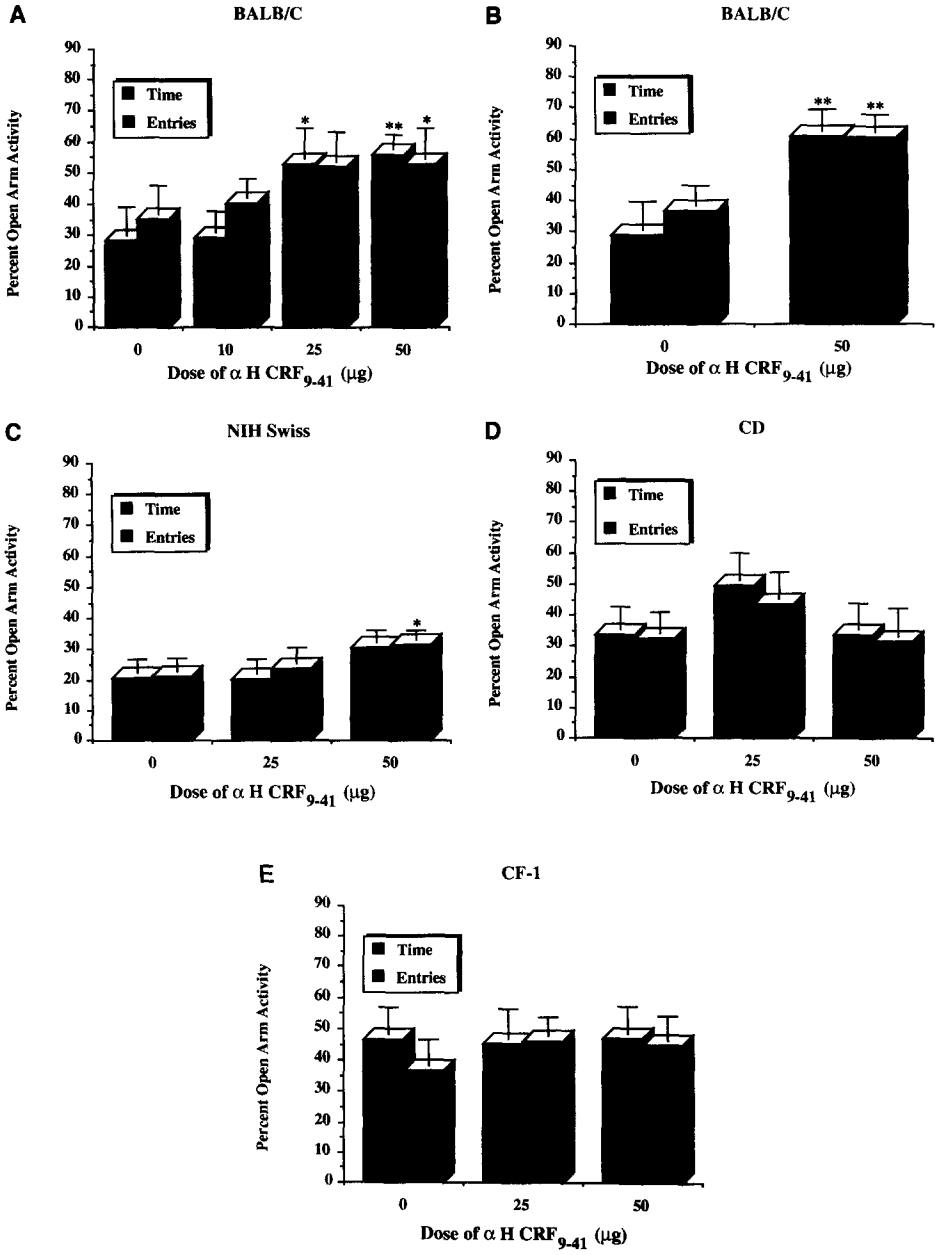

FIG. 1. Percent time spent in and percent entries into the open arms of the elevated plus-maze following ICV administration of αH CRF_{9-41} in four strains of mice. BALB/C (A and B), NIH Swiss (C), CD (D), and CF-1 (E) mice were administered antagonist (10–50 μg) 1 h prior to a 5-min observation period on the plus-maze. See Methods section for a detailed description of procedure. Displayed are the group means (8–10 mice/group) ± SEMs. $^{**}p < 0.05$ compared to vehicle controls. $^{*}p < 0.10$ compared to vehicle controls.

TABLE 1

EFFECT OF αH CRF_{9-41} AND DIAZEPAM (DZP) ON TOTAL NUMBER OF ARM ENTRIES IN THE ELEVATED PLUS-MAZE IN FOUR STRAINS OF MICE

Treatment	Total No. Arm Entries (Mean ± SEM)	Treatment	Total No. Arm Entries (Mean ± SEM)
	BALB/C		
Vehicle (ICV)	15.6 ± 1.9	Vehicle (IP)	11.5 ± 2.2
αH CRF_{9-41}		DZP	
10 μg	17.9 ± 1.2	1.0 mg/kg	23.4 ± 2.7*
25 μg	18.1 ± 2.6	2.0 mg/kg	19.7 ± 3.8
50 μg	23.3 ± 2.0*	4.0 mg/kg	20.9 ± 2.4*
	NIH Swiss		
Vehicle (ICV)	15.4 ± 2.2	Vehicle (IP)	24.7 ± 2.9
αH CRF_{9-41}		DZP	
25 μg	20.0 ± 2.1	1.0 mg/kg	39.7 ± 2.5*
50 μg	25.3 ± 1.9*	2.0 mg/kg	35.4 ± 2.5*
	CD		
Vehicle (ICV)	16.5 ± 1.4	Vehicle (IP)	19.0 ± 1.6
αH CRF_{9-41}		DZP	
25 μg	16.0 ± 2.1	1.0 mg/kg	35.5 ± 2.2*
50 μg	16.3 ± 2.7	2.0 mg/kg	34.0 ± 3.5*
		4.0 mg/kg	31.3 ± 7.3
	CF-1		
Vehicle (ICV)	23.0 ± 2.7	Vehicle (IP)	14.4 ± 2.6
αH CRF_{9-41}		DZP	
25 μg	19.6 ± 2.6	1.0 mg/kg	20.3 ± 3.1
50 μg	22.7 ± 2.5	2.0 mg/kg	15.7 ± 2.3
		4.0 mg/kg	17.3 ± 4.6

*$p < 0.05$ compared to vehicle.

ment (22). Additionally, the locomotor response to CRF in a novel environment was examined in these four strains of mice. Finally, CRF receptor binding in the forebrain was examined in each strain.

METHODS

Subjects

Male BALB/C, NIH Swiss, CF-1, and CD mice (Harlan Sprague–Dawley, Indianapolis), which weighed 20 g upon arrival, served as subjects. Mice were housed 10 per cage in a colony maintained on a 14-h light/10-h dark cycle for two weeks prior to the experiments. All testing took place during the light portion of the cycle. Standard laboratory chow and water were available ad lib. Each animal was used in one experiment only.

Procedure

ICV Treatment. Peptides were administered ICV as previously described (11). A 26-gauge stainless steel cannula, 3 mm in length, was inserted into the ventricle at the intersection of midline and a line parallel to the anterior tip of the ear. Peptide or vehicle was infused over a 10-s period in a 5-μl volume. The accuracy of this procedure was examined by visualization of dye in the ventricular system.

Elevated plus-maze test. Each mouse received an ICV (5 μl) injection of either 25 or 50 μg αH CRF_{9-41} (Peninsula, Belmont, CA) or vehicle (0.1% bovine serum albumin [BSA] in saline) 1 h prior to being tested on the elevated plus-maze. The maze, composed of two opposing open (40 × 10 cm) and two opposing enclosed (50-cm-high walls) arms, was elevated 50 cm above the floor.

For testing, each mouse was individually placed onto the center of the maze and behavior was recorded for 5 min. During this time the number of entries into and the duration of time spent in each arm of the maze was recorded. Two measures of open arm activity were calculated: 1) percent time spent in the open arms = time spent in the open arms/time spent in all arms, and 2) percent entries into open arms = number of entries into open arms/number of entries into all arms. Additionally, total number of entries into all arms was used as a measure of activity in a novel environment.

In a second set of experiments, mice from each strain received an injection (IP) of diazepam (DZP) or saline vehicle 30 min prior to being tested in the plus-maze. This was done to ensure that all strains were sensitive to the effects of a known anxiolytic under the testing conditions employed. Following DZP (1.0–4.0 mg/kg) administration, behavior was recorded as described above.

Locomotor activity test. The effect of ovine CRF (Peninsula) on locomotor activity in a novel environment was tested in each mouse strain. CRF (0.1–1.0 μg) or BSA vehicle was administered ICV in a 5-μl volume. One hour later mice were

individually placed into an Opto-Varimex (Columbus Instruments) activity chamber. The breaking of photobeams (8 × 8 array) was recorded by a Compaq computer. The primary measure of activity was distance (cm) traveled in the 30-min session.

CRF receptor binding. In a separate set of experiments, mice from each strain were used to determine the density of CRF binding sites in the forebrain. Saturation analysis of specific [^{125}I]tyrosine^{0} ovine CRF (DuPont NEN, Boston) binding to receptors in a membrane preparation of mouse forebrain was examined using a modification of the method described by DeSouza (7). Briefly, fresh forebrain tissue (48 000 × *g* twice-washed tissue pellet) was suspended in buffer [50 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (Hepes) containing 10 mM $MgCl_2$, 2 mM ethylene glycol Bis-(β-aminoethyl ether) N,N,N'-tetraacetic acid (EGTA), 0.1 mM bacitracin, 100 KIU/ml aprotinin, and 0.1% bovine serum albumin; pH 7.0] and incubated with 12 concentrations of [^{125}I]CRF (0.05–20 nM; 20 Ci/mmol) for 2 h at room temperature in a final assay volume of 0.5 ml. Nonspecific binding was defined by 10^{-6} M ovine CRF. The binding reaction was terminated by centrifugation, and radioactivity in washed tissue pellets was measured in an LKB gamma counter. The K_d and B_{max} values were calculated using LIGAND, a nonlinear curve fitting program (18).

Data analysis. For the elevated plus-maze test, percent time and percent entries into the open arms as well as total number of arm entries were subjected to separate one-way analyses of variance (ANOVAs) with dose of αH CRF_{9-41} or DZP as between-subjects factors. Differences in distance traveled by each group in the 30-min locomotor test were analyzed by one-way ANOVA with dose of CRF as the between-subjects factor. For both sets of behavioral experiments the differences between the effects of individual doses and vehicle treatment were assessed with separate variance *t* tests. Strain differences in B_{max} and K_d values for CRF receptor binding were also analyzed by ANOVA.

RESULTS

Elevated Plus-Maze Test

Percent time spent in and percent entries into the open arms of the elevated plus-maze following the ICV administration of αH CRF_{9-41} in four strains of mice are shown in Fig. 1. In BALB/C mice, αH CRF_{9-41} produced a significant overall effect on percent time spent in the open arms of the maze, $F(3, 28) = 3.41$, $p < 0.05$ (Fig. 1A). Subsequent *t* tests revealed that 50 μg αH CRF_{9-41} significantly increased percent time spent in the open arms ($p < 0.02$). While 25 μg of antagonist resulted in a marginal effect (two-tailed *t* test, $p < 0.05$), 10 μg was without effect. In this experiment, 50 μg

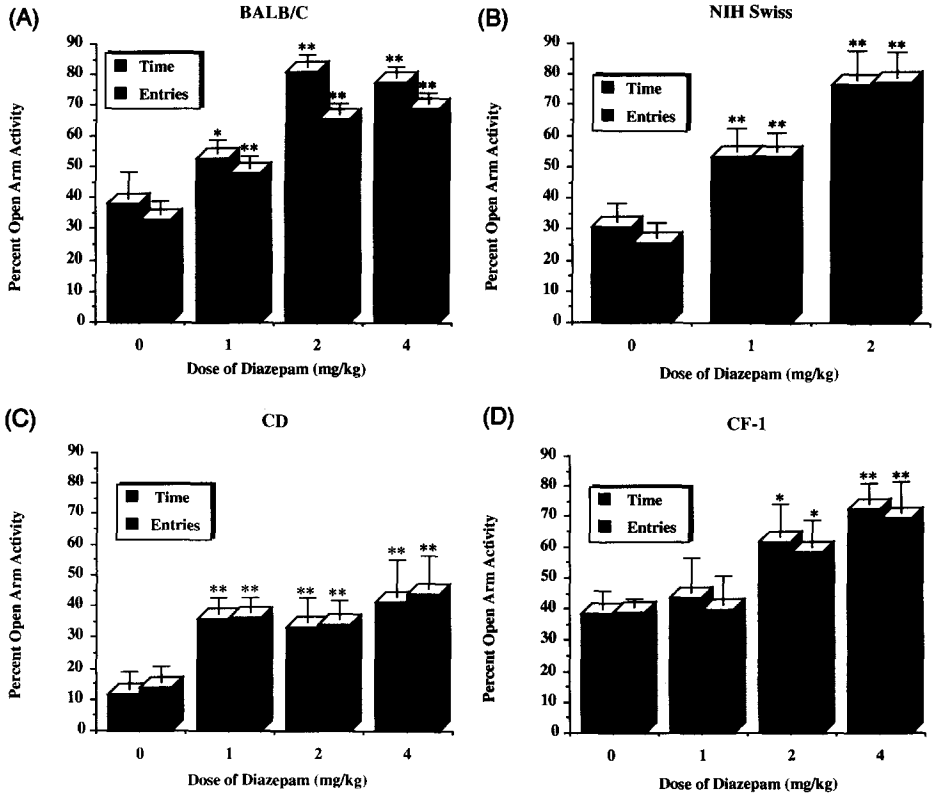

FIG. 2. Percent time spent in and percent entries into the open arms of the elevated plus-maze following administration (IP) of diazepam (DZP) in four strains of mice. BALB/C (A), NIH Swiss (B), CD (C), and CF-1 (D) mice were administered DZP (1–4 mg/kg) 1 h prior to a 5-min observation period on the plus-maze. See Methods section for a detailed description of procedure. Displayed are group means (8–10 mice/group) ± SEM. $^{**}p < 0.05$ compared to vehicle controls. $^{*}p < 0.10$ compared to vehicle controls.

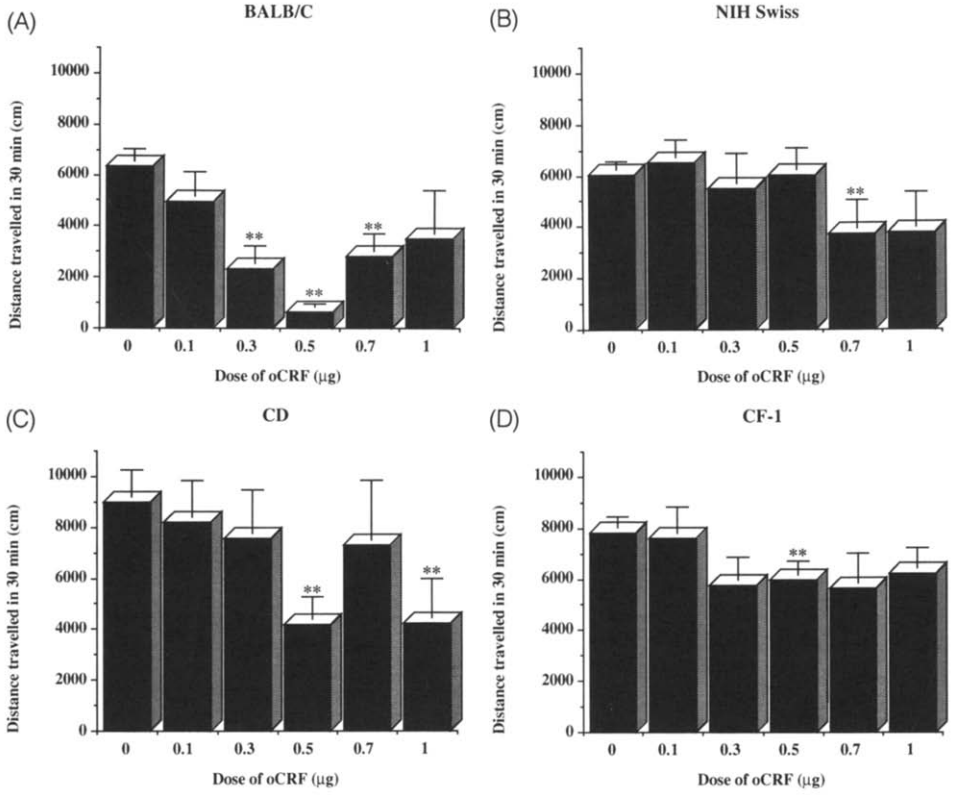

FIG. 3. The effect of CRF ICV on distance travelled (cm) in an activity chamber in four strains of mice. BALB/C (A), NIH Swiss (B), CD (C), and CF-1 (D) mice, naive to the activity chambers, were administered CRF (0.1–1.0 μg) 1 h prior to a 30-min session. The group means (8–10 mice/group) are displayed. $^{**}p < 0.05$ compared to vehicle controls.

αH CRF_{9-41} also resulted in a marginal increase in percent entries into the open arms of the maze (two-tailed t test, $p < 0.05$). Additionally, as shown in Table 1, 50 μg αH CRF_{9-41} significantly increased total number of arm entries ($p < 0.05$). In a second experiment with BALB/C mice, 50 μg αH CRF_{9-41} significantly increased both percent time spent in, $F(1, 14) = 8.41, p < 0.02$, and percent entries into the open arms, $F(1, 14) = 7.82, p < 0.02$ (Fig. 1B).

The open arm activity of NIH Swiss, CD, or CF-1 mice (Fig. 1C–E) was not significantly altered by ICV treatment with αH CRF_{9-41}. However, administration of 50 μg of antagonist resulted in a significant increase in total number of arm entries in NIH Swiss mice ($p < 0.02$) (Table 1).

In all strains tested, both percent time spent in and percent entries into the open arms were significantly higher in DZP-treated than in vehicle-treated mice (Fig. 2). In BALB/C mice, ANOVA revealed an effect of DZP on both percent time spent in the open arms, $F(3, 36) = 10.60, p < 0.001$, and percent entries into the open arms, $F(3, 36) = 16.78, p < 0.0001$ (Fig. 2A). Additionally, BALB/C mice injected with DZP made a significantly greater number of total arm entries than those injected with vehicle, $F(3, 36) = 3.63, p < 0.05$ (Table 1). DZP also had a significant effect on all three measures in NIH Swiss mice: percent time spent in open arms, $F(2, 21) = 8.32, p < 0.01$; percent entries into open arms, $F(2, 21) = 15.60, p < 0.001$; and total number of arm entries, $F(2, 21) = 5.10, p < 0.02$ (Fig. 2B). In this strain, only the effects of the two lowest doses of DZP were analyzed, since the highest dose tested (4.0 mg/kg) resulted in sedation such that NIH Swiss mice failed to enter any arms of the maze. In CD mice, all doses of DZP significantly increased both percent time spent in and percent entries into the open arms (Fig. 2C). As all doses of DZP similarly affected the percent time measure, the overall ANOVA was only marginally significant, $F(3, 27) = 2.7, p = 0.06$. Thus, the lowest dose tested may have been maximally effective in CD mice with respect to this measure. In CD mice, DZP significantly increased both percent entries into the open arms, $F(3, 27) = 3.46, p < 0.05$, and total number of arm entries, $F(3, 27) = 3.86, p < 0.02$. An overall significant effect of DZP on percent time, $F(3, 27) = 2.98, p < 0.05$, and percent entries, $F(3, 27) = 3.46, p < 0.02$, was also found in CF-1 mice (Fig. 2D).

Locomotor Activity Test

The effects of CRF ICV on distance traveled (cm) in a novel environment in the four strains of mice are displayed in Fig. 3. Although at least one of the four doses of CRF tested significantly decreased locomotor activity in each strain (as revealed by t tests), an overall significant effect was only found in BALB/C mice, $F(5, 51) = 15.90, p < 0.001$ (Fig. 3A). The lowest doses of CRF which produced significantly less locomotor activity (t test, $p < 0.05$) than vehicle ICV in each strain are as follows: 0.03 μg (BALB/C); 0.07 μg (NIH Swiss); and 0.05 μg (CD and CF-1). Following ICV vehicle treatment, BALB/C mice were less active than CD mice ($p < 0.02$) and CF-1 mice ($p < 0.05$) but were no different from NIH Swiss ($p > 0.05$).

CRF Receptor Binding

There was a trend toward decreased CRF receptor density (B_{max}) in forebrain of BALB/C mice as compared to the other strains; however, no significant differences in radioligand affinity (K_d) or CRF receptor density (B_{max}) in the forebrain were found among the four mouse strains (Table 2).

DISCUSSION

The CRF receptor antagonist αH CRF_{9-41} significantly increased percent open arm activity of BALB/C mice in the elevated plus-maze. This result suggests that αH CRF_{9-41} is anxiolytic in BALB/C mice in this test. At the doses tested, αH CRF_{9-41} had no effect on percent open arm activity in other tested strains: NIH Swiss, CD, or CF-1 mice. The lack of effect of αH CRF_{9-41} in these three mouse strains may have been due to a partial agonist effect of the peptide which counteracted the antagonist action. However, at the doses tested, no behavioral evidence for a partial agonist action of αH CRF_{9-41} was seen in the BALB/C mice in this test. Alternatively, the differential behavioral effects of αH CRF_{9-41} among the four strains may indicate that BALB/C mice are more sensitive to CRF receptor blockade under stressful circumstances than the other three strains tested. Among six mouse strains tested by Shanks et al. (22), the BALB/C showed the greatest increase in plasma corticosterone following foot shock. Thus, a CRF antagonist may have a more profound behavioral effect on BALB/C mice than on mice which are less responsive to stress. Additional evidence that the sensitivity of the subject is important in revealing the anxiolytic activity of αH CRF_{9-41} in the plus-maze test comes from a study in rats in which it was found that αH CRF_{9-41} attenuated the "anxiogenic" effect of ethanol withdrawal in the plus-maze (2). However, in ethanol-naive rats the CRF antagonist produced no effects. In rats, αH CRF_{9-41} alone has been shown to be anxiolytic in paradigms which assess behavioral effects of foot shock or fear of foot shock (15,25). Presumably these conditions are more stressful than those used for the elevated plus-maze test, in which behavior depends on the tendency of rodents to avoid open, elevated space. Thus, the anxiolytic effect of a CRF antagonist may depend on the sensitivity of the subject and the severity of the stress imposed by the experimental conditions.

DZP increased percent open arm activity in all four of the mouse strains tested. Thus, each strain was sensitive to the effects of a benzodiazepine anxiolytic. This indicates that under the conditions employed in the present studies, it was possible to detect an effect of an anxiolytic in each strain. DZP also increased total number of maze arm entries in BALB/C, NIH Swiss, and CD mice. This effect stands in contrast to the known sedative effect of DZP and indicates that, in the elevated plus-maze, an anxiolytic may increase total activity. The result may be due to the fact that the plus-maze was a novel environment in which an anxiolytic may be expected to increase exploration. In BALB/C and NIH Swiss mice, αH CRF_{9-41} (50 μg) also increased total number of arm entries. This effect of the CRF antagonist may have also been due to an anxiolytic action.

In the second set of experiments reported here, BALB/C, NIH Swiss, CD, and CF-1 mice were tested for locomotor activity following the administration of CRF ICV. In this way, the responsiveness of the four strains to the agonist was also examined. In all four strains CRF reduced locomotor activity in a novel test environment. This result is in agreement with those showing that CRF decreased locomotor activity of rats in a novel environment (23). In the present experiments, the effect of CRF was greater and the minimal effective dose lower in BALB/C mice than in the other three strains. These results suggest that BALB/C mice were more sensitive to central administration of the CRF agonist as well as the CRF antagonist. BALB/C mice were less active than CD or CF-1 mice following vehicle treatment. This indicates that the BALB/C strain may have been more sensitive to the stressful novel environment than the CD or CF-1 mice. However, BALB/C and NIH Swiss mice were equally active following vehicle treatment, even though BALB/C were more responsive to CRF, indicating that baseline locomotor activity may not predict sensitivity to CRF.

One possible explanation for the relative increased sensitivity to CRF as assessed by locomotor activity and the enhanced responsiveness to an antagonist observed in the BALB/C strain is a difference in activity of the endogenous CRF pathways which could be reflected in either amount or affinity of CRF receptors. However, assessment of CRF receptor density and affinity in forebrain homogenates using standard radioligand binding methods did not reveal any significant differences between BALB/C and the other mice strains in this regard. Although CRF receptor density in the forebrain did not differ among the four mouse strains, a number of other mechanistic factors may have contributed to the differential behavioral sensitivity to CRF and αH CRF_{9-41}. One possibility is that CRF receptor density in other brain regions is different among the four strains. Alternatively or additionally, there may be a strain-dependent effect on the ability of CRF to stimulate adenylate cyclase or otherwise affect second messenger function. It is also possible that the behavioral effects of CRF and the CRF antagonist are due to indirect actions on non-CRF neurotransmitters for which differential behavioral sensitivity may exist. Finally, the stress-induced release of endogenous CRF may be different among the four mouse strains. Thus, behavioral sensitivity to CRF may be under the control of numerous factors which deserve investigation.

The results of the present experiments indicate that αH CRF_{9-41} is anxiolytic in the plus-maze test in a mouse strain (BALB/C) which may be particularly sensitive to environmental stress (22). Additionally, BALB/C mice were more sensitive to the locomotor-suppressing effect of CRF than the other strains tested. If BALB/C mice are genetically predisposed to increased behavioral responsivity to stress, and if endogenous CRF has a role in this predisposition, the present behavioral results would be expected. BALB/C mice may provide a good model with which to study the interaction between stress and CRF, and with which to investigate the contribution of CRF to stress-induced behavioral changes. Such a model would contribute to an understanding of the role of CRF in stress-related clinical disorders such as anxiety and depression.

TABLE 2

[^{125}I] CRF BINDING IN FOREBRAIN TISSUE OF FOUR MOUSE STRAINS

Strain	K_d (nM)	B_{max} (fmols/mg pro)
BALB/C	1.36 ± 0.20	219 ± 39
NIH Swiss	1.31 ± 0.17	288 ± 25
CD	1.83 ± 0.40	271 ± 63
CF-1	1.18 ± 0.17	275 ± 14

Values represent the mean ± SEM of data obtained from three to five mice.

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