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Corticotropin-Releasing Factor Stimulates Accumulation of Adenosine 3′,5′-Monophosphate in Rat Pituitary Corticotrophs

Abstract. *The presence of synthetic ovine corticotropin-releasing factor leads to a rapid and marked stimulation of adenosine 3′,5′-monophosphate accumulation in an enriched population of rat pituitary corticotrophs in primary culture. The increase, observed as early as 60 seconds after the addition of corticotropin-releasing factor, suggests that changes in the intracellular concentration of the cyclic nucleotide coincide with or precede the secretion of adrenocorticotropic hormone in response to corticotropin-releasing factor.*

Vale *et al.* (*1*) recently elucidated the structure of an ovine peptide, corticotropin-releasing factor (CRF), with potent adrenocorticotropic hormone (ACTH)–releasing activity in vivo and in vitro in the rat. This knowledge has opened new avenues for studying the control of adrenocortical activity and for achieving a better understanding of the mechanisms controlling the pituitary response to stress. The first suggestion that adenosine 3′,5′-monophosphate (cyclic AMP) acts as an intracellular mediator of pituitary ACTH secretion was based on the observation that theophylline, an inhibitor of cyclic nucleotide phosphodiesterase, stimulates ACTH release in intact pituitary glands (*2*). In addition, cyclic AMP derivatives are powerful stimuli of ACTH secretion in intact pituitaries (*2*) and in cultured pituitary cells (*3, 4*). Although the observation of a stimulatory effect of theophylline and cyclic AMP derivatives on ACTH release indicated that the cyclic nucleotide has a role in the control of ACTH secretion, definitive proof of the role of the adenylate cyclase system had to be obtained by

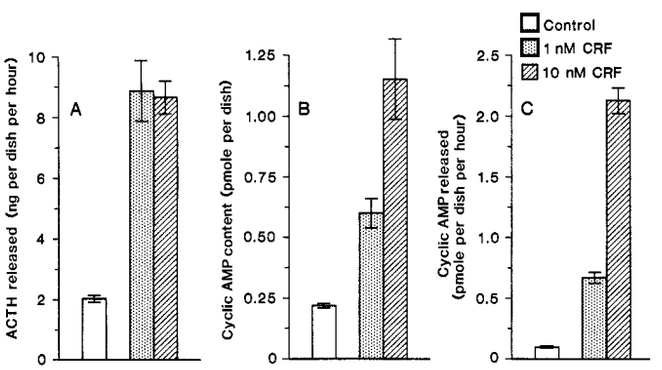

Fig. 1. Effect of synthetic ovine CRF on ACTH release and on cyclic AMP content and release in rat corticotrophs. ACTH and cyclic AMP were measured by specific radioimmunoassays (*4, 9*) after a 1-hour incubation period in the absence or presence of 1 or 10 n*M* CRF. Anterior pituitary cells were enzymatically dispered as described (*10*). Adult female Sprague-Dawley rats (Charles River CD strain) were obtained from Canadian Breeding Farms and ovariectomized. Two weeks later they were killed and their pituitary glands were removed. An enriched population of corticotrophs was prepared by centrifugation for 9 minutes at 65*g* through a continuous gradient of a 3 to 7.5 percent solution (weight to volume) of Ficoll (Pharmacia) in sterile Hepes buffer. The collected cells were plated in Eagle's minimum essential medium (Dulbecco's modification), containing 10 percent horse serum and 2.5 percent fetal calf serum, at a density of 2×10^5 cells per milliliter in Flow dishes with multiple wells. The cell fraction used contained 30 percent corticotrophs, 1 percent thyrotrophs, 50 percent mammotrophs, and 14 percent somatotrophs. This represents an approximately threefold enrichment of corticotrophs. Cell numbering was performed (*11*) after immunostaining for rat ACTH, luteinizing hormone, follicle-stimulating hormone, prolactin, thyrotropin, and growth hormone with antiserums provided by A. F. Parlow. After 4 days in culture, the cells were washed and incubated for the various time periods with the indicated concentrations of CRF prepared 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 with HPLC (*12*). Radioimmunoassay data were analyzed with a program derived from that of Rodbard and Lewald (*13*). Statistical significance was measured using the multiple-range test of Duncan and Kramer (*14*). All assays were performed in duplicate on samples from triplicate dishes. Data are means ± standard errors.

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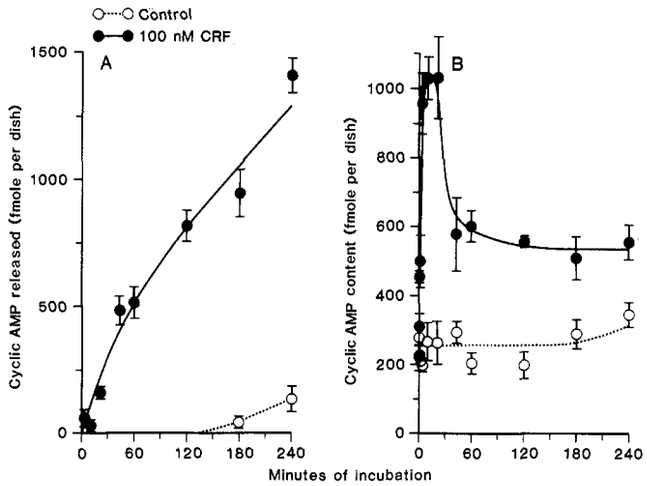

Fig. 2. Time course of the effect of 100 n*M* CRF on cyclic AMP release and content in rat corticotrophs. The experiment was performed as described in the legend to Fig. 1, except that the cells were grown on collagen to minimize the contribution of fibroblasts to cyclic AMP levels.

measurements of changes in cyclic AMP levels or adenylate cyclase activity. The present study demonstrates the potent stimulatory effect of CRF on cyclic AMP accumulation in rat corticotrophs.

Enriched rat corticotrophs were incubated for 1 hour in the absence or presence of 1 or 10 n*M* CRF. In cultures incubated with 1 n*M* CRF, ACTH release was approximately 3.5 times higher than in control cultures, and cyclic AMP content and release were two and seven times higher, respectively (Fig. 1). The tenfold higher concentration of CRF did not provide a stronger stimulation of ACTH release than 1 n*M* CRF, but did increase cyclic AMP content and release above the control values by factors of 5 and 20, respectively.

Since the ACTH response to stress occurs within 2 minutes in vivo (*5*) and in vitro (*6*), we studied the time course of action of CRF on cyclic AMP levels at short time intervals after addition of the peptide. A cyclic AMP content 100 percent higher than the control value was observed 60 seconds after the addition of 100 n*M* CRF to the ACTH-secreting cells; a (maximal) 400 percent difference was measured at 4 minutes (Fig. 2B). Cyclic AMP content then remained approximately constant for 20 minutes before decreasing to a plateau 100 percent higher than the control values between 60 and 240 minutes of incubation. The rapid effect of CRF on cyclic AMP is illustrated in Fig. 2A: while basal cyclic AMP levels are undetectable for up to 120 minutes in the absence of CRF, they can be measured as soon as 4 minutes after addition of the peptide, the increase remaining linear for up to 60 minutes and reaching a level ten times higher than the control level after 240 minutes.

The present data clearly show that synthetic ovine CRF leads to a rapid and marked stimulation of cyclic AMP accumulation in an enriched population of rat corticotrophs. The 100 percent stimulation of cyclic AMP content as early as 60 seconds after addition of the peptide strongly suggests that the changes in cyclic AMP accumulation coincide with or precede ACTH release induced by CRF. This agrees with the rapid response of ACTH release to different stressful stimuli in vivo (*5*) as well as in vitro in dissociated pituitary cells after addition of a hypothalamic extract containing ACTH-releasing activity (*6*). That changes in cyclic AMP accumulation are due to a stimulation of adenylate cyclase activity rather than to inhibition of cyclic nucleotide phosphodiesterase is supported by the finding of a similar effect of CRF in the presence of inhibitors of cyclic nucleotide phosphodiesterase (theophylline or isobutyl methylxanthine).

The fact that maximal stimulation of ACTH release occurs in the presence of 1 n*M* CRF, while a maximal effect on cyclic AMP levels is observed at a tenfold higher concentration of the peptide, suggests that the cyclic nucleotide is compartmentalized and that each compartment has different biological significance. In analogy with other systems, these data suggest that an elevation of cyclic AMP in a small cellular compartment in pituitary corticotrophs, not reflected by measurements of total cellular cyclic AMP, is sufficient for maximal peptide-induced cellular activation. This effect of CRF can be compared with the finding that increased release of thyrotropin and ACTH induced by thyrotropin-releasing hormone (TRH) and lysine-vasopressin, respectively, can occur at low concentrations in the absence of detectable changes in cyclic AMP levels while higher concentrations of the peptides are accompanied by increased cyclic AMP levels (*7*).

The present data show that CRF, like two other hypothalamic releasing hormones, luteinizing hormone–releasing hormone (LHRH) and TRH (*7*, *8*), stimulates cyclic AMP accumulation in the pituitary gland. The stimulatory effect of CRF on cyclic AMP levels is, however, seen earlier and is of greater magnitude than that observed with LHRH and TRH. Since CRF does not stimulate the release of adenohypophyseal hormones other than ACTH (*1*), it is likely that the changes in cyclic AMP levels reflect specific changes in the corticotrophs. Although intracellular mechanisms other than cyclic AMP are likely to be implicated in the control of ACTH-secreting cell activity, the present data indicate that the cyclic nucleotide is an intracellular mediator of CRF action.

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