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# Differential Behavioral Actions of Corticotropin-Releasing Factor (CRF)

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VELDHUIS, H. D. AND D. DE WIED. *Differential behavioral actions of corticotropin-releasing factor (CRF).* PHARMACOL BIOCHEM BEHAV 21(5) 707–713, 1984.—In order to elucidate the involvement of the 41 amino-acid residue corticotropin-releasing factor (CRF) in the modulation of brain functioning, the behavioral profile of the releasing hormone was determined using tests for spontaneous behavior, grooming, active and passive avoidance behavior. Intracerebroventricular (ICV) administration of CRF in a dose that does not stimulate the pituitary-adrenal axis, resulted in an activation of open-field behavior, as measured by ambulation and rearing activities. Also grooming activity was significantly enhanced after central application of CRF. In hypophysectomized rats, which show an impaired shuttle-box avoidance acquisition, CRF restored acquisition for the duration of the treatment. Paradoxically, extinction of pole-jumping active avoidance behavior of intact rats was facilitated by the releasing factor, even in adrenalectomized animals. Passive avoidance behavior was affected bidirectional: higher doses of CRF (300 ng), given subcutaneously (SC), attenuated passive avoidance retention, probably via activation of the pituitary-adrenal system resulting in high corticosterone levels. Lower doses (30 ng), however, which were also given SC did not stimulate pituitary-adrenal activity, and facilitated retention of passive avoidance behavior. Central administration of CRF in very low doses (30 pg) had the same effect as higher doses of CRF given SC, i.e., inhibition of passive avoidance retention. Taken together, the data indicate that CRF can affect behavior via a direct action on the central nervous system. The question remains whether this activity is an intrinsic property of CRF, or mediated by the release of hormones or neuropeptides.

Corticotropin-releasing factor Open-field behavior Grooming Avoidance behavior Plasma-corticosterone level

IN 1981 Vale and coworkers reported the elucidation of a molecular structure of an ovine hypothalamic corticotropin releasing factor (CRF), which represents a 41-amino acid containing polypeptide with potent ACTH and β-endorphin releasing activity [25,27].

Besides releasing activity, CRF also acts within the brain to stimulate sympathetic outflow [7], which in turn influences cardiovascular function [16], and/or results in the development of hyperglycemia [7]. Furthermore, CRF administration by the intracerebroventricular route resulted in an enhanced behavioral effect on novelty, as measured in an open field, on grooming and ingestive behavior [6, 21, 26].

The incentive that CRF not only acts on the release of several hormones (ACTH, β-endorphin) but also could be the first mediator of the stress response [23], stimulated us to characterize the activity of CRF on several behaviors associated with stress. For this reason the effects of CRF were investigated on spontaneous behavior in a novel environment (open-field), grooming, and on passive and active avoidance behaviors. CRF was administered peripherally as well as intracerebroventricularly so as to differentiate peripheral from central effects and the participation of other hormonal (pituitary-adrenal) systems in its behavioral influences. In view of the intrinsic corticotropin releasing activity peripheral plasma corticosterone levels were monitored after SC and ICV injections of CRF in order to establish possible indirect effects.

## METHOD

### *Animals*

Male Wistar albino rats, weighing 140–180 g at the onset of the experiments, were obtained from the Central Breeding Laboratories of TNO, Zeist, The Netherlands. Housing conditions consisted of a constant temperature of 22°C at a 14 hr light:10 hr dark cycle, with free access to food and tap water.

### *Surgery*

Bilateral adrenalectomy was performed through a dorsal approach under ether anesthesia. After the operation the animals were placed in groups of five with 0.9% saline as drinking water. Sham-operated control animals went through the same operative procedure, but the adrenals were left intact; they were given normal tap water.

Hypophysectomy was performed via the transauricular route under ether anesthesia [20]. Loss of body weight, adrenal atrophy and macroscopic inspection of the sella turcica served as criteria for the completeness of hypophysectomy. Only data from completely hypophysectomized rats were used in the present study. Sham-hypophysectomized animals were operated in the same way, except that the pituitary of these animals was not aspirated.

For intracerebroventricular (ICV) administration, a pol-

yethylene cannula was implanted into one of the lateral cerebral ventricles under Hypnorm (Philips Duphar B.V., Amsterdam, The Netherlands) anesthesia [13]. Animals were allowed to recover from the cannulating operation for at least six days. The correct positioning of the place of the tip of the cannula was checked macroscopically by injection of methylene blue after the experiments had been completed.

*Treatments*

Synthetic CRF (a gift from Dr. F. Labrie) was dissolved in 0.9% saline at 0°C. Injections were given subcutaneously (SC) in the neck, or intracerebroventricularly (ICV) in a volume of 0.5 ml respectively 1 μl. Control animals received the same volume of the vehicle (saline). All behavioral tests involved blind procedures where the experimenter did not know the treatment codes.

*Open Field Behavior*

The open field apparatus consisted of a walled circular arena (radius 40 cm, height 31 cm). The floor was divided into oblong blocks with an 8 cm radius circle in the centre for scoring ambulatory activity. The room remained dark during testing and 60 W lamp 40 cm from the floor served as the light source. The animals were subjected to a 5 min test in the open field. Each animal was placed individually in the center of the apparatus. The number of floor units crossed (ambulation, divided in the outer and in the inner part of the open field), the rearings (also in the outer and inner part), the frequency and duration of grooming episodes and the defecation were recorded [33]. Treatments were given intracerebroventricularly 1 hr prior to the open-field test.

*Grooming*

Grooming behavior was recorded as described before [17]. Briefly, five days prior to the experimental session, a plastic cannula (see above) was implanted into one of the lateral cerebral ventricles. After injection of CRF or the vehicle, the rats were placed individually into a novel environment, i.e., glass boxes (24×12.5×14 cm) in a low noise room, and recording of grooming began 15 minutes thereafter. Recording of the behavior was performed by the 15th sec sampling procedure, i.e., the observer determined every 15th sec for 50 min whether or not the rat displayed one of the following elements: vibrating, washing, grooming, scratching, paw licking and tail licking. In the 50 min observation period a maximum of 200 positive 15th sec grooming scores could be obtained.

*Active Avoidance in a Shuttlebox*

Avoidance conditioning was performed in a shuttlebox. The procedure was slightly different from that described previously [9]. The sound of a buzzer served as the conditioned stimulus (CS) for 5 sec prior to the unconditioned stimulus (US) of a scrambled electric footshock (0.05 mA) delivered through the grid floor of the shuttlebox. When the rat had crossed the barrier within 5 sec of CS presentation, the CS was immediately terminated and the response was recorded as a conditioned avoidance response (CAR). If the rat failed to make the response within 20 sec after the onset of CS, both CS and US were terminated. A total of ten conditioning trials were given daily for 14 days. The average intertrial interval was 60 sec, varying between 40 and 80 sec. The total number of CAR's scored by each animal during the first seven and the second seven sessions of ten trials served as the index of avoidance behavior. Sham-operated rats were treated daily with saline and hypophysectomized animals received saline or peptide treatment SC from day 1–7 of avoidance conditioning 1 hr before each session.

TABLE 1

EFFECT OF SC AND ICV ADMINISTRATION OF CRF ON PLASMA CORTICOSTERONE LEVELS IN THE RAT

| Treatment* | n | Plasma corticosterone (μg/100 ml)† |
|---|---|---|
| SC | | |
| saline | 5 | 6.50 ± 1.12 |
| 30 ng CRF | 7 | 6.52 ± 1.65 |
| 300 ng CRF | 6 | 11.57 ± 0.64‡ |
| 1000 ng CRF | 5 | 13.24 ± 0.55‡ |
| ICV | | |
| saline | 5 | 1.93 ± 0.60 |
| 0.3 ng CRF | 5 | 2.49 ± 0.74 |
| 3 ng CRF | 6 | 2.70 ± 0.53 |
| 30 ng CRF | 6 | 4.66 ± 1.06 |
| 300 ng CRF | 5 | 11.12 ± 2.42‡ |

*CRF was administered SC or ICV 1 hr prior to sacrifice.
†Mean ± S.E.M. is given.
‡$p<0.05$ vs. saline.

*Active Avoidance in a Pole-Jumping Situation*

Active avoidance in a pole-jumping situation was carried out as described previously [10,29]. Rats were conditioned to avoid the US of an electric footshock (0.20 mA, AC) by jumping onto a pole (diameter 1.5 cm) located in the center of a box (30×30×40 cm). The CS was a light signal. The US was applied if an avoidance response had not occurred within 5 sec after the onset of the CS. The CS remained on during presentation of the US for maximal 20 sec. Ten acquisition trials were given daily with an intertrial interval of 60 sec, ranging between 40 and 80 sec. The 4 day acquisition paradigm used in these pole-jumping experiments was designed to produce a prolonged extinction in the saline-injected rats. Acquisition training for 4 days was followed by extinction sessions at day 5. Ten non-reinforced trials were presented per session in which the CS was terminated immediately after the rat had jumped onto the pole within 5 sec (CAR) or after 5 sec in the absence of avoidance. Those animals which made 8 or more avoidances at the first extinction session on day 5 were used for further experimentation. The rats received peptide or saline treatment SC immediately after the end of the first extinction session and two more extinction sessions were run at 2 and 4 hours after the first one.

*Passive Avoidance Behavior*

Animals were trained in a step-through type one-trial learning passive avoidance test [1]. The experimental apparatus consisted of an illuminated platform attached to a large, dark compartment equipped with a grid floor. After habituation to the dark compartment (2 min) rats were placed on the platform and allowed to enter the dark compartment, which they normally did within 15 sec. On the next day after three

TABLE 2

EFFECT OF ICV TREATMENT WITH CRF ON OPEN-FIELD BEHAVIOR OF RATS

| Treatment* | Amb. wall† | Amb. centre | Rearing wall | Rearing free | Grooming | Defecation |
|---|---|---|---|---|---|---|
| saline | 119.8 ± 7.5 | 4.5 ± 0.9 | 5.3 ± 0.7 | 2.7 ± 0.8 | 14.5 ± 4.4 | 2.7 ± 1.0 |
| 0.1 ng CRF | 147.5 ± 11.3 | 9.5 ± 1.1 | 6.0 ± 0.6 | 3.0 ± 1.7 | 12.3 ± 3.6 | 3.5 ± 0.8 |
| 1 ng CRF | 109.3 ± 4.1 | 10.2 ± 2.0 | 6.8 ± 0.7 | 2.2 ± 0.7 | 19.2 ± 3.7 | 3.5 ± 0.9 |
| 10 ng CRF | 140.3 ± 10.7 | 15.0 ± 1.7‡ | 10.2 ± 0.9§‡ | 3.5 ± 1.3 | 10.7 ± 1.3 | 2.3 ± 0.8 |

*Treatments were given intracerebroventricularly 1 hr prior to the open-field test.
†Mean ± S.E.M. is given; n=6.
‡$p<0.01$ vs. saline (Newman-Keuls multiple range test).
§$p<0.05$ vs. 0.1 ng and 1 ng CRF (Newman-Keuls multiple range test).

more trials, unavoidable scrambled footshock (0.35 mA, 2 sec) was delivered through the grid floor of the dark compartment (learning trial). Rats were removed from the shock box 10 sec after termination of the shock. Passive avoidance latencies were tested 24 hr and 48 hr following the learning trial; the rat was placed on the platform and the latency to enter the dark compartment was measured up to a maximum of 300 sec. Treatment with CRF or saline were given immediately after the learning trial (post-learning treatment) or one hour prior to the retention test (pre-retention treatment), SC and/or ICV.

*Plasma Corticosterone Determinations*

Corticosterone levels were measured in plasma after dilution in 0.2% ethylene glycol and heat inactivation (30 min, 80°C). A radioimmunoassay based on an antiserum against corticosterone-21-hemisuccinate was used [30].

*Statistics*

Analysis of variance (ANOVA, SPSS program) and post hoc comparisons of group means evaluated with the Student Newman-Keuls Multiple Range Test (SNK, SPSS program) were used for analysis of parametric data. Mann-Whitney U-test was employed for non-parametric data [24].

## RESULTS

*CRF Effects on Plasma Corticosterone Levels*

In order to establish the possible role of stimulation of the pituitary-adrenal axis as one of the causes for the effects of CRF on behavior, the influence of several doses of CRF, administered SC and ICV, on plasma corticosterone was determined (Table 1). After peripheral administration CRF in doses of 300 and 1000 ng significantly elevated plasma corticosterone levels. Central administration of CRF in doses ranging from 0.3 ng to 300 ng revealed that the highest dose used, i.e., 300 ng significantly increased plasma corticosterone levels, measured 1 hr after administration. Thus, from this experiment it can be concluded that administration of CRF either SC or ICV in doses of 300 ng or more, leads to a stimulation of the pituitary adrenal system and as a consequence, behavioral effects could be ascribed to circulating pituitary and/or adrenal hormones instead of a direct effect of CRF.

*Open Field Behavior*

Subcutaneous administration of CRF 1 hr prior to the open field test in doses ranging from 10 ng to 1000 ng did not affect any of the indices scored in the open field test during a 5 min observation period (data not shown). However, using the ICV route of administration a dose-dependent increase in ambulation in the inner part and rearing in the outer part was observed (Table 2). The highest dose tested, i.e., 10 ng ICV significantly increased both parameters. With this dose, no effect was found on grooming or defecation.

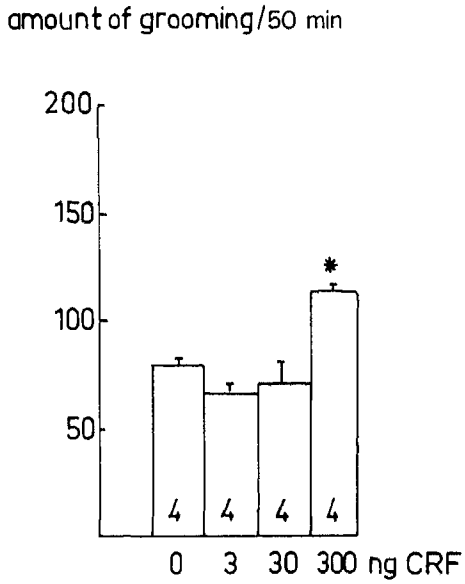


FIG. 1. The effect of intraventricular injection of CRF (3, 30 and 300 ng) and saline on grooming in the rat. The total positive 15th sec grooming scores are given (Mean±SEM of 4 animals per group). *$p<0.001$ vs. saline and other CRF treated groups (Student Newman-Keuls multiple range test).

*Grooming*

In Fig. 1 the total positive 15th sec scores are given. The data clearly show that in a 50 min observation period ICV administration in a dose of 300 ng significantly increased novelty induced grooming activity, $F(3,12)=16.21$, $p<0.001$. During the observation period no stretching and yawning syndrome (SYS) could be found, as observed after intracranial administration of ACTH and ACTH-derivatives [17].

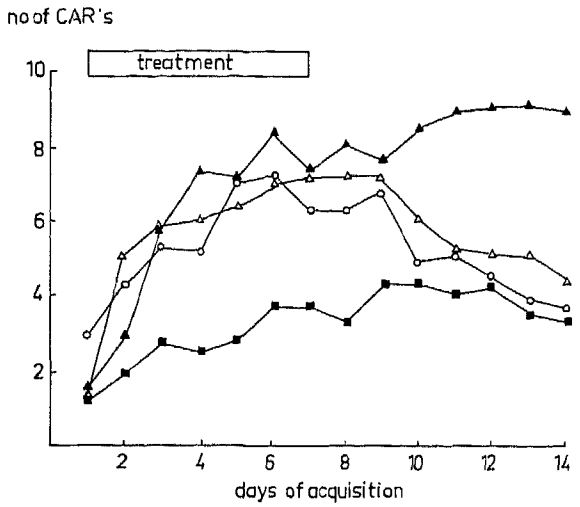


FIG. 2. Avoidance acquisition of hypophysectomized rats and sham-operated rats in a shuttle-box (US 0.05 mA). Groups of 6–7 hypophysectomized rats were treated subcutaneously 1 hr before the daily session on days 1–7 with saline (■) or CRF in a dose of 200 ng (○) or 600 ng (△). Sham-operated rats were treated SC with saline (▲). The number of CAR's is plotted versus the day of training.

*Active Avoidance Behavior*

Hypophysectomized rats, treated SC with saline exhibit poor avoidance acquisition in a shuttlebox, using a low shock intensity of 0.05 mA, and their performance does not rise above the 45% level throughout the 14 day period of training [12,15]. Treatment with 200 or 600 ng CRF SC daily for 7 days produced a gradual increase in avoidance acquisition during the first 7 days. Acquisition behavior of these rats was comparable to that of the sham-operated, saline treated animals. Termination of the treatment resulted in a progressive decrease of avoidance performance to the 40–50% level (Fig. 2).

In Fig. 3 the total number of CAR's during the first 7 days (daily treatment) and the second 7 days (no treatment) are shown. Treatment of hypophysectomized rats daily with 200 or 600 ng CRF resulted in a total number of CAR's not significantly different from that of sham-operated control animals, and significantly different from that of hypophysectomized saline treated rats, $F(3,20)=15.52$, $p<0.01$. After cessation of the treatment, during acquisition days 8–14, a significantly lower total number of CAR's was found for the CRF-treated rats in comparison to the sham-operated animals. However, rats treated with the highest dose, i.e., 600 ng, made a significantly higher number of CAR's during days 8–14 than hypophysectomized rats treated with saline (Fig. 3).

As can be seen in Fig. 4, SC treatment with 300 ng or 1000 ng CRF immediately after the first extinction session significantly facilitated extinction of the pole-jumping avoidance response 2 and 4 hr later. It is known that adrenal steroids facilitate extinction of active avoidance behavior [28]. Thus, the possibility that the observed facilitation was mediated by activation of the pituitary-adrenal activation as the result of CRF treatment was studied further. Intact animals underwent acquisition training during 4 days. On day 5 they were subjected to bilateral adrenalectomy. After a 2-day recovery period, the animals were given re-acquisition training for 3 days, and subsequently subjected to an extinction procedure

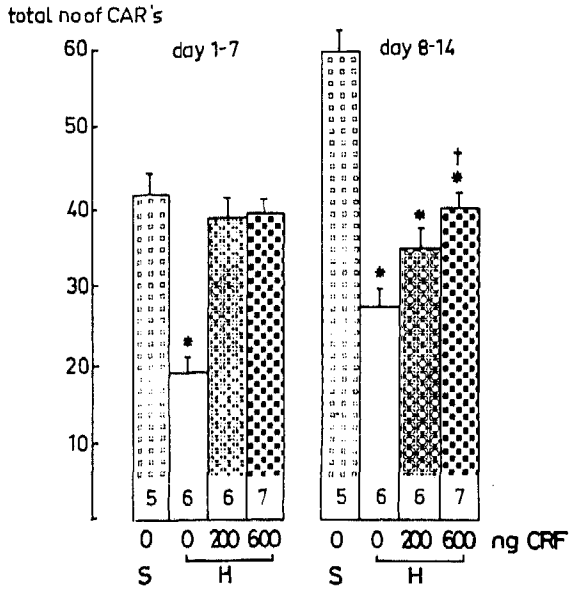


FIG. 3. Effect of CRF on total number of CAR's during the first 7 days (daily SC treatment) and the second 7 days (no treatment) of shuttle-box avoidance acquisition (data of experiment presented in Fig. 2). The numbers in the columns refer to the number of rats tested. Mean±SEM are shown. *$p<0.01$ vs. sham-operated control animals (S) (Student Newman-Keuls multiple range test). †$p<0.05$ vs. saline treated hypophysectomized rats (H) (Student Newman-Keuls multiple range test).

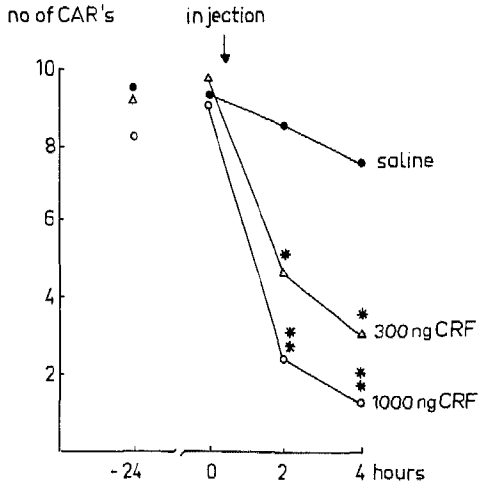


FIG. 4. Effect of CRF on the rate of extinction of pole-jumping avoidance behavior in rats. Rats were injected SC directly after the first extinction session and were tested 2 and 4 hr later. Groups consisted of 6 rats. *$p<0.025$ vs. saline injected animals. **$p<0.001$ vs. saline injected animals.

as described for intact rats. As shown in Fig. 5, administration of 1000 ng CRF SC also induced facilitation of extinction of pole-jumping avoidance behavior in adrenalectomized rats.

*Passive Avoidance Behavior*

The results of subcutaneous administration of CRF 1 hr prior to retention testing are summarized in Table 3. CRF

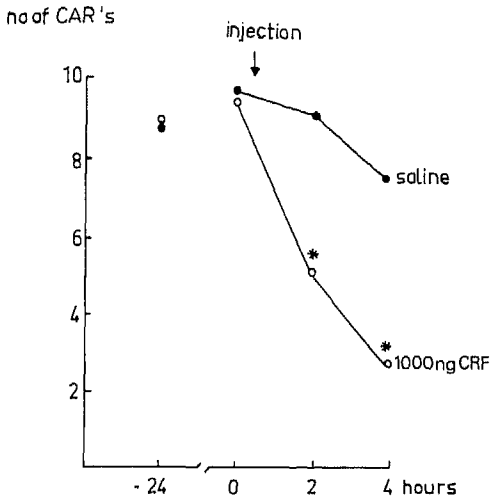


FIG. 5. Effect of CRF on the rate of extinction of pole-jumping avoidance behavior in adrenalectomized rats. Rats were injected SC directly after the first extinction session and were tested 2 and 4 hr later. Groups consisted of 6 rats. *$p<0.05$ vs. saline injected animals.

TABLE 3

EFFECT OF SC TREATMENT WITH CRF ON RETENTION OF ONE-TRIAL LEARNING PASSIVE AVOIDANCE RESPONSE IN RATS

| Treatment | n | 24 hr Retention test† |
|---|---|---|
| saline | 17 | 81 |
| 3 ng CRF* | 6 | 32 |
| 10 ng CRF | 6 | 110 |
| 30 ng CRF | 17 | 224‡ |
| 100 ng CRF | 11 | 39 |
| 300 ng CRF | 11 | 6§ |
| 1000 ng CRF | 11 | 14§ |

Differences between peptide and saline-treated rats are expressed as the level of statistical significance (Mann-Whitney U-test) as follows: ‡$p<0.025$; §$p<0.01$.

*CRF was administered subcutaneously 1 hr prior to retention testing.

†Median latency in sec.

TABLE 4

EFFECT OF ICV TREATMENT WITH CRF ON RETENTION OF ONE-TRIAL LEARNING PASSIVE AVOIDANCE RESPONSE IN RATS

| | n | 24 hr Retention test† | 48 hr Retention test |
|---|---|---|---|
| Post learning treatment* | | | |
| saline | 8 | 104 | 86 |
| 30 pg CRF | 8 | 21§ | 48 |
| 300 pg CRF | 8 | 32‡ | 55 |
| 3 ng CRF | 8 | 119 | 101 |
| 30 ng CRF | 8 | 140 | 108 |
| Pre-retention treatment* | | | |
| saline | 9 | 70 | 54 |
| 30 pg CRF | 9 | 8¶ | 8‡ |
| 300 pg CRF | 10 | 27§ | 60 |
| 3 ng CRF | 9 | 80 | 68 |
| 30 ng CRF | 8 | 111 | 134‡ |

Differences between peptide and saline treated rats are expressed as the level of statistical significance (Mann-Whitney U-test) as follows: ‡$p<0.05$; §$p<0.025$; ¶$p<0.01$.

*CRF was administered intracerebroventricularly in a volume of 1 $\mu$l either directly after the learning trial (upper part) or 1 hr prior to retention testing (lower part).

†Median latency in sec.

had a dual effect on the retention 24 hr after the learning trial. A low dose (30 ng) significantly facilitated passive avoidance behavior, while higher doses, i.e., 300 and 1000 ng attenuated this behavior. No effect could be found at any dose of CRF used when the animals were retested 48 hr after the learning trial (data not shown).

Post-learning as well as pre-retention treatment with CRF intracerebroventricularly induced a dose-dependent attenuation of passive avoidance behavior (Table 4). Treatment with 30 pg as well as 300 pg ICV significantly attenuated passive avoidance, while higher doses, i.e., 3 and 30 ng did not have a significant effect. The lowest dose (30 pg) showed a significant attenuated passive avoidance behavior after the 48 hr retention test, while the highest dose, 30 ng, significantly facilitated passive avoidance behavior at the 48 hr retention test only.

## DISCUSSION

The present results indicate that CRF can affect behavioral processes in different ways. It stimulated acquisition of shuttle-box avoidance behavior and facilitated extinction of pole-jumping avoidance behavior. Moreover, it had a bimodal effect on passive avoidance behavior, which depended on the dose and the route of administration. These differential effects of CRF might be caused by the simultaneous release of ACTH (corticosteroids) and endorphins

after peripheral as well as central administration. In fact, plasma corticosterone levels were increased following peripheral and central CRF administration. Thus, to establish the intrinsic effects of CRF on behavior, one should eliminate effects that could be the result of the action of ACTH, corticosteroids, and other neuropeptides.

Generally, corticosteroids, such as corticosterone, cortisol and the synthetic glucocorticoid dexamethasone do not affect learning of active avoidance responses [2,3]. Retention of learned behavioral responses as studied in active and passive avoidance situations is profoundly influenced by glucocorticoids. Corticoids facilitate extinction of active avoidance responses [3, 11, 18, 28]. Passive avoidance retention is suppressed by higher doses of corticosteroids [4, 5, 19], while low doses facilitated avoidance retention [19]. Although glucocorticoids specifically restore exploratory behavior in a novel environment in adrenalectomized rats [31,32], intact animals are not affected by such treatments.

The influence of ACTH and related peptides on behavior are in general opposite to those found after corticosteroid administration. Thus, ACTH and related peptides facilitate acquisition and delay extinction of active avoidance behavior and facilitate passive avoidance behavior (for an extensive review see [14]). Furthermore, excessive grooming can be enhanced after ICV adminisered ACTH, but not after systemic application [17,34]. Open field behavior of intact animals is not influenced by ACTH and related peptides [33].

Concerning endorphins, no effect has been found on acquisition of active avoidance behavior. Extinction of pole-jumping avoidance behavior, however, is profoundly influenced. $\beta$-Endorphin and $\alpha$-endorphin delayed extinction of pole-jumping behavior, while $\gamma$-endorphins had an opposite effect, i.e., facilitated extinction. Passive avoidance behavior is facilitated by $\alpha$-endorphin and attenuated by $\gamma$-endorphin (for references see [14]).

CRF administered ICV in a dose that does not stimulate the pituitary-adrenal axis, was shown to activate some indices of open-field behavior, i.e., ambulation and rearing components. Interestingly, SC administration of CRF did not cause an effect on open-field activity. Sutton and coworkers [26] who focussed on specific effects of CRF after ICV administration observed identical results in the open field test with the 10 ng dose. Interestingly, those authors found, using a 100 times higher dose of CRF ICV, which could stimulate the pituitary-adrenal axis, a general decrease in open field activity as measured by ambulation and rearing. The stimulatory effect seen in the present experiments following low doses of CRF (10 ng) might therefore be an intrinsic behavioral effect of the releasing hormone.

Relatively high doses of CRF (300 ng), injected ICV, induced grooming activity. Also others found enhanced grooming using much higher amounts of CRF [21], which was shown to be independent of pituitary-ACTH release. In our study the absence of the typical ACTH-dependent stretching and yawning syndrome (SYS) also indicates a CRF action, independent of pituitary-ACTH release.

Hypophysectomized rats, which were shown to have an impaired acquisition of shuttlebox avoidance behavior, chronically treated with CRF in doses of 200 and 600 ng, exhibited a restored avoidance learning during treatment. Cessation of treatment resulted in disappearance of the peptide effect. This effect resembles the temporary restoration of avoidance acquisition of hypophysectomized rats treated with ACTH and related peptides. This effect could therefore be due to centrally released ACTH. In this respect this study indicates that CRF may release ACTH from stores in the CNS.

Irrespective of the presence or absence of the adrenals administration of CRF caused a dose-dependent facilitation of extinction of pole-jumping avoidance behavior. The doses of CRF which induced this effect could activate the pituitary-adrenal system and corticosteroids facilitate extinction of pole-jumping avoidance behavior. Nevertheless, the effect could not be mediated by the adrenal cortex, nor by ACTH, since this hormone delays extinction of pole-jumping behavior.

Peripheral administration of CRF in doses known to stimulate pituitary-adrenal activity inhibited passive avoidance behavior. Since corticosterone administration in amounts known to produce similar blood corticosterone levels as CRF in doses of 100 and 1000 ng, affected passive avoidance behavior in a similar way, the effect of these higher doses of CRF could be ascribed to increased levels of corticosterone. However, a low dose (30 ng) had the opposite effect and facilitated passive avoidance behavior.

Central administration of CRF in doses of 30 and 300 pg, administered ICV either immediately after the learning trial or before the retention test, inhibited passive avoidance behavior. This also could not be explained by an action via pituitary-ACTH release, since ACTH and related peptides facilitate passive avoidance retention, and the doses used are too low to activate the pituitary-adrenal system.

The present data indicate that CRF has certain intrinsic behavioral effects via a direct action on the central nervous system. In might be that shorter amino-acid sequences contained in the CRF molecule convey a direct influence on neural mechanisms underlying behavior. However, the different behavioral responses suggest the influence of additional factors, for example as a result of the release of pituitary and brain peptides (combinations such as ACTH, $\beta$-endorphin, $\gamma$-MSH or others). This seems to be a possibility since CRF has recently been found by Peterfreund and Vale [22] to stimulate somatostatin secretion from cultured brain cells. However, nothing is known so far about the release of these hormones by CRF from POMC-containing terminals in the brain. Assuming the role CRF could have in the mediation of the stress response via the release of peptides from the pituitary and the brain, the present data prompt further investigations, in which a separation between endocrine and central activities of CRF should be established. For instance, it has already been shown that the C-terminal free carboxyl analog of CRF is inactive in vitro in releasing peptides from the pituitary [27]. Also, it had been reported that this CRF-analog did not influence locomotor activity as does the complete CRF molecule [26]. Studies with such CRF-derivatives, which are devoid of endocrine activities are necessary to determine the intrinsic neurotropic action of CRF. It is possible that in view of the differential behavioral actions of CRF, the releasing hormone acts as a precursor hormone for shorter fragments. CRF is a large polypeptide, containing 41 amino acids and it could well be that via biotransformation behaviorally active peptides could be generated, in the same way as has been shown for pro-opiomelanocortin and the neurohypophyseal hormones (for reviews see e.g., [8,14]).

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