Corticotropin-Releasing Factor Stimulates Secretion of Melanocyte-Stimulating Hormone from the Rat Pituitary

Abstract. *Administration of synthetic ovine corticotropin-releasing factor led to rapid, parallel increases in adrenocorticotropin and α-melanocyte–stimulating hormone concentrations in rat plasma. Prior treatment with dexamethasone almost completely blocked the adrenocorticotropin response but not the increase in melanocyte-stimulating hormone. These data demonstrate that corticotropin-releasing factor is a potent stimulator not only of adrenocorticotropin secretion from the corticotrophs of the anterior pituitary gland but also of peptide secretion from the intermediate lobe. Such data suggest that melanocyte-stimulating hormone and β-endorphin play a role in the physiological response to stress.*

Recent elucidation of the structure of ovine corticotropin-releasing factor (CRF) (*1*) opens new avenues for studying the mechanisms controlling the pituitary adrenocortical response to stressful stimuli. This peptide is a potent stimulator of adrenocorticotropic hormone (ACTH) secretion in the rat in vivo as well as in adenohypophysial cells in vitro (*1*).

Since not only the corticotrophs of the anterior lobe of the pituitary gland but also the cells of the intermediate lobe secrete the same series of peptides derived from pro-opiocortin (*2, 3*), we studied the effect of CRF on plasma ACTH, which is derived mainly from the adenohypophysis, and α-melanocyte–stimulating hormone (α-MSH), which is secreted almost exclusively by the intermediate lobe. To further discriminate between the effects of CRF at the level of the anterior and intermediate lobes, we used the property of glucocorticoids to specifically inhibit peptide secretion of adenohypophysial origin (*4*). We found that CRF is a potent stimulator of peptide secretion, not only in the corticotrophs of the adenohypophysis but also in the homogeneous cell population of the intermediate lobe (*5*).

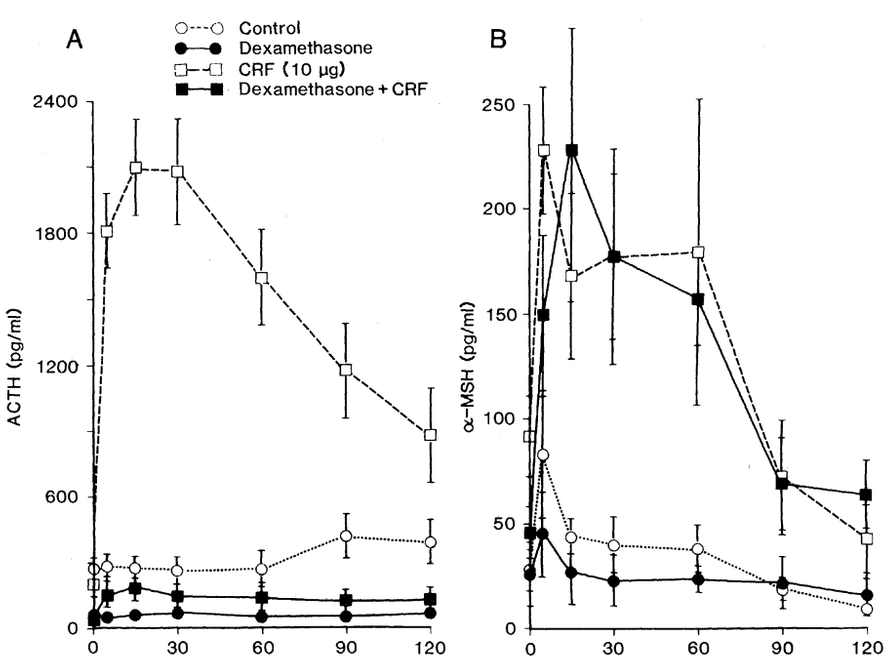

Fig. 1. Effect of CRF on plasma ACTH and α-MSH and effect of dexamethasone on plasma ACTH and α-MSH and the response to CRF. The subjects were adult female Sprague-Dawley rats ovariectomized 2 weeks before the experiment began. A catheter was inserted into the right superior vena cava under Surital (50 mg/kg, intraperitoneally) anesthesia 2 days before so that the experiment could be performed on undisturbed, freely moving animals. CRF was synthesized by solid-phase methods and purified by preparative reverse-phase high-performance liquid chromatography (HPLC). Homogeneity was determined by analytical HPLC on 300-Å C_{18} silica columns and by peptide mapping of enzymatic digests on HPLC (*10*). Blood samples (0.8 ml) were drawn at the indicated time intervals after injection of CRF for measurement of plasma ACTH and α-MSH by radioimmunoassay (*11*). Data are means ± standard errors for duplicate determinations of samples obtained from eight to ten animals per group. Radioimmunoassay data were analyzed with a program derived from Rodbard and Lewald (*12*). Statistical significance was measured by means of the multiple range test of Duncan-Kramer (*13*).

As illustrated in Fig. 1A, intravenous administration of 10 μg of synthetic ovine CRF in rats led to an approximately sixfold increase in the concentration of ACTH in plasma after 5 minutes. The ACTH level reached a plateau at 15 and 30 minutes, followed by a progressive decrease toward basal levels. CRF had a similar stimulatory effect and time course of action on plasma α-MSH (Fig. 1B).

Since 99 percent of pituitary α-MSH is contained in the intermediate lobe while 90 percent of pituitary ACTH is present in the anterior lobe (*6*), these results suggest a stimulatory effect of CRF on both lobes. Glucocorticoids are specific inhibitors of peptide secretion (ACTH, β-lipoprotein, β-endorphin, α-MSH, and related peptides) originating exclusively in the anterior pituitary and have no effect on the secretory activity of the intermediate lobe (*4*). Therefore, we studied the effect of dexamethasone on the ACTH and α-MSH responses to CRF. Administration of 100 μg of dexamethasone 15 and 3 hours before the injection of CRF led to a 75 percent inhibition of basal plasma ACTH ($P < .01$, Duncan-Kramer multiple-range test) and a slight but nonsignificant inhibition of basal α-MSH. The most important finding, however, was a 95 percent decrease in the ACTH response to CRF ($P < .01$), while the α-MSH response remained unchanged (Fig. 1).

Clearly, CRF is a potent stimulator not only of adenohypophysial corticotrophs but also of cells of the intermediate lobe. Both pituitary lobes are known to secrete in different amounts the same peptides derived from pro-opiocortin (*3*). This is supported by the earlier demonstration of a common precursor for ACTH, β-lipoprotein, and other related peptides (*2*). As mentioned above, ACTH and α-MSH are the predominant secretory products of the anterior and intermediate lobes, respectively.

While the stimulatory effect of CRF on ACTH secretion by anterior lobe corticotrophs can be explained by a direct action at the pituitary level (*1*), the equally potent action on the pars intermedia cells may be either direct or secondary to an action at a suprapituitary level. The activity of the pars intermedia cells is known to be stimulated by a β_2-adrenergic system and inhibited by a dopaminergic receptor (*7*). In the event of a direct action of CRF, this peptide would be added to β-adrenergic agonists as a new stimulator of pars intermedia cells.

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The potent stimulatory effect of CRF on the secretion of pars intermedia peptides—mainly α-MSH and β-endorphin—could have major implications for our understanding of the physiological response to stress. It may indicate a role of α-MSH or circulating β-endorphin (*8*). Like thyrotropin-releasing hormone, which stimulates the release of thyrotropin and prolactin, and like somatostatin, which inhibits the secretion of growth hormone, prolactin, and thyrotropin (*9*), CRF is a hypothalamic releasing peptide with multiple sites of action.

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Rat Pro-Opiomelanocortin Contains Sulfate

Abstract. *Intermediate lobes isolated from rat pituitary glands incorporated [^{35}S]sulfate into pro-opiomelanocortin and other adrenocorticotropic hormone–containing peptides. Incubation of intermediate lobes in medium containing the arginine analog canavanine inhibited the cleavage of pro-opiomelanocortin into smaller products. Pro-opiomelanocortin that accumulated in the presence of canavanine was also sulfated.*

In recent studies sulfate has been detected covalently linked to oligosaccharides on alpha and beta subunits of the pituitary glycoprotein hormones lutropin and thyrotropin (*1*, *2*) but not on human chorionic gonadotropin. The amino acid sequences of pituitary and placental alpha subunits are the same within a species. This raises the question of whether sulfation of lutropin and thyrotropin occurs as the result of an unusual capacity of pituitary cells for processing oligosaccharides or because these structurally related hormones (*3*) contain unique recognition sequences for sulfation. We sought to determine whether the pituitary glycoprotein pro-opiomelanocortin (POMC) (*4*, *5*), which bears no structural relation to the thyrotropin-gonadotropin family and is synthesized in cells other than gonadotrophs and thyrotrophs, contains sulfate.

Metabolic labeling of POMC with [^{35}S]sulfate was examined in the intermediate lobe of the rat pituitary, where POMC is the major product, accounting for up to 30 percent of total protein synthesis (*4*). When intermediate lobes were incubated for 5 hours with [^{35}S]methionine or [^{35}S]sulfate, several labeled products were precipitated by antiserum to adrenocorticotropic hormone (ACTH) (lanes 1 and 3 in Fig. 1) but not by nonimmune serum (lanes 2 and 4 in Fig. 1). Methionine-labeled products had apparent molecular weights by electrophoresis of 35K, 22K, 20K, 14K, and 5K (K = 1000). Based on the known mobilities of ACTH-containing peptides in the intermediate lobe (*4*, *5*), we suggest that the 35K protein corresponds to POMC, the 22K and 20K products to the NH_2-terminal segment of POMC containing ACTH, the 14K fragment to glycosylated ACTH (the molecular weight estimation of this small peptide is aberrantly high due to the oligosaccharide), and the 5K fragment to nonglycosylated ACTH. The 35K, 22K, and 14K proteins were labeled with sulfate.

Pro-opiomelanocortin contains three glycosylation sites, but the one in ACTH is glycosylated in only a fraction of the POMC molecules (*4*, *5*). Presumably this contributes to the electrophoretic heterogeneity of proteins that contain ACTH domains: POMC (30K and 35K), NH_2-terminal fragment (20K and 22K), and ACTH (5K and 14K). In the case of POMC, only the highest molecular weight form was sulfated (POMC could be resolved into multiple bands by electrophoresis for longer times).

Proteolysis of POMC occurs at pairs of basic amino acid residues (Arg-Lys, Lys-Arg, or Arg-Arg), as does proteolysis of precursors of many other peptide hormones, such as insulin, gastrin, glucagon, somatostatin, and parathyroid hormone (*5*). Incubation of intermediate lobes with canavanine, an arginine analog, blocked the cleavage of POMC (lanes 2 and 3 in Fig. 2A). Moreover, the mobility of POMC was decreased by canavanine, causing an increase in apparent molecular weight from 35K to 38K. We have seen similar shifts in mobility in other peptides containing amino acid analogs. Presumably, the mobility shift reflects structural changes in the protein which are attributable to the incorporated analog (*6*). It is also possible that processing of the oligosaccharide chains may be modified by the incorporated analog. Processing of proinsulin and proglucagon can be inhibited by canavanine (*7*).

Partial sequence analysis of the 38K product synthesized in the presence of canavanine was performed after labeling with [^{3}H]leucine and [^{35}S]methionine to determine whether the NH_2 terminus of POMC was altered by incorporation of canavanine (Fig. 2B). The occurrence of leucine residues at positions 3 and 11 and the absence of methionine at the NH_2 terminus confirmed that the product has the same sequence as POMC (*8*) and is devoid of its signal peptide.

In the presence of canavanine the only sulfate-labeled product precipitated by antiserum to ACTH was POMC, and its mobility was changed by canavanine in the same way as that of the methionine-labeled product (lanes 4 and 5 in Fig. 2A). These results provide further evidence that the sulfate-labeled products seen in Fig. 1 are POMC and other ACTH-containing peptides.

The sites to which sulfate is linked in the ACTH-related species have not yet been ascertained. Some forms of gastrin have sulfate linked to the phenolic group of tyrosine, and these molecules have been detected in the intermediate and posterior lobes of the pituitary (*9*). Two considerations suggest that sulfate is not linked to tyrosine in the ACTH-related species. Acid extractions of tissue before

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