Pharmacology Biochemistry and Behavior, Vol. 44, pp. 925–930, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Further Characterization of the Behavioral Effects of Peripherally Administered Corticotropin-Releasing Factor in Guinea Pigs

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Received 6 July 1992

BECKER, L. A. AND M. B. HENNESSY. *Further characterization of the behavioral effects of peripherally administered corticotropin-releasing factor in guinea pigs.* PHARMACOL BIOCHEM BEHAV 44(4) 925–930, 1993.—Guinea pig pups were either not injected (NI) or given SC injection of either saline vehicle (SAL) or 14 μg of corticotropin-releasing factor (CRF). In an isolation test, mean number of vocalizations and several measures of locomotor activity were markedly lower for CRF pups than for NI or SAL controls. CRF pups defecated less than did SAL pups. No differences were found among conditions for self-grooming. Behavioral freezing was shown by only two pups in the entire study. Significantly more CRF pups displayed piloerection, eye-closing, and a characteristic crouched stance than did controls. In a defensive withdrawal test, no differences among conditions were found for the proportion of pups entering a darkened chamber or for the latency to enter the chamber; however, CRF pups entered the chamber significantly fewer times during the 60-min test than did controls. There were no differences among conditions in the distance swum or number of turns made in a forced-swim test. These results replicate our earlier findings that peripheral injection of CRF suppresses vocalizing and a measure of locomotor activity in isolated guinea pig pups and identifies a number of additional behavioral effects. Of central interest here, the results indicate that the suppression of vocalizing and locomotion during isolation is not due to an increase in competing stress-related behavior or to diminished motor capacity.

CRF Isolation Defensive withdrawal Forced swim Guinea pig

CORTICOTROPIN-releasing factor (CRF) and high-affinity binding sites for CRF have been located in various regions of the CNS and periphery (1,7,20,24). CRF appears to be a critical component in the body's diverse responses to stressors. At the pituitary, CRF stimulates release of ACTH (corticotropin) and β-endorphin (22). Centrally administered CRF produces autonomic activation, stimulates central catecholaminergic systems, and leads to various behavioral responses characteristic of animals exposed to aversive stimuli (5,6,10,28). Scores of studies have now reported stress-related behavioral changes following central CRF administration; however, only a few studies have found behavioral effects of CRF given by peripheral routes. In adult rhesus monkeys, IV CRF produced a number of behavioral changes, most notably, an increase in vocalizing and "lying down" behavior, and a decrease in self-grooming (17). In adult rats, effects of SC CRF on avoidance performance have been observed (29) and intracardial (IC) and IV CRF were found to decrease behavioral measures of nociception (3,11). The nociceptive effect did not appear to be mediated by opioids (3). In their discussion, Ayesta and Nikolarakis (3) note that rats receiving IC CRF also exhibited "lying down" behavior similar to that previously reported in monkeys (17).

Recently, we found that 7 and 14 μg of SC CRF suppressed the vocalizing and locomotor activity of guinea pig pups during a 30-min period of isolation in a novel environment (12). Behavior was not suppressed following injection of ACTH, and the CRF effect was not reversed with naloxone, indicating that the behavioral suppression was not secondary to actions of ACTH, glucocorticoids, or β-endorphin. Because CRF is a 41-amino-acid peptide, it is unlikely that there was extensive penetration of injected CRF across the blood-brain barrier. Moreover, 5 μg CRF infused through an indwelling cannula directly into a lateral ventricle did not suppress the vocalizing of isolated guinea pig pups (15). Thus, the peripherally injected CRF probably was not acting at a central receptor to suppress behavior. It is possible that receptors that have been identified in the periphery, in particular those in the adrenal medulla and sympathetic ganglia (1), were involved in mediating the effect.

One obstacle in understanding the effects of SC CRF is the lack of information regarding the internal state associated

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with the reduction in vocalizing and locomotor activity. That is, the frequency of these behaviors theoretically could have declined because the pups were calmed because they were "freezing" in response to a heightened level of "stress," because they had been rendered incapable of significant muscular activity, or through some other means altogether. To begin to address this issue, the present study examined the effect of SC CRF on various behavioral measures to provide a behavioral context for the previously observed suppression of vocalizations and locomotor activity.

There were four specific purposes of this study. The first was to investigate the effect of CRF on additional stress-related behavioral measures. Because CRF has been found to produce various physiological and behavioral responses typical of animals in stressful situations (10), one explanation for our findings of suppressed vocalizing and locomotor activity could be that the peripheral injection of CRF led to a stress-like state and emission of associated behavior (e.g., behavioral freezing), which was incompatible with vocalizing and locomotor activity. To assess the effect of CRF on stress-related behavior, freezing, self-grooming, and defecation were scored in an isolation test. Increased self-grooming in rats has been reported both in stressful situations (16) and following *central* CRF administration (19) [though as noted above, IV CRF reduced self-grooming in rhesus monkeys (17)]. Aversive stimuli, such as exposure to a brightly lit open field, affects the frequency of defecation in rodents (2,9). In guinea pigs, as opposed to rats and mice, exposure to an open field tends to inhibit defecation (26). We also examined the guinea pig pups in a defensive withdrawal task. A heightened stress-like state was expected to result in greater initial withdrawal from, and less subsequent exploration of, a novel open arena. Our task was modeled after that of Takahashi, Kalin, Vanden Burgt, and Sherman (25), who found evidence that elevated *central* CRF enhanced withdrawal and decreased exploration in rats.

The second purpose of the study was to determine whether the reduced locomotor activity of CRF-injected guinea pig pups reflected reduced motor capacity. That is, did the CRF render pups incapable of vigorous motor activity? We used a modified version of the forced-swim test developed by Rylands (23) specifically to assess the effect of drugs on locomotor activity in guinea pigs. Based upon Rylands' (23) observation that both swimming speed and the tendency to reverse direction in a brief swim test are sensitive measures of drug-induced motor impairment, we measured both the distance pups swam and the number of times they reversed direction in a 30-s test.

The third purpose was to assess in greater detail the influence of CRF on locomotor activity in the isolation test. In our earlier work (12), the measure of locomotor activity was the number of times that pups crossed lines dividing the floor of the test cage into four sections. This measure showed considerable variation even across groups of pups that had not been injected prior to testing. Further, although the 14-μg dose of CRF virtually eliminated line crossings, pups appeared to be exhibiting some locomotor activity within a single section of the cage. Therefore, the present study employed two additional measures of locomotor activity that would be sensitive to locomotion occurring within a single section of the cage: the total duration and number of bouts of locomotor activity.

The fourth purpose of the present study was to document our informal observation that pups injected with CRF tended to exhibit piloerection, eye-closing, and a "crouched" stance. Accordingly, we noted whether or not pups exhibited each of these characteristics in the isolation test.

METHOD

Animals

Animals were 37 preweaning albino guinea pigs (*Cavia porcellus*) of the Hartley strain from 14 litters bred in our laboratory. Each lactating female and her litter were housed in a clear, polycarbonate, maternity cage (48.3 × 38.1 × 20.3 cm). Water and guinea pig chow were freely available. Diets were supplemented with alfalfa. The colony room was maintained on a 12h L : 12h D cycle (lights on 0700 h). The day of birth was designated day 0. Litters larger than three pups were culled to three. Only litters of two or three were used in this experiment.

Conditions and General Test Procedures

Approximately equal numbers of male and female pups were assigned to each of three conditions: no injection (NI), saline vehicle injection (SAL), and 14 μg CRF injection (CRF). To control for litter effects, no more than one pup from a litter was assigned to any one condition. For each test, SAL and CRF pups were injected SC (0.2 ml vol) in the nape of the neck and then returned to the home cage for 60 min prior to testing. Pups in the NI condition were not disturbed prior to testing. The dose and pretreatment interval were chosen because they resulted in the clearest suppression of vocalizing and locomotor activity (line crossings) in our earlier study (12). CRF solution was maintained at −80°C and injected within 5 min of thawing. To minimize disturbance to the litter on any one day, each test was conducted over a several day range and no more than two pups from the same litter were tested on the same day. The interval between tests was kept constant for each pup.

Isolation Test

On days 20–21, the experimental animal was transported (< 10 s) in a carrying cage from the colony room to the test room located in the same laboratory suite as the colony room. Here, the pup was placed alone into a clear, empty, uncovered, polycarbonate cage (47.5 × 23.8 × 20.0 cm) for 30 min. All pups were placed at one end and facing a long wall of the cage. The cage was located on a table under standard fluorescent room lighting. An observer behind one-way glass recorded the number of pup vocalizations ["whistles" (4)] on a hand counter. To assess locomotor activity, the number of line crossings and bouts of locomotion, as well as the total duration of locomotion were recorded. Other behavioral measures examined were the total duration of grooming and freezing (immobility of entire body for at least 60 s) and the number of bouts of defecation. (It was not possible to accurately count the number of fecal boli because pups commonly consume boli during defecation.) Finally, the presence or absence of piloerection, eye-closing, and a crouched stance were noted during the 30-min test. The crouched stance involves holding the body close to the ground with the head tucked into the body.

Initially, all continuous variables other than vocalizing were scored using an event recorder. However, the first animals from all conditions that were tested vocalized at an unusually low rate. Because the presence of the event recorder was the only change in the test room from earlier experiments, we suspected that low-amplitude noise emitted by this device was inhibiting vocalizing. Therefore, we removed the event recorder and began to record behavior with a check sheet (frequency measures) and stopwatches (duration measures).

The same pattern of vocalizing across conditions was seen with and without the event recorder, but the number of vocalizations more than doubled, increasing to levels comparable to those of earlier experiments. Therefore, only data from pups tested following removal of the event recorder were included in analyses for the isolation tests (n = 7–8/group).

Defensive Withdrawal Test

On days 22–23, pups were tested in the defensive withdrawal apparatus, which consisted of an open area (55.9 × 55.9 × 11.5 cm) with wooden floor and Plexiglas walls and an attached enclosed wooden chamber (24.1 × 24.1 × 18.4 cm). The open area and darkened chamber were connected by a passageway (12 × 12 cm). The floor of the apparatus was covered to a depth of 6 mm with wood chips that were replaced between tests. The tests were conducted under dim lighting provided by two 60-W incandescent bulbs hanging 1 m above and 1 and 2 m to the side of the center of the open area. Dim lighting was used because pilot data indicated that this would lengthen the initial latency to enter the darkened chamber and thereby minimize "ceiling" effects. The pup was placed into the apparatus 30 cm from, and facing toward, the darkened chamber entrance. Behavior was recorded for 60 min with a low-light videocamera (Panasonic Model WV1550) and time-lapse video recorder (Panasonic Model AG-6010S). The proportion of pups entering the darkened chamber and the latency to initially enter the chamber served as measures of withdrawal, and the total number of entries into the chamber was used to assess exploration.

Forced-Swim Test

On days 23–24, the pup was tested in the swim apparatus (289.6 × 8.0 × 45.7 cm) following the general procedures of Rylands (23). The apparatus, which was constructed of wood, and water-sealed with a plastic liner, was filled with water (21°C) to a depth of 20 cm so that the pup could not touch bottom. The pup was placed into the apparatus 91 cm from one end and facing in the opposite direction. The test lasted 30 s and was conducted under standard fluorescent lighting. An observer traced the path of the animal with a pencil on a scaled drawing of the apparatus. From the tracing, the distance swum and number of turns made were derived. Turning almost always consisted of a 180° rotation; those few occasions on which pups turned 360° were also scored as single turns.

Data Analysis

Similar patterns of results were observed for males and females. Therefore, for ease of presentation gender is not included as a variable here. For continuous variables, analyses of variance (ANOVAs) followed by Newman-Keuls posthoc tests were the analyses of choice. When data violated assumptions for parametric tests, nonparametric Kruskal-Wallis ANOVAs by ranks followed by Mann-Whitney U-tests were used. For dichotomous variables, significance was assessed with the Fisher exact probability test.

RESULTS

Isolation Test

The number of vocalizations emitted by pups varied significantly across conditions, $F(2, 19) = 11.97$, $p < 0.01$. As expected, CRF pups vocalized less than did NI ($p < 0.01$) or SAL ($p < 0.01$) controls (Fig. 1). All measures of locomotor activity also varied across conditions [line crossings, $H = 10.98$, $p < 0.01$; duration of locomotion, $H = 13.28$, $p < 0.01$; number of bouts of locomotor activity, $F(2, 19) = 6.30$, $p < 0.01$]. For each of these measures, CRF pups were less active than were pups of either the NI or SAL control groups (all $ps < 0.05$), which, in turn, did not differ from each other.

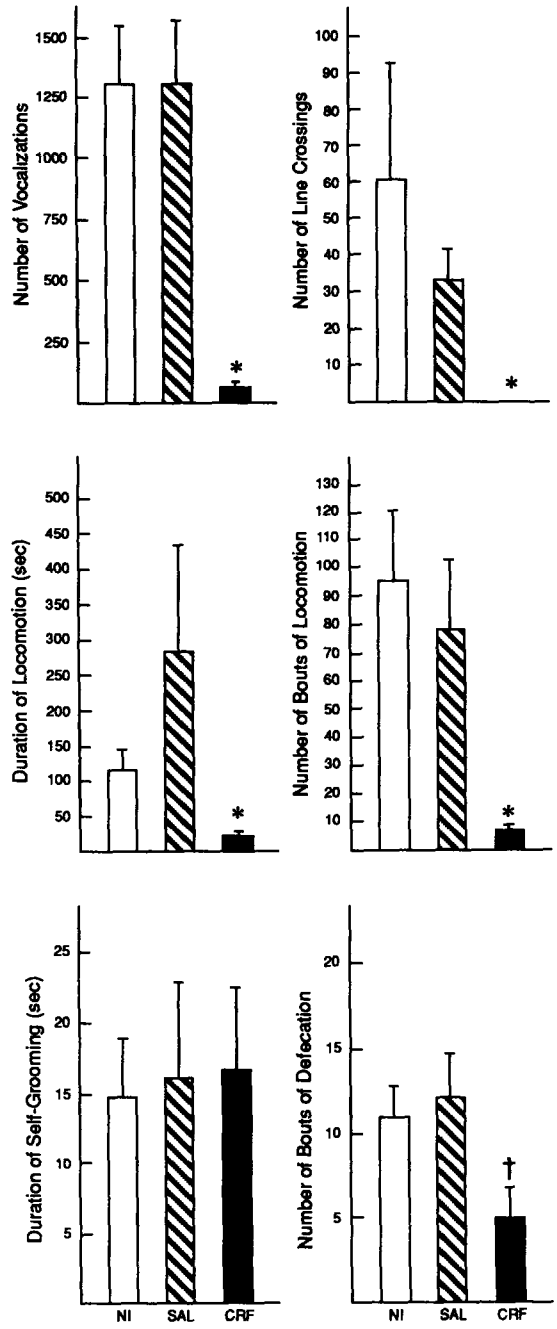

FIG. 1. Mean and SE (vertical lines) for vocalization, locomotion, self-grooming, and defecation measures for noninjected (NI), saline-injected (SAL), and corticotropin-releasing factor injected (CRF) pups in the isolation test. *Differs from NI and SAL, $ps < 0.05$. † Differs from SAL, $p < 0.01$.

TABLE 1

PROPORTION OF NI, SAL, AND CRF PUPS THAT EXHIBITED PILOERECTION, EYE-CLOSING, AND A CROUCHED STANCE IN THE ISOLATION TEST

	Condition		
Measure	NI	SAL	CRF
Piloerection	0/7	0/7	8/8*
Eye-closing	0/7	0/7	6/8*
Crouching	1/7	1/7	8/8*

*Differs from NI plus SAL, $p < 0.01$.

There were no line crossings by any CRF pups; yet, as illustrated in Fig. 1, the other two measures of locomotor activity showed that CRF pups did exhibit some, albeit minimal, locomotor activity within the section of the cage in which they remained.

Behavioral freezing was a rare event, occurring only twice in the isolation tests: once in a CRF pup and once in an NI control. Pups of all conditions groomed themselves, and there was no difference across conditions in the number of seconds spent grooming (Fig. 1). The overall effect for the number of bouts of defecation was significant, $F(2, 19) = 3.50$, $p < 0.05$. This was due to CRF pups defecating less than did SAL pups (Fig. 1).

Piloerection, eye-closing, and a crouched stance all were observed frequently in CRF animals. A greater proportion of CRF pups exhibited each of these characteristics than did controls ($ps < 0.01$, CRF vs. NI plus SAL, Table 1).

Defensive Withdrawal Test

There was no significant difference across conditions for either the proportion of pups entering the darkened chamber or the latency to initially enter (pups not entering were assigned a latency of 3,600 s; Table 2). However, the variable behavior of CRF pups deserves comment. Whereas 10 CRF pups entered the chamber upon release from the experimenter's hand (8 of 10 within 4 s, 1 in 26 s, and 1 in 462 s), the remaining 2 pups moved upon release to a corner of the Plexiglas walls of the open area, where they stayed for the remainder of the 60-min test. These two pups exhibited piloerection, eye-closing, and crouching (those in the chamber could not be observed). Like the two CRF pups remaining in the open area, those that entered the chamber displayed little subsequent activity, resulting in a significant effect across conditions for the measure of total number of entries into the chamber, $H = 17.90$, $p < 0.01$ (Table 2). CRF pups exhibited fewer total entries than did either NI ($p < 0.01$) or SAL ($p < 0.01$) controls.

Forced-Swim Test

All pups swam and remained afloat for the entire test. There was no significant difference across conditions for either the total distance swum or the number of turns made (Table 3). Interestingly, there was a nonsignificant tendency for CRF pups to swim *farther* than did controls.

DISCUSSION

SC CRF suppressed the vocalizing and crossing into different sections of the isolation test cage by guinea pig pups, which replicates our earlier results (12). CRF-injected pups did not exhibit prolonged bouts of behavioral freezing. Rather, they showed a small amount of locomotor activity within the section of the cage in which they remained and exhibited normal levels of self-grooming. In all, we found no evidence that an increase in stress-related behavior interfered with the emission of vocalizations or locomotor activity.

Further, there was no clear evidence that CRF induced a stress-like state. In addition to the lack of effects on the measures of freezing and self-grooming in the isolation test, there was no effect of CRF on withdrawal measures in the defensive withdrawal test. CRF pups did defecate less than SAL pups during isolation, and they made fewer total entries into the enclosed chamber of the defensive withdrawal apparatus than did controls. However, reduced defecation might be either secondary to reduced overall activity or a result of a direct action of CRF on gastrointestinal motility [although in rats peripherally administered CRF stimulates rather than inhibits fecal excretion; (30)]. Similarly, the small number of entries into the enclosed chamber by CRF pups seems at least as likely to be a manifestation of suppressed locomotor activity as an indication of a diminished tendency to explore the apparatus. The finding that two CRF pups remained in a corner of the open area for essentially the entire test session suggests that the effect on locomotor activity was powerful, competing even with the tendency to withdraw into the darkened chamber. In sum, without supporting evidence from other measures, it appears that the reduced defecation and total number of entries into the darkened chamber by CRF pups reflect factors other than an increase in an internal stress-like state.

The results of the forced-swim test indicate that CRF pups were capable of vigorous locomotor activity. Therefore, it

TABLE 2

PROPORTION OF PUPS THAT ENTERED DARKENED CHAMBER, MEAN (±SE) LATENCY TO ENTER, AND MEAN (±SE) NUMBER OF ENTRIES MADE FOR NI, SAL, AND CRF PUPS

	Condition		
Measure	NI	SAL	CRF
Proportion entering	12/12	11/11	12/14
Latency to enter	279.3(±269.2)	210.9(±172.9)	553.4(±346.5)
Number of entries	12.8(±3.4)	10.2(±2.7)	1.6(±0.4)*

*Differs from both NI and SAL, $p < 0.01$.

TABLE 3

MEAN (±SE) cm SWUM AND TURNS MADE BY NI, SAL, AND CRF PUPS

	Condition		
Measure	NI	SAL	CRF
Distance swum	614.7(±31.0)	609.6(±56.1)	709.1(±35.1)
Number of turns	6.5(±0.5)	5.3(±0.6)	4.8(±0.7)

does not appear that the suppression of vocalizing and locomotion in the isolation test were the result of reduced motor capacity.

The present findings confirm our informal observation that SC CRF produced piloerection, eye-closing, and a crouched stance. The meaning of these effects is unclear. One possibility is that they reflect a state of malaise, although if this is the case the malaise does not appear to be accompanied by fever because rectal temperature of pups previously was found not to be affected by 14 μg of SC CRF (12). The eye-closing and crouched stance bear some resemblance to effects of peripherally administered CRF in other species. Orth et al. (21) reported that some of their human volunteers "dozed intermittently or constantly throughout the experiment." Because guinea pig pups often appear to sleep in a crouched stance, the eye-closing and crouched stance observed in CRF pups in the present experiment could reflect increased sleepiness following CRF injection. Similarly, the crouched stance may correspond to the "lying down" behavior previously reported in rats and monkeys following exogenous peripheral CRF (3,17). However, these possibilities are only speculative at this point. We cannot be certain, for instance, that the observed eye-closing is not the result of ptosis rather than sleepiness. Moreover, whether the characteristics observed here are directly related to the suppression of vocalizing and locomotor activity also remains uncertain.

In our earlier work, we found that 7 and 14 μg CRF suppressed vocalizing and locomotor activity whereas 0.07 and 0.7 μg had no effect (12). The 7- and 14-μg doses are comparable on a body weight basis to doses found to produce behavioral effects following peripheral injection in other species [(3,17); guinea pig pups of the ages tested here weigh approximately 265–300 g]. One potential mechanism for behavioral effects of peripheral CRF is through effects on blood pressure. In some species, peripherally administered CRF has been reported to reduce blood pressure (e.g., 17,27), an effect that could contribute to the behavioral suppression observed in the present study. However, this does not appear to be the case. In recently completed work, we found that 14 μg CRF had no systematic effect on the blood pressure of guinea pig pups 60 min later (Williams, Nolan, and Hennessy, unpublished). Another possibility is that CRF acts at known peripheral CRF binding sites, such as those identified in the adrenal medulla and sympathetic ganglia (1). Perhaps stimulation of these receptors results in activation of afferent fibers that inhibit central systems underlying behavior.

In rat pups, emission of ultrasonic vocalizations by individual animals during isolation was found to be positively correlated with a measure of nociception (18). Because peripherally administered CRF has been shown to diminish nociception in rats (3,11), it is possible that the suppression of vocalizations (and perhaps of locomotor activity) in guinea pig pups is related to analgesia induced by CRF.

During prolonged isolation, infant guinea pigs and monkeys have been found to show a gradual reduction in vocalizing and a simultaneous increase in plasma cortisol levels (8,13,14). Because injections of CRF, but not ACTH, suppressed the vocalizing of guinea pig pups, and because this reduction was not naloxone reversible, we previously suggested that high and/or sustained elevations of CRF may contribute to the reduced vocalizing that normally occurs during prolonged isolation (12). At the present time, we cannot determine the extent to which the effects of peripherally injected CRF reflect physiological vs. pharmacological actions; nonetheless, the findings of the current study shed some light on this issue by indicating that the CRF-induced suppression of vocalizations and locomotor activity is not due to either the elicitation of incompatible stress-related behavior or to diminished motor capacity.

ACKNOWLEDGEMENTS

The authors thank Michael Williams for assistance with the apparatus. This work was supported by Grant BNS-8822240 and a Research Experiences for Undergraduates supplemental award from the National Science Foundation. The study constitutes the senior thesis of L.A.B., who is now at the Department of Psychology, Binghamton University.

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