Effect of the Long-term Administration of Corticotropin-releasing Factor on the Pituitary-Adrenal and Pituitary-Gonadal Axis in the Male Rat

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Abstract

The effect of the continuous exposure to ovine corticotropin-releasing factor (oCRF) was measured in adult male rats. The intravenous infusion of 0.75 nmol oCRF/h to intact rats over a 24-h period was accompanied by a peak of ACTH and corticosterone secretion that occurred during the first 90 min of administration of the releasing factor, followed by a decrease to lower, but still above control, values. Additionally, corticotropin-releasing factor (CRF)-treated rats had decreased plasma testosterone levels. The subcutaneous administration of 0.075 or 0.75 nmol oCRF/h to intact rats for 7 d also resulted in elevations of both plasma ACTH and corticosterone levels comparable to those measured after a 24-h exposure to the releasing factor, as well as dose-related hypertrophy of the adrenals and increases in pituitary ACTH content. In these animals, CRF markedly inhibited luteinizing hormone (LH) (but not follicle-stimulating hormone [FSH]), testosterone, and PRL secretion and decreased seminal vesicle weights. All the effects of CRF were mimicked by exogenously administered ACTH. By contrast, with the exception of FSH secretion, which was slightly elevated by CRF, neither CRF nor ACTH were able to significantly modify reproductive parameters in adrenalectomized animals, which suggests that the elevation of circulating levels of adrenal steroids induced by peripherally administered CRF represents major mediators of CRF-induced inhibition of fertility.

These results indicate that in the rat, the continuous stimulation of the pituitary-adrenal axis by peripherally administered CRF causes some degree of desensitization of the pituitary-adrenal axis, but is still accompanied by persistent elevations of the circulating levels of both ACTH and corticosteroids. The increased secretion of adrenal steroids by CRF-treated rats is believed to participate in the disruption of reproductive parameters observed in these rats.

Introduction

The development of response attenuation during prolonged exposure to a stimulus has been documented for a number of biological events. It is well established, for example, that the repeated administration of the hypothalamic factor, gonadotropin releasing hormone, results in the disruption of reproductive functions, a phenomenon that seems to be due in part to the response attenuation of both pituitary and gonad (review in 1).

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The ACTH response to corticotropin-releasing factor (CRF)[1] by cultured (2) or superfused (3) pituitary cells decreases during continuous exposure to CRF. Although desensitization has been demonstrated in in vitro (2, 3) as well as in certain in vivo experiments (4), we have observed in the course of toxicology studies involving daily injections of CRF to rats over a 1-wk period, that this treatment resulted in hypertrophied adrenals (4). Such results suggest that ACTH release remained elevated in those animals, and are in agreement with reports indicating that repeated stimulation of the hypothalamic-pituitary-adrenal axis by stress (5–10) or by serial injections of CRF (4) also does not lead to abolition of the ACTH secretory response.

In addition to its stimulatory action on the pituitary-adrenal axis (11, 12), we have found that CRF would markedly inhibit luteinizing hormone (LH) release when injected into the lateral ventricle of male or female rats, and that this effect was not mediated through steroids of adrenal or gonadal origin, or through increased activity of the corticotroph (13). We have, therefore, examined the effect of the continuous peripheral administration of CRF over a 24-h or 7-d period on both the pituitary-adrenal and pituitary-gonadal axis in both intact and adrenalectomized rats, and compared it with the effect of long-term infused ACTH.

Methods

Animals. Adult Sprague-Dawley male rats (230–250 g) were used throughout this study. They were fed Purina Chow (Ralston Purina Co., St. Louis, MO) and water ad lib., and housed under a 12-h light/12-h darkness regimen (lights on at 0700 h). When appropriate, adrenalectomy was performed under ether anesthesia via the lumbar approach, 6–7 d before the start of the experiment.

For the experiment dealing with exposure of CRF for 24 h, the animals were equipped with indwelling jugular and femoral catheters (12). The cannulas placed in the femoral artery were used for serial blood sampling and the cannulas placed in the jugular vein were connected to Sage infusion pumps (A. H. Thomas Co., Philadelphia, PA) that delivered 0.2 ml/h. The cannulas were protected by a metal coil and placed on a swivel. Surgery was performed 24 h before the start of the experiment. On the day of the assay, a first blood sample was taken, followed by starting of the infusion. Subsequent blood samples were obtained at the times described under Results, and immediately replaced by an equivalent volume of erythrocytes (resuspended in normal saline) from donor rats. CRF was dissolved in 0.04 M phosphate-buffered saline with 0.1% bovine serum albumin (BSA) and 0.01% ascorbic acid, and placed in 10-ml disposable glass syringes.

For the experiment dealing with exposure to the peptides over a 7-d period, oCRF or ACTH (Cortrosyn), dissolved in 0.04 M phosphate-buffered saline containing 0.1% BSA and 0.01% ascorbic acid, were

1. *Abbreviations used in this paper:* CRF, corticotropin-releasing factor; FSH, follicle-stimulating hormone; HR, heart rate; LH, luteinizing hormone; MAP, mean arterial pressure; oCRF, ovine corticotropin-releasing factor; PRL, prolactin.

placed in Alzet osmotic minipumps (model 2001, Alza Inc., Palo Alto, CA). The minipumps have a capacity of 225 μl and a nominal pumping rate of delivery rate was 1 μl/h. They were loaded to their maximum capacity, so that they would be completely exhausted in 8 d. The Alzet pumps were inserted dorsally under the skin during light ether anesthesia. In previous experiments, we have also connected the pumps to the jugular vein, and obtained comparable results. The stability of CRF in the Alzet pumps was checked at the end of the experiment by radioimmunoassay (14) and bioassay (3) of the remaining content of the pumps. Neither assay indicated any significant loss in either immuno- or bioactivity.

At the end of the 7-d experiment, the animals were decapitated, and the adrenals, testes, seminal vesicles (emptied), and ventral prostates were freed of fat and weighed to the nearest 0.1 mg. The pituitaries were placed in 1 ml 0.1 N HCl/1 N HoAc, boiled for 5 min, homogenized, and frozen.

Hormone assays. Plasma ACTH (12), corticosterone (12), prolactin (PRL) (3), testosterone (1), and CRF (14) levels were measured as previously described. Plasma LH values were measured by the ovine-ovine procedure of Niswender et al. (15). Pituitary ACTH content was measured after dilution of the samples with the radioimmunoassay buffer.

Hormones. oCRF was synthesized by solid phase methodology (16) and provided by Dr. Jean Rivier, Salk Institute. Cortrosyn [ACTH-(1-24)] was purchased from Organon, Inc., West Orange, NJ.

Cardiovascular parameters. Heart rate and mean arterial pressure were measured according to Fisher et al. (17).

Statistical analysis. Differences between treatments were analyzed by analysis of variance.

Results

Effect of intravenous administration of oCRF to intact rats for 24 h. Plasma ACTH levels of intact rats receiving 0.75 nmol oCRF/h for 24 h reached peak values (range, 0.690–0.985 ng/ml) by 45–90 min after the beginning of the infusion, then declined to lower values (range, 0.240–0.355 ng/ml) for the remainder of the experiment (Fig. 1). Corticosterone secretion showed a peak increase at 45–90 min (range, 0.141–0.335 μg/ml), then decreased to levels ranging from 0.115 to 0.148 μg/ml from 10 to 24 h after the treatment. Control animals showed typical circadian rhythms (Fig. 1) with plasma ACTH and corticosterone levels that were significantly lower than those of CRF-treated rats at all times (ACTH, ≤50–75 pg/ml; corticosterone, ≤0.012–0.063 μg/ml). Plasma testosterone levels, measured at the end of the experiment only, were 2.54±0.32 ng/ml for control rats and 1.21±0.27 ng/ml for CRF-treated rats ($P \leq 0.05$). Plasma PRL levels were 5.95±0.52 ng/ml for control rats and 5.63±0.47 ng/ml for CRF-treated rats ($P > 0.05$).

Effect of subcutaneous administration of oCRF or ACTH to intact rats for 7 d. The administration of 0.075 nmol oCRF/h to intact rats was not accompanied by significantly elevated levels of circulating CRF (control, ≤0.150 ng/ml; 0.075 nmol oCRF/h, 0.177±0.027 ng/ml; $P > 0.05$). This treatment caused a marked increase in pituitary ACTH content, and a moderate elevation in plasma ACTH and corticosterone values, but no significant changes in adrenal weights (Table I). Additionally, it induced a slight decrease in seminal vesicles' weights, but no alteration of testes or ventral prostate weights despite a significant lowering of testosterone secretion (Table II). Exposure to 0.75 nmol oCRF/h, which corresponded to markedly elevated values of circulating CRF (3.114±0.663 ng/ml), was accompanied by a significant increase in pituitary

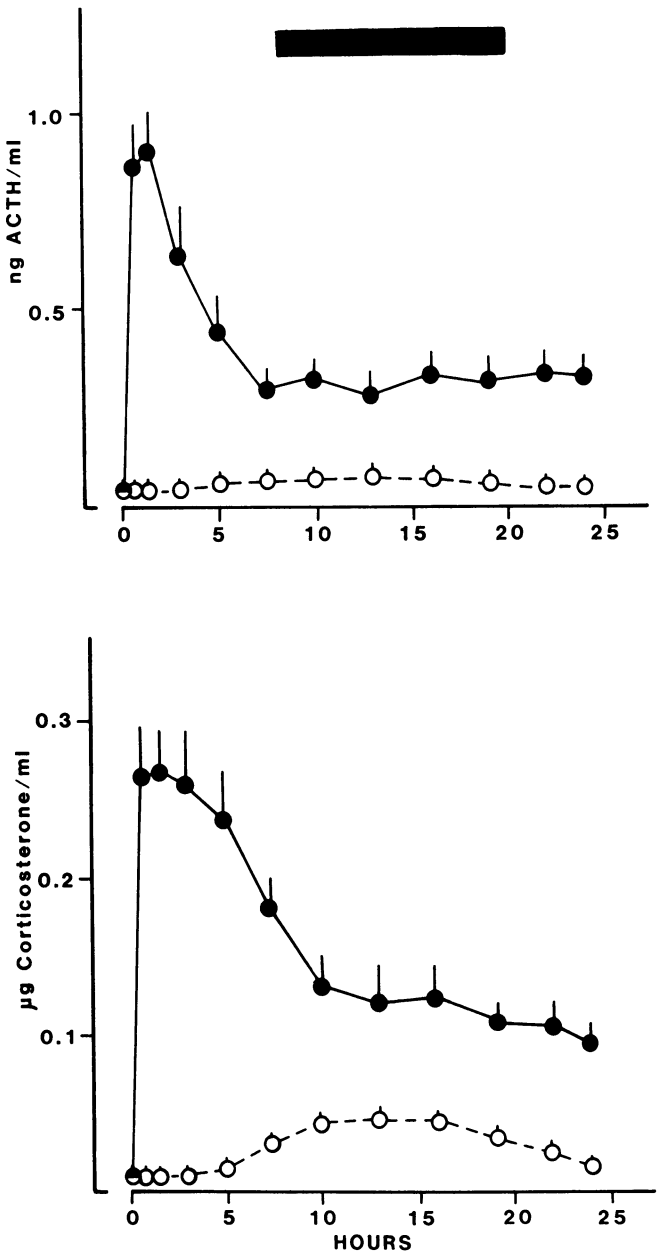

Figure 1. Effect of the intravenous infusion of the vehicle or 0.75 nmol oCRF/h on ACTH and corticosterone secretions by intact rats over a 24-h period. ○, control rats; ●, CRF-treated rats. Each point represents the mean±SEM of six–nine animals. ■ indicates the period of darkness.

ACTH content, adrenal weights, and plasma ACTH and corticosterone levels (Table I) as well as decreases in plasma testosterone values and the weights of androgen-dependent organs (Table II). Plasma LH levels were significantly lowered by the higher dose of CRF, but there were no significant changes in FSH secretion.

In these intact rats, the continuous exposure to 10, 30, or 100 mU ACTH/h was accompanied by dose-related increases in circulating levels of ACTH and corticosterone (Table I), as well as decreases in plasma LH, testosterone values, and seminal vesicle weights (Table II). Both CRF and ACTH caused a significant decrease in PRL secretion (Table III).

Table I. Effect of the Administration of oCRF or ACTH for 7 d on the Hypothalamic-Pituitary-Adrenal Axis of Intact Rats

Treatment	N	ACTH	ACTH pituitary content	Corticosterone	Adrenals
		pg/ml	*μg/pit*	*ng/ml*	*mg*
Control	10	51.3±2.6*	1.54±0.12	17.8±3.2	41.2±3.3
0.075 nmol oCRF/h	10	194.9±19.1‡	2.20±0.15‡	28.2±7.4§	41.4±4.0‖
0.75 nmol oCRF/h	10	347.8±56.2‡	2.68±0.24‡	122.3±15.8‡	58.3±5.3‡
10 mU ACTH/h	10	198.3±10.5‡	ND	21.0±2.5‖	38.9±2.4‖
30 mU ACTH/h	6	396.7±14.4‡	ND	105.3±14.64‡	51.4±4.8‖
100 mU ACTH/h	6	834.3±50.6‡	ND	293.7±31.4‡	133.3±10.2‡

* Data are presented ±SEM. ND, not determined; ‡ $P \leq 0.01$; § $P \leq 0.05$; ‖ $P > 0.05$.

Mean arterial pressure (MAP) and heart rate (HR) were measured in order to document possible cardiovascular changes during the mid-course of the experiments. Four control rats and four rats receiving 0.75 nmol CRF/h were monitored before the start of the experiment (day 0), as well as on days 3 and 7 of the infusion. Mean HR for control animals on days 0, 3, and 7 was 349.2±8.5, 355.0±8.4, and 350.9±7.6 beats/min; their MAP was 110.3±4.9, 107.5±3.2, and 109.1±4.2 mmHg, respectively. Mean HR of CRF-treated rats was 335±10.2, 329±11.3, and 330±10.5 beats/min, and their MAP was 109.5±3.7, 107.5±3.2, and 105.4±6.3 mmHg. These differences were not statistically significant. These animals were not included in the results of Tables I and II.

Effect of administration of oCRF or ACTH to adrenalectomized rats for 7 d. The administration of 0.75 nmol oCRF/h to adrenalectomized rats produced a slight, though not statistically significant elevation in plasma ACTH levels, but no alteration in pituitary ACTH content. There was a slight ($P > 0.05$) decrease in plasma LH and testosterone values, as well as the weights of androgen-dependent organs, and a small but significant ($P \leq 0.01$) increase in plasma follicle-stimulating hormone (FSH) levels. The infusion of 100 mU ACTH/h, which markedly increased circulating levels of ACTH, did not modify testosterone release or the weights of sex organs (Table IV). Both CRF and ACTH decreased plasma PRL levels (Table III).

Discussion

In the rat, repeated as well as continuous stresses are usually accompanied not only by unimpaired multiple increases in ACTH secretion, but also by a state of hyper-responsiveness of the adrenocortical system (5–10). This suggests that elevated levels of plasma corticosteroid do not necessarily prevent the corticotrophs' responsiveness to hypothalamic activation.

Human pathological conditions also arise, which are characterized by increased corticosteroid as well as ACTH release, and the question as to whether CRF secretion is elevated in such cases is not yet resolved. Our results indicate that in the rat, the long-term exposure to exogenous CRF results in persistently high levels of circulating ACTH as well as corticosterone. This suggests that in the presence of elevated levels of CRF, a new set point for ACTH and corticosterone is reached, and raises the possibility that certain states of adrenal hyper-responsiveness, such as Cushing's disease and alcoholic pseudo-Cushing's, might be associated at some stage with higher output of CRF. Note, however, that though the infusion of CRF for 24 h or 7 d resulted in markedly elevated levels of plasma ACTH and corticosterone by the end of the experiment, these values were significantly lower than those present during the early part of the infusion. This observation indicates that though the secretory rate of the pituitary and the adrenals can remain elevated during continuous exposure of the rat to CRF, it nevertheless exhibits, as would have been expected from in vitro studies (2, 3), some desensitization. Note that since Schulte et al. (18) have reported that the continuous administration of low doses of CRF did not consistently elevate ACTH and cortisol release in man and in nonhuman primates, it is possible that the ability of the pituitary-adrenal axis to maintain its responsiveness to CRF is species-specific.

Comparison of the effects of CRF and ACTH indicates that doses of CRF and exogenously administered ACTH that result in comparable levels of circulating ACTH have different

Table II. Effect of the Administration of oCRF or ACTH for 7 d on Reproductive Parameters of Intact Rats

Treatment	N	LH	FSH	Testosterone	Testes	SV	VP
		ng/ml	*ng/ml*	*ng/ml*	*gm*	*mg*	*mg*
Control	10	1.07±0.09	73.7±5.3*	2.74±0.38	3.12±0.06	243.7±17.6	203.2±14.7
0.075 nmol oCRF/h	10	0.92±0.08*	73.1±4.6*	1.69±0.32‡	2.99±0.07*	194.9±17.3‡	173.1±11.9*
0.75 nmol oCRF/h	10	0.38±0.03§	74.5±5.1*	0.57±0.33§	2.98±0.07*	105.3±8.8§	136.0±14.2§
10 mU ACTH/h	10	0.79±0.05‡	ND	1.45±0.25‡	3.04±0.12*	203.8±16.2‡	183.1±14.66*
30 mU ACTH/h	6	0.67±0.04§	ND	1.28±0.27§	3.05±0.14*	205.7±10.3‡	185.7±12.3*
100 mU ACTH/h	6	0.49±0.03§	ND	0.90±0.34§	3.08±0.13*	161.0±10.5§	191.4±13.5*

SV, seminal vesicles; VP, ventral prostate; * $P > 0.05$; ‡ $P \leq 0.05$; § $P \leq 0.01$; ND, not determined.

Table III. Effect of oCRF or ACTH on PRL Secretion

Treatment	N	PRL
		ng/ml
Intact rats, control	8	7.16±1.37
Intact rats, 0.075 nmol oCRF/h	7	6.41±1.17*
Intact rats, 0.75 nmol oCRF/h	7	3.67±0.63‡
Intact rats, 30 mU ACTH/h	6	4.10±0.53‡
Intact rats, 100 mU ACTH/h	6	3.81±0.60‡
ADX rats, control	9	8.69±0.68
ADX rats, 0.75 nmol oCRF/h	9	5.50±0.57‡
ADX rats, 100 mU ACTH/h	9	5.94±0.63‡

ADX, adrenalectomized.
* $P > 0.05$.
‡ $P \geq 0.01$.

effects on testosterone secretion. At present, we cannot rule out that high doses of CRF might exert some direct inhibitory action on the testes, which might at least partially account for the more marked lowering of androgen release observed in CRF-treated rats.

As has been extensively documented in a number of species including man (11, 18–22), the acute intravenous administration of CRF causes peripheral vasodilation and hypotension, and an accompanying stress-induced activation of the hypothalamic-pituitary-adrenal axis. It seems improbable, however, that the CRF-induced inhibition of reproductive functions was solely due to a nonspecific stress effect, since it was not observed in adrenalectomized rats, in which CRF causes changes in cardiovascular parameters that are comparable to those measured in intact animals (23).

Prolonged exposure to stress has been reported to exert deleterious effects on reproductive functions (23–31), but there is still controversy concerning the mechanisms involved. Some investigators have suggested that stress-induced increased levels of circulating corticosteroids and/or adrenal androgens would decrease pituitary responsiveness to GnRH, and therefore LH secretion (32–36), or exert a direct inhibitory action on steroidogenesis (31, 33, 37, 38). Others have reported that ACTH, but not corticosterone, could delay puberty (39), interrupt pregnancy (40–42), and inhibit ovulation (43). We have recently observed that the intracerebroventricular administration of CRF markedly lowered LH secretion in gonadectomized/adrenalectomized rats, while acutely large doses of peripherally administered CRF were without effect (13). We had additionally suggested that this inhibition was not mediated through an increase in ACTH secretion, since blockade of the action of CRF on the corticotrophs by dexamethasone did not interfere with CRF-induced lowering of LH release (13). The results presented here indicate that high amounts of CRF administered subcutaneously would significantly inhibit testosterone secretion and decrease ventral prostate weights. The observation that this effect can be at least partially mimicked by ACTH in intact, but not adrenalectomized rats, coupled with the inability of peripherally administered CRF to modify reproductive parameters in the absence of circulating steroids of adrenal origin, suggests that adrenal steroids represent major direct modulators of the deleterious action on reproductive parameters of peripherally injected CRF. Indeed, our observation that CRF could lower plasma LH levels in intact, but not adrenalectomized rats, supports the hypothesis previously proposed by Mann et al. (35) that long-term ACTH infusions would inhibit LH secretion through adrenal androgen-mediated changes in pituitary activity. Our data, however, do not permit us to rule out that adrenal glucocorticoids may have also exerted a direct inhibitory effect on the testes (37).

Finally, our results indicate that the peripheral administration of CRF and ACTH caused some inhibition of PRL secretion in both intact and adrenalectomized rats. These findings are in contrast with reports that increases in circulating levels of ACTH have been shown to stimulate PRL release in female rats (44), but agree with the observation by Collu et al. (45) that chronic stress causes a marked decrease in plasma PRL levels. Since CRF has little or no direct effect on the mammotroph (3), but increases adrenal steroids shown to inhibit PRL secretion (46), it might either have acted through a modification of the steroid milieu or have penetrated areas of the brain involved in the control of PRL release.

We conclude that in the intact rat, the continuous stimulation of the pituitary-adrenal axis by CRF, though causing some degree of desensitization, is nevertheless accompanied by persistent elevations of the circulating levels of both ACTH and corticosteroids, as well as by disruption of reproductive parameters. Whether these observations in the rat can be related to various pathological conditions seen in man needs to be further investigated.

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Table IV. Effect of the Administration of oCRF or ACTH for 7 d to Adrenalectomized Rats

	N	ACTH	ACTH pituitary content	LH	FSH	Testosterone	Testes	SV	VP
		ng/ml	*μg/pit*	*ng/ml*	*ng/ml*	*ng/ml*	*g*	*mg*	*mg*
Control	10	2.19±0.30	4.98±0.18	1.12±0.10	89.4±9.9	2.87±0.39	3.33±0.11	277.2±17.4	240.7±20.3
0.75 nmol oCRF/h	10	2.83±0.32*	5.17±0.46*	1.05±0.08*	133.3±11.2‡	2.18±0.20*	3.19±0.07*	251.5±20.5*	224.1±26.5*
100 mU ACTH/h	10	4.38±0.41‡	ND	0.98±0.09*	ND	2.93±0.29*	3.28±0.05*	272.5±19.8*	241.1±20.7*

SV, seminal vesicles; VP, ventral prostate; * $P > 0.05$; ‡ $P \leq 0.01$; ND, not determined.

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