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Proconflict Effect of $ACTH_{1-24}$: Interaction With Benzodiazepines

MARIA G. CORDA,[1] MARZIA ORLANDI AND WALTER FRATTA*

*Department of Experimental Biology, Chair of Pharmacology and *Department of Neuroscience, University of Cagliari, Cagliari, Italy*

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CORDA, M. G., M. ORLANDI AND W. FRATTA. *Proconflict effect of $ACTH_{1-24}$: Interaction with benzodiazepines.* PHARMACOL BIOCHEM BEHAV **36**(3) 631–634, 1990.—The intracerebroventricular injection of $ACTH_{1-24}$ (0.1–10 μg) produced a dose-dependent decrease of punished licking periods in the conflict test in rats. The same treatment failed to modify unpunished behavior. A similar effect was produced by α-MSH (0.25–5 μg), whereas $ACTH_{4-10}$ and $ACTH_{11-24}$ were ineffective in doses up to 10 μg. The proconflict effect of $ACTH_{1-24}$ was completely antagonized by diazepam (1.5 mg/kg IP) and partially by the benzodiazepine receptor antagonist Ro 15-1788 (5 mg/kg IP). The results are in line with a possible "anxiogenic" action of $ACTH_{1-24}$.

ACTH α-MSH Benzodiazepines Conflict test Anxiety Rat

SEVERAL lines of evidence suggest that the adrenocorticotropic hormone ACTH mediates important behavioral and biochemical responses to stress [for a review see (9)]. Exposure to experimentally induced stressful situations activates the pituitary-adrenal system leading to release of ACTH and steroids from the anterior pituitary and the adrenal cortex respectively (18). In intact rats the intracerebroventricular (ICV) administration of ACTH has been found to produce excessive grooming, a rodent behavioral response to stressful situations, and to inhibit social contacts in the social interaction test, an effect indicative of anxiogenic properties (10, 12, 14, 16). In addition, $ACTH_{4-10}$, a fragment of ACTH, has been shown to antagonize the anxiolytic effect of benzodiazepines in rats (20). The purpose of the present study was to further examine the stress-like behavioral effects of ACTH in rats. To this aim we evaluated the effect of the ICV administration of $ACTH_{1-24}$ in the Vogel's conflict test, an experimental situation very sensitive to states of stress or fear (5,21). We also compared the effects of $ACTH_{1-24}$ with those produced by $ACTH_{4-10}$ and $ACTH_{11-24}$ (two fragments of $ACTH_{1-24}$) and by melanocyte stimulating hormone, α-MSH, since these peptides mimic the effects of $ACTH_{1-24}$ in a number of behavioral and biochemical tests [for a review see (1, 8, 9)].

Finally, we determined whether $ACTH_{1-24}$-induced effects in the conflict test could be attributed to the "anxiogenic" properties of this peptide by attempting to antagonize these effects with the anxiolytic drug diazepam.

METHOD

Animals and Surgery

Male Sprague-Dawley CD rats (Charles River, Como, Italy) weighing 150–180 g were housed under conditions of controlled temperature and lighting (light period: 0800–2000 hr) and were given free access to food and water.

Rats were anaesthetized with chloral hydrate (350 mg/kg IP) and positioned in a David Kopf stereotaxic instrument. A 7 mm, 23 gauge stainless steel guide cannula was aimed 1 mm above the lateral ventricle and secured to the skull with two stainless steel screws and dental acrylic cement. The coordinates for the operation were: 1 mm posterior to bregma, ±2 mm lateral and 3.5 mm below the surface of the skull at the point of entry, with the toothbar elevated 5 mm from the interaural plane. At the end of the surgery a 30 gauge stylet was placed inside the cannula. Correct placement of the cannula was verified postmortem by injecting 10 μl of methylene blue ICV and checking for diffusion through the ventricle.

Conflict Test

Conflict testing was performed as previously described (5), one week after surgery. Briefly, groups of 16–24 rats were deprived of water 60 hr prior to the conflict session. The rat to be tested was placed in a clear Plexiglas box (28 × 28 × 20 cm), with a stainless steel grid floor. This box was enclosed in a sound-attenuated ventilated chamber (Lafayette Instruments, IN). Water was provided through a stainless steel drinking tube extending 1 cm into the box, 3 cm above the floor. The drinking tube and the grid floor were connected to a constant-current shock generator and to a drinkometer. The shock generator was controlled by a Timex Sinclair 1000-computer which delivered one shock (0.20 mA), lasting 0.5 sec, every 3 sec of cumulative drinkometer output. This 3 sec period of cumulative drinking was termed "licking period."

[1]Requests for reprints should be addressed to Maria G. Corda, Dept. of Experimental Biology, Chair of Pharmacology, University of Cagliari, Via Palabanda 12, 09123 Cagliari, Italy.

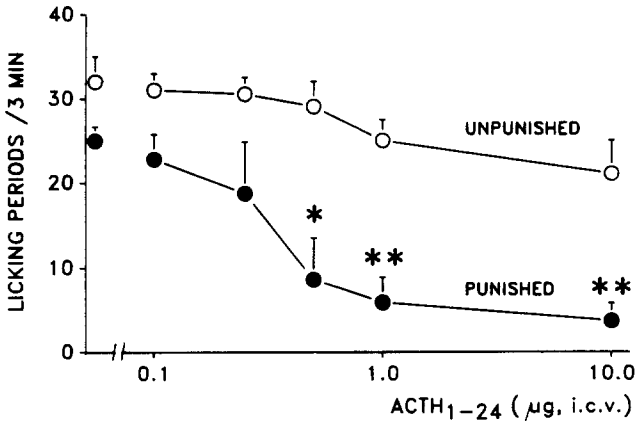

FIG. 1. Dose-response curve for the proconflict effect of $ACTH_{1-24}$. $ACTH_{1-24}$ (0.1–10 μg) was administered ICV 40 min before the test. Licking periods were measured during unpunished (empty circles) or punished (full circles) sessions. Each point represents the mean ± S.E.M. of 4–6 rats per group. *$p<0.01$, **$p<0.001$ compared to the respective saline-treated groups (Duncan's multiple range test).

Unpunished drinking was measured in a separate group of animals with the current intensity set to 0 mA. Experiments were carried out between 1100 and 1800 hr. One hour before the test, each rat was placed in the test chamber and their latency to drink was measured; the animals with a latency of more than 3 min were discarded. Drugs were injected at different times before the test. At the time of the test each rat was allowed to habituate to the new environment by leaving it in the test chamber for 15 min. At the end of this period the drinking tube was inserted into the cage and the animal was allowed to lick for 3 sec (one licking period) before the first shock was delivered. A 3-min test period started at the end of the first shock. The total number of licking periods were recorded by the computer.

Drugs

On the day of the test rats received an intracerebroventricular (ICV) injection of vehicle (5 μl saline) or $ACTH_{1-24}$ (0.1–10 μg), $ACTH_{4-10}$ (2.5–10 μg), $ACTH_{11-24}$ (2.5–10 μg) or α-MSH (0.25–5 μg). Injections were made 40 min before the test by replacing the stylet wire with an 8 mm stainless steel cannula attached to a PE 10 tubing (filled with the infusate) and to a 10 μl Hamilton syringe. The injection time was 30 sec. Following injection, the stylet wire was replaced. Twenty min after ICV injection a separate group of rats was injected intraperitoneally (IP) with diazepam (1.5 mg/kg) or Ro 15-1788 (5 mg/kg). Both drugs were suspended in distilled H_2O with 100 μl of Tween 80/5 ml and were injected in a volume of 3 ml/kg of body weight. $ACTH_{1-24}$, $ACTH_{4-10}$, $ACTH_{11-24}$ and α-MSH were kindly donated by Dr. L. Maitre (Ciba-Geigy, Basel, Switzerland). Diazepam and Ro 15-1788 were kindly supplied by Hoffmann-La Roche, Basel, Switzerland.

Statistics

The main effects of drugs were analyzed by one-way analysis of variance (ANOVA) and, where applicable ($p<0.05$), post hoc comparison of the means was carried out using the Duncan's multiple range test.

RESULTS

The ICV administration of $ACTH_{1-24}$ induced a dose-dependent (0.1–10 μg) decrease in the number of punished licking periods (correlation coefficient of regression analysis = .94). As shown in Fig. 1, the effect was statistically significant after a dose of 0.5 μg and the maximal inhibition was produced by a dose of 1 μg, $F(5,20)=5.3$, $p<0.005$. The same doses of $ACTH_{1-24}$ were unable to modify unpunished behavior, $F(5,18)=2.0$, $p>0.1$.

TABLE 1

EFFECT OF α-MSH ON CONFLICT TEST

Treatment	Dose (μg/5 μl)	Licking Periods/3 Min: Unpunished	Licking Periods/3 Min: Punished
Saline		27.1 ± 2.5	22.2 ± 2.7
α-MSH	0.25	—	15.5 ± 4.5
α-MSH	1	24.0 ± 3.1	9.4 ± 4.3*
α-MSH	2.5	21.0 ± 2.3	5.0 ± 1.1†
α-MSH	5	19.1 ± 2.7	8.3 ± 1.8†

Saline or α-MSH was administered ICV 40 min before the test. Licking periods were measured during unpunished or punished sessions. Each value represents the mean ± S.E.M. of 4–6 rats per group. *$p<0.05$, †$p<0.005$ vs. the respective saline-treated group (Duncan's multiple range test).

A similar proconflict effect was produced by the ICV administration of α-MSH. In fact, a 58% and 78% decrease in the number of punished licking periods was observed with a dose of 1 and 2.5 μg, respectively, $F(4,21)=4.2$, $p<0.01$ (Table 1). The suppression of punished licking produced by α-MSH occurred at doses that failed to change unpunished behavior. In contrast, $ACTH_{4-10}$ and $ACTH_{11-24}$, two fragments of ACTH, did not affect punished behavior in doses up to 10 μg (Table 2).

To test whether the effect of $ACTH_{1-24}$ on conflict test was sensitive to the action of anxiolytic benzodiazepines, in subsequent experiments diazepam (1.5 mg/kg) was administered IP 20 min after the peptide. As shown in Fig. 2, diazepam completely antagonized the proconflict effect of $ACTH_{1-24}$. Interestingly, a significant blockade of the $ACTH_{1-24}$ effect was also observed after the administration of 5 mg/kg of the benzodiazepine receptor antagonist Ro 15-1788, $F(5,43)=7.7$, $p<0.0001$.

It is worth noting that at the doses used in these experiments neither diazepam nor Ro 15-1788 modified punished licking, either in the proconflict test (Fig. 2) or in the anticonflict test (current intensity for the shock set at 1 mA, results not shown).

DISCUSSION

Previous studies have shown that the intracerebroventricular

TABLE 2

EFFECT OF $ACTH_{4-10}$ AND $ACTH_{11-24}$ ON CONFLICT TEST

Treatment	Dose (μg/5 μl)	Shocks/3 Min
Saline		26.9 ± 3.1
$ACTH_{4-10}$	2.5	25.1 ± 2.6
$ACTH_{4-10}$	10	27.8 ± 2.1
$ACTH_{11-24}$	2.5	24.0 ± 3.2
$ACTH_{11-24}$	10	23.5 ± 2.7

Saline or peptides was administered ICV 40 min before the test. Licking periods were measured during the punished session (1 licking period = 1 shock). Each value represents the mean ± S.E.M. of 4 rats per group.

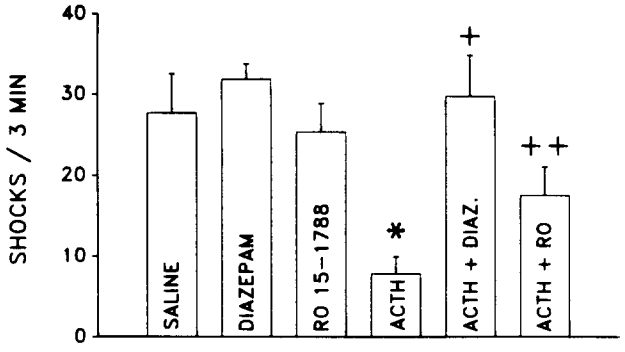

FIG. 2. Proconflict effect of $ACTH_{1-24}$: Antagonism by diazepam and Ro 15-1788. $ACTH_{1-24}$ (1 μg) was administered ICV 40 min before the test. Diazepam (1.5 mg/kg) or Ro 15-1788 (5 mg/kg) were given IP 20 min after $ACTH_{1-24}$. Licking periods were measured during the punished session (1 licking period = 1 shock). Each value represents the mean ± S.E.M. of 8–9 rats per group. *$p<0.001$ vs. saline; +$p<0.001$; ++$p<0.05$ vs. ACTH alone (Duncan's multiple range test).

administration of $ACTH_{1-24}$ produces behavioral changes consistent with increased arousal or "anxiety" (10, 12, 14, 16). The present study extends these findings by examining the effects of $ACTH_{1-24}$ on a widely accepted model of anxiety, the modified Vogel's conflict test (5,21). $ACTH_{1-24}$ produced a marked reduction in the number of licking periods during punishment. This effect is not due to a decrease in food-motivated behavior since it occurs at doses that failed to change unpunished licking. It is also unlikely that the action of $ACTH_{1-24}$ in the conflict test is due to an increase in pain sensitivity. In fact, the maximal decrease in the number of punished licking periods was observed at a dose of 1 μg, which has no hyperalgesic effect in the tail-flick test [(19), our unpublished results]. Thus, the proconflict effect of $ACTH_{1-24}$ is likely to reflect an increased fear or "anxiety." This conclusion is in line with the results of File and Clark (12) and Niesink and Van Ree (16) on the "anxiogenic" effect of $ACTH_{1-24}$ in the social interaction test in rats. Accordingly, other behavioral effects of $ACTH_{1-24}$ have been interpreted as the result of increased arousal or fear, such as the enhanced acquisition of avoidance responses, the induction of grooming behavior and the reduction of exploration in a novel situation (8,14).

Our results show that the entire aminoacidic sequence of $ACTH_{1-24}$ is not essential in inducing proconflict effect. In fact we have obtained similar results by injecting α-MSH, an endogenous product derived by the cleavage of $ACTH_{1-39}$, whose amino acid sequence corresponds to that of $ACTH_{1-13}$. Moreover, we found that the sequence $ACTH_{11-24}$ is ineffective on our model up to the dose of 10 μg, supporting the idea that the active sequence is contained within the structure of α-MSH. It is known that the shortest sequence of ACTH or α-MSH-like peptides for several central actions is represented by the $ACTH_{4-10}$ or in some cases by the even shorter $ACTH_{4-7}$ (7,8). However, some discrepancy exists on the anxiogenic effectiveness of $ACTH_{4-10}$. In fact, File and Clark (12) found this peptide active in the social interaction test, while Vellucci and Webster (20) reported $ACTH_{4-10}$ as inactive in the Geller-Seifter rat conflict test. Our data are in agreement with the latter report. In fact, in our test we found that $ACTH_{4-10}$ is inactive up to the dose of 10 μg. These findings indicate that "anxiety" measured in different experimental conditions is not equivalent and may involve different neuronal substrates.

Our study has also shown that the proconflict effect of $ACTH_{1-24}$ is completely antagonized by diazepam. This result confirms that exogenously administered $ACTH_{1-24}$ produces an increase in "anxiety" that can be reversed by classical antianxiety drugs. Interestingly, also Ro 15-1788 was able to partially antagonize the proconflict effect of $ACTH_{1-24}$. Ro 15-1788 is a benzodiazepine receptor antagonist almost devoid of intrinsic activity (15). However, in particular experimental conditions it has been shown to have partial agonist or inverse agonist properties (3, 4, 6, 13, 22). Thus, it may be proposed that in our experimental situation (i.e., high levels of fear or "anxiety" induced by $ACTH_{1-24}$), Ro 15-1788 behaves as a partial agonist at the benzodiazepine receptor. In agreement with this hypothesis, it has been suggested that "anxiety" or stress-enhancing compounds such as corticotropin releasing factor (CRF) and amphetamine may enhance the partial agonist properties of Ro 15-1788 in certain test situations (2).

Alternatively, it is tempting to speculate that $ACTH_{1-24}$ may produce "anxiety" by interfering with the function of an endogenous anxiogenic ligand for benzodiazepine receptors. In fact, compounds that bind to benzodiazepine receptors and produce proconflict effects in rats have been recently extracted and purified from brain (11,17). If this were the case, then Ro 15-1788 would antagonize the effect of $ACTH_{1-24}$ by blocking the binding of this hypothetic endogenous ligand to benzodiazepine receptors.

In conclusion, this study shows that $ACTH_{1-24}$ in rats has a proconflict effect which is mimicked by α-MSH, is blocked by diazepam and is reduced by Ro 15-1788. These findings give further support to the view that $ACTH_{1-24}$ produces behavioral effects consistent with an increased fear or "anxiety" in rats.

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