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Corticotropin releasing factor increases the adrenocortical responsiveness to adrenocorticotropin[1]

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Summary. In the course of studying the plasma adrenocorticotropic hormone (ACTH) and corticosterone responses to synthetic corticotropin releasing factor (CRF), we noted some disparity in the responses. A higher dose (20 µg compared with 5 µg per rat i.a.) produced an equal plasma ACTH but greater plasma corticosterone response in adult male rats. Thus, we examined the possibility that CRF increases adrenocortical responsiveness to ACTH. CRF significantly ($p < 0.0005$) increased the plasma corticosterone response to ACTH in rats pretreated with dexamethasone. Thus, synthetic CRF increases corticosterone secretion in rats not only by stimulating ACTH secretion, but also by increasing the adrenocortical responsiveness to ACTH.
Key words. Rat; adrenocortical responsiveness; ACTH, plasma; corticosterone, plasma; corticotropin releasing factor (CRF).

Glucocorticoid secretion from the adrenal cortex is regulated by ACTH which in turn is controlled by hypothalamic corticotropin-releasing factor (CRF). The primary role of CRF in regulating pituitary-adrenocortical secretion has been well established, and recently, a 41-amino acid peptide that fulfills many of the criteria of a physiological CRF has been purified from ovine hypothalamic extracts, sequenced[2,3] and subsequently synthesized[3-5]. Although the effects of this synthetic CRF to increase plasma ACTH are readily apparent[4,6-8], we noted some disparity in the effects of CRF to increase plasma ACTH and corticosterone. Thus, we examined the possibility that CRF increased adrenocortical responsiveness to ACTH.

Materials and methods. Adult male Sprague-Dawley rats (Charles River, CD) weighing 280–320 g were caged individually in an environmental room at 23 °C with lights on from 09.00–21.00 h for 7 days before use in an experiment. Rats were cannulated[9] 2 days before experimentation to allow systemic administration of drug and blood sampling without stress. Blood (1.5 ml for ACTH plus corticosterone or 0.25 ml for corticosterone) was collected on ice and replaced after each sampling with an equal volume of heparinized saline solution. Blood was centrifuged at 4000 × g for 20 min at 4 °C, and plasma was stored at −70 °C for subsequent determination of ACTH[10] and corticosterone[11]. Synthetic human ACTH, kindly provided by the National Pituitary Agency, NIAMDD, was used for standard and iodination; the antibody used (R1543 raised against porcine ACTH and not cross-reacting with αMSH) was kindly provided by D. Orth. Human plasma was used as the source of corticosteroid-binding globulin in the corticosterone assay. Data were analyzed using 2-way analysis of variance to compare the effects of drug, time, and interaction between drug and time[12].

A study was carried out to examine the effect of synthetic CRF (Peninsula, San Carlos, CA) on plasma concentrations of ACTH and corticosterone. Animals ($n = 6$/group) received CRF, 5 or 20 µg/rat i.a.; blood was withdrawn immediately before and 5, 20 and 30 min after drug administration. The plasma ACTH and corticosterone responses are shown in figure 1. There were significant increases in plasma concentrations of both ACTH and corticosterone after intraarterial administration of both doses of CRF compared to either the basal concentrations or the corresponding hormonal responses after intraarterial injection of saline. The plasma corticosterone responses after i.a. administration of 5 and 20 µg of CRF were further compared using a 2-way analysis of variance. No differences were noted in the effects of the 2 doses of CRF on plasma ACTH; however, the plasma corticosterone response to 20 µg of CRF was significantly ($p < 0.025$) potentiated when compared to the plasma corticosterone response after intraarterial administration of 5 µg of CRF.

A second study was carried out to determine the hypothesis that the potentiated plasma corticosterone response to 20 µg of CRF seen in the previous study resulted from an effect of CRF to increase the adrenocortical responsiveness to ACTH. This

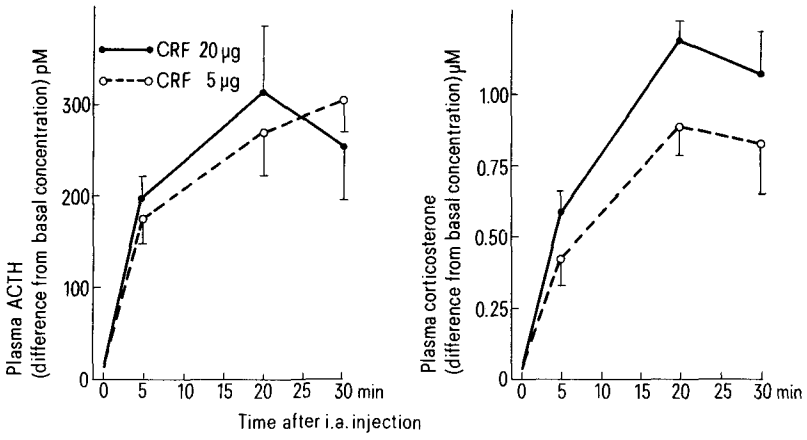

Figure 1. Plasma ACTH and corticosterone responses to synthetic CRF. The plasma corticosterone but not the ACTH response to 20 µg of CRF was significantly ($p < 0.025$ using ANOVA) potentiated when compared to the responses to 5 µg of CRF.

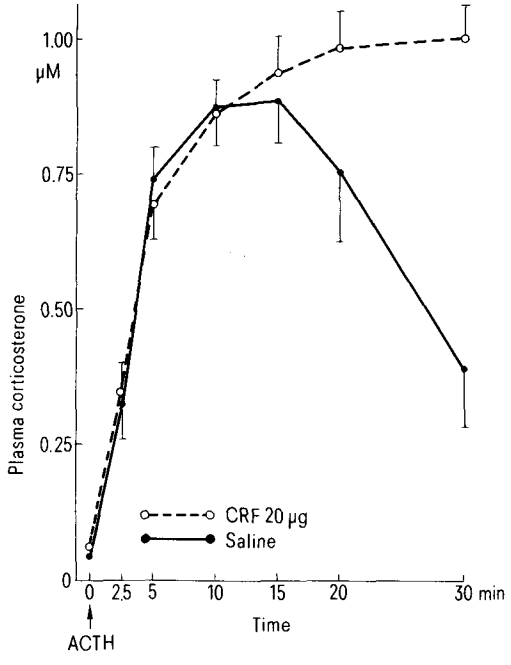

Figure 2. Effect of CRF on the adrenocortical responsiveness to ACTH. The plasma corticosterone response to exogenous ACTH in dexamethasone-treated rats was significantly prolonged ($p < 0.0005$ using ANOVA) in rats given CRF 20 µg when compared with saline-injected controls.

study examined the effect of CRF on the plasma corticosterone response to a given dose of exogenous ACTH in rats in which endogenous ACTH secretion was suppressed by prior administration of dexamethasone. This study comprised 3 groups of rats (n = 6–8/group) all of which received dexamethasone (Hexadrol, Organon, West Orange, NJ), 250 µg/kg i.a., 90 min before i.a. administration of ACTH (Cortrosyn, Organon, Toronto, Canada), 4 mU/kg. Two groups of rats received CRF, 1 or 20 µg/rat, and one group received saline vehicle i.a. just prior to administration of ACTH, and blood was withdrawn immediately before CRF injection and 2.5, 5, 10, 15, 20 and 30 min after administration of ACTH. The test dose of ACTH (4 mU/kg) produced significant increases in plasma corticosterone in all 3 groups as shown by 2-way analysis of variance. CRF, 20 µg, significantly ($p < 0.0005$) increased the plasma corticosterone response to ACTH in dexamethasone-pretreated rats when compared to the response in saline-treated animals (fig. 2). This increase in corticosterone response was dependent upon an increased duration, not an increased amplitude, of response. CRF, 1 µg, produced a plasma corticosterone response to ACTH that was intermediate between the saline and CRF, 20 µg, response curves (data not shown), but not significantly different from the response seen in saline-treated rats.

Results and discussion. The results of our study demonstrate that the pharmacologic administration of CRF increases corticosterone secretion in rats not only by stimulating ACTH secretion, but also by increasing the adrenocortical responsiveness to ACTH. A recent attempt by Rivier et al.[6] to demonstrate an effect of CRF to increase adrenocortical responsiveness to ACTH was unsuccessful. Several extrapituitary effects of CRF including increased mean arterial blood pressure, heart rate[13], plasma catecholamines, plasma glucose[14,15], oxygen consumption, motor activity[14] grooming[16] and decreased amount of rearing and food consumption[16] have been reported. All of these effects have been related to an action of CRF in brain. The data of the present study provide evidence for an extrapituitary effect of CRF outside the central nervous system. In an analogous fashion, another hypothalamic releasing factor, luteinizing hormone releasing hormone, has been shown to induce ovulation in hypophysectomized rats in the absence of serum luteinizing hormone[17].

Humoral factors in addition to ACTH may be involved in the regulation of adrenocortical function. Pedersen and Brownie[18] reported that a pituitary factor other than ACTH may account for the stress-induced increase in activity of cholesterol ester hydroxylase, the rate-limiting enzyme in the steroidogenic pathway in the rat adrenal cortex. The pituitary proopiomelanocortin-derived peptides, α-MSH[19,20] and β-endorphin[21], have been found to stimulate corticosterone synthesis in isolated rat adrenal cells and to alter adrenocortical responsiveness to ACTH. Our data raise the possibility that CRF stimulates glucocorticoid secretion either by a direct effect at the adrenal cortex or through the mediation of a dexamethasone-nonsuppressable humoral or neural factor. The physiological relevance of this CRF effect and the mechanisms involved await resolution.

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Pineal function cannot prevent the occurrence of castration cells in male rats

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Summary. The relationship between the pineal gland and the pituitary gland was investigated in male rats. The results indicate that the hypothalamo-adenohypophysial-gonadal axis is affected by the pineal gland, but the appearance of castration cells following gonad ablation may be only slightly modified by alterations in pineal gland function.
Key words. Rat pineal; rat pituitary; castration cells; functional activation of pineal; gonadectomy.

It is believed that the pineal gland produces some anti-gonadotrophic substances, e.g., melatonin, arginine vasotocin (AVT), etc., and alters the secretion of gonadotrophins via the hypothalamus and/or adenohypophysis[1-5]. Above all, Martin and Klein[6] reported that melatonin can act directly on the pituitary to suppress the release of LH induced by LHRH and that melatonin may act both at the hypothalamic level and at the pituitary level to regulate LH secretion.

It is known that pinealectomy sometimes results in pituitary hypertrophy[7], and usually enhances the pituitary contents[5-8]. However, to our knowledge, there are few reportes concerning the relationship between the pineal and the ultrastructural features of gonadotrophs. Clementi et al.[9] demonstrated a remarkable dilation of the endoplasmic reticulum (ER) associated with cellular hyperfunction following pinealectomy.

The present study was performed in order to learn whether a functionally activated pineal gland, induced by blinding and olfactory bulbectomy in male rats, prevents the occurrence of castration cells or modifies the ultrastructure of gonadotrophs following gonadectomy in the rat.

Materials and methods. 25 Male rats of a Wistar-derived strain were obtained at 35 days of age, separated into 5 groups with 5 animals in each, and surgically prepared for the experiment: group 1 was sham-operated (control-group); group 2 was gonadectomized (G-group); group 3 was gonadectomized, blinded and olfactory bulbectomized (GB-group); group 4 was gonadectomized, blinded, olfactory bulbectomized and pinealectomized (GBP-group); group 5 consisted of rats which were gonadectomized, blinded, olfactory bulbectomized, and injected with 5 µg melatonin mixed 10% gelatin solution twice a day for 1 month (GBM-group). All animals were anesthetized with nembutal during the operations. The animals were sacrificed after 1 month by decapitation following anesthesia with ether. The anterior pituitary glands were removed and cut into halves. One half was fixed in Bouin's solution for immunocytochemistry and the other was fixed in 2% paraformaldehyde and 3% glutaraldehyde in 0.1 M cacodylate buffer for 2 h and then in 1% osmic acid for 1 h prior to processing for EM examination. For immunocytochemistry, the unlabeled antibody method of Sternberger et al.[10] was used to identify gonadotrophs. Antiserum to rat LH was a gift from NIADDK, NIH, USA.

Results. The control group (sham-operated) showed no alteration of ultrastructure of gonadotrophs in the anterior pituitary (fig. 1) as compared with that of normal intact rats. Removal of testes for 30 days induced numerous hypertrophic gonadotrophs as seen in figure 2; likewise, removal of testes, olfactory bulbs and eyeballs for 30 days brought about various

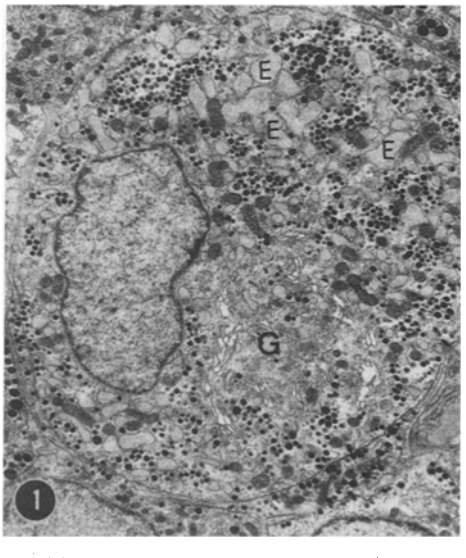

Figure 1. A typical gonadotroph (Type-I gonadotroph) of the rat. E: Endoplasmic reticulum, G: Golgi body. × 4500.