CRF-STIMULATED BIOSYNTHESIS OF ACTH IN A CELL-FREE SYSTEM FROM RAT ANTERIOR PITUITARIES

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Summary: A cell-free system consisting of ribosomes,pH 5 enzymes and supernatant prepared from rat anterior pituitaries was found to be active in the incorporation of 3H-serine into ACTH. The rate of biosyntesis of ACTH,in a cell-free system as, measured by the incorporation of radioactive amino acid, and the rate of biological activity were markedly increased by the addition of CRF. The synthesis of ACTH was significantly inhibited by puromycin and RNAase but was not significantly inhibited by actinomycin D and DNAase.

It has been established that CRF stimulates not only biosynthesis and release of ACTH but also differentiation of chromophobes into acidophils in a tissue culture(1).

The biosynthesis of ACTH using a cell-free system consisting of ribosomes, pH 5 enzymes, and supernatant fractions was described for the first time by Rao et al.(2).

As will be reported in another paper, rat anterior pituitary cultured for 2 weeks in a medium containing CRF(0.30 μg/2ml) produced 14 times more ACTH than the sample cultured in a medium without CRF.(Ishikawa and Goto, in preparation.)

On the other hand, the cell-free system from rat anterior pituitary cultured for one day in a medium containing CRF(0.30 μg/2ml) was found to synthesize 8 times more ACTH than the system derived from cultures without CRF.(Ishikawa and Goto, in preparation.)

In order to clarify the site of action of CRF, the effect of CRF in a cell-free system is examined in the present paper using a system similar to that of Adiga et al.(3).

Abbreviations used: ACTH, adrenocorticotropic hormone; CRF, corticotropin releasing factor; GTP, guanosine triphosphate; ATP, adenosine triphosphate; PEP, phospho(enol)pyruvate; PK, pyruvate kinase; DOC, deoxycholate.

Materials and Methods: L-(3H)serine-T(G)(250 mCi/mM) was obtained from Japan Radioisotope Association. β-Mercaptoethanol, GTP,ATP,PEP,PK,Bentonite, and DOC were products of Sigma Chemical Co.LTD. Ribonuclease(RNAase) and deoxyribonuclease(DNAase) were obtained from Worthington Biochemical Corp., actinomycin D was obtained from Merk & Corp., and puromycin was obtained from Nutritional Biochemical Corp..

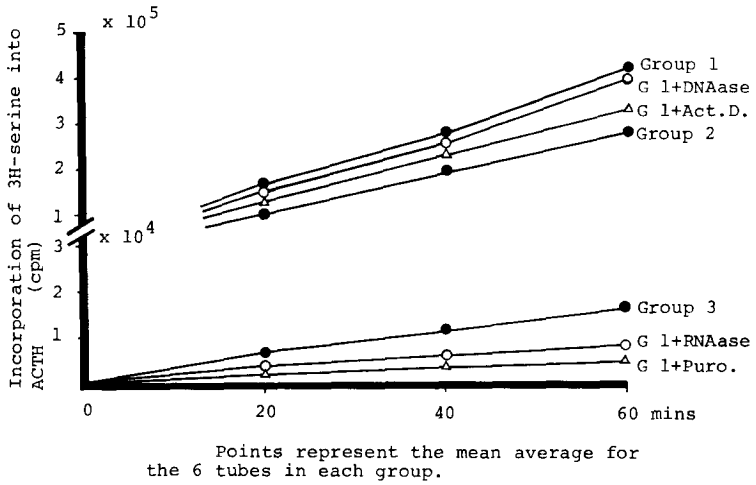
Preparation of CRF: CRF was extracted according to the method of Dariwal et al.(4)from porcine stalk median eminence. The activity of CRF was examined by the method of Arimura et al.(5). In order to determine whether or not a biological activity of ACTH is present in CRF, the bioassay method of Ohtsuka et al.(1) was used. Although CRF activity was present, there was no ACTH activity.

Preparation of constituents of the pituitary cell-free system :

This procedure was carried out following Adiga's method(3).

Incorporation conditions of 3H-serine into ACTH: The entire procedure was carried out by the method of Adiga et al.(3). Each tube contained 3H-serine(10 μCi); ATP(5 μ moles); GTP(1 μ mole); PEP(2.5 μ moles); Tris buffer pH 7.6(20 m moles); and amino acid mixture(0.05 ml). For convenience all components were combined into a o.2 ml"cocktail"mixture.

Three groups of components were assembled as incubation



Points represent the mean average for the 6 tubes in each group.

Fig. 1 The effect of DNAase, RNAase, actinomycin D and puromycin on the synthesis of ACTH by CRF in a cell-free system

media. Each tube contained ribosomal suspension, 0.2 ml(0.76 mg RNA); pH 5 enzyme, 0.1 ml(5 mg protein); and "cocktail solution," 0.2 ml. The only difference among the three groups was the CRF content: Group 1 contained CRF(0.10 µg/0.7 ml); Group 2 contained CRF(0.05 µg/0.7 ml); and Group 3 contained no CRF. In each case, the final volume of the incubation medium was 0.7 ml after the addition of medium M(6). The mixtures were incubated for 20, 40 and 60 mins at 37°C.

The reaction was stopped by adding 0.1 ml of 50% trichloroacetic acid(TCA). Serine incorporation into ACTH was measured using the method of Adiga et al.(3). Radioactivity was measured in a Packard Tri-Carb scintillation spectrometer.

Assay of ACTH: ACTH from the radioactive spot corresponding to the ACTH position on the standard sample was extracted overnight using 0.1 N-HCl. The hormonal activity of the extract was assayed by the in vitro method of Ohtsuka et al.(1).

Biosynthesis of protein: Radioactive protein was then isolated from the precipiteted materials by the method of Adiga et al.(3).

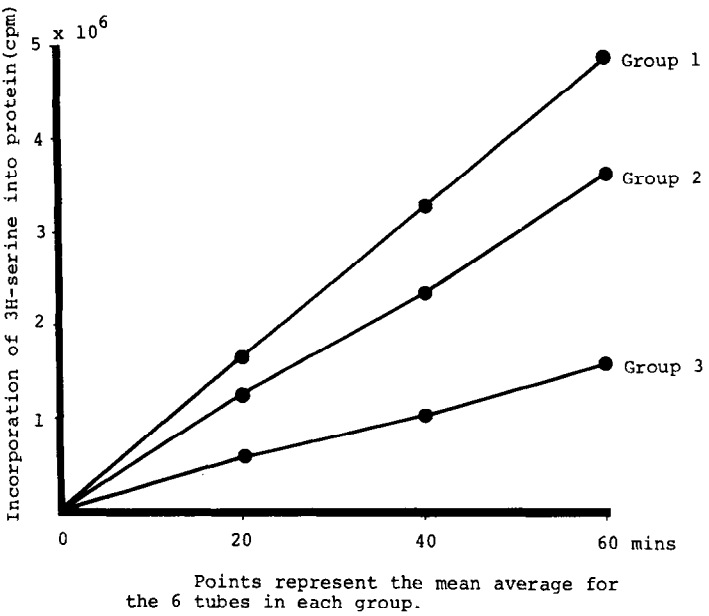


Fig. 2 Effect of CRF on the incorporation of 3H-serine into protein in a cell-free system

Determination of protein and ribonucleic acid(RNA): Protein was determined according to the method of Lowry et al.(7). Ribosomal ribonucleic acid(r-RNA) was measured as described by Fleck and Munro(8), except that bovine serum albumin was used as carrier instead of cell sap(6).

Results: In Figure 1, radioactivity of 3H-serine in ACTH in a cell-free system without CRF was approximately 1.6 x 10^4 cpm after 60 min of incubation. With addition of CRF(0.05 µg/0.7ml medium and 0.10 µg/0.7 ml medium) into the cell-free system, the radioactivity increased to 2.8 x 10^5 and 4.2 x 10^5 cpm respectively during 60 min incubation period. The activity was approximately 15 to 26 times as much as in a cell-free system containing no CRF. Protein biosynthesis was also stimulated by the addition of CRF; however, the rate was only 2 or 3 times higher than the rate without CRF(Fig. 2).

Table 1 Biological activity of ACTH* synthesized in a cell-free system with and without CRF

	Dose of CRF (ug/0.7 ml)	ACTH (mIU) 20 min	40 min	60 min
Group 1	0.10	3.2 ± 0.5**	6.1 ± 0.7	13.5 ± 2.0
Group 2	0.05	1.4 ± 0.2	2.5 ± 0.3	7.1 ± 0.5
Group 3	0	0.2 ± 0.01	0.2 ± 0.03	0.3 ± 0.03

* ACTH activity in 0.7 ml incubation medium was measured.

** Figures represent the mean average for the 6 tubes in each group. mean ± S. E.

Incorporation was significantly inhibited by puromycin (20 µg/ml) and RNAase(2 µg/ml)(Fig. 1),but actinomycin D(15 µg/ml) and DNAase 100 µg/ml) caused little inhibition(Fig. 1). This indicates that ACTH synthesis is similar to the usual process of protein synthesis.

The activity of biosynthesized ACTH was 0.3 mIU after 60 min incubation in Group 3 and 13.5 mIU with the addition of CRF in Group 1(Table 1). This indicates that the biological activity of ACTH in a cell-free system increases with the addition of CRF.

Discussion: The present study was undertaken to pinpoint the site of CRF action which induces the production of ACTH in a cell culture. From Fig. 1 it is clear that the stimulation of ACTH synthesis by the addition of CRF in a cell-free system is caused by certain influences on the process of protein synthesis. As reported elsewhere, actinomycin D, which inhibits RNA synthesis of the pituitary gland in culture, did not inhibit the production of ACTH stimulated by the addition of CRF. This indicates that CRF operates in the stage after transcription of RNA.

This paper describes the effect of CRF on ACTH synthesis. Since the effect of CRF on protein synthesis is not yet clear, it is not known whether or not CRF can protect polysomes against enzymatic degradation or stimulate the increase components in a cell-free system.

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