Inhibition of ACTH Induced Cyclic AMP Synthesis in Isolated Rat Adrenal Cells by NPS-ACTH[1] (36774)

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Chemical modification of the single tryptophan residue in ACTH by reaction with *o*-nitrophenyl sulfenyl chloride results in the loss of the lipolytic activity of the hormone in isolated rat fat cells (1). Moreover, the *o*-nitrophenyl sulfenyl derivative of ACTH (NPS-ACTH) was shown to be a specific inhibitor of ACTH induced stimulation of lipolysis in rat fat cells as well as adenylate cyclase in rat fat cell ghosts (2). In view of the role of cyclic AMP in mediating the action of ACTH in the rat adrenal gland (3), the effects on NPS-ACTH on the synthesis of cyclic AMP in adrenal cells are of considerable interest. The stimulation of cyclic AMP synthesis by ACTH and NPS-ACTH in isolated rat adrenal cells prelabeled with ^{3}H-adenine has been investigated and the results are presented in this communication.

Materials and Methods. ACTH (4) and NPS-ACTH (1) were prepared as previously reported. ^{3}H-adenine and ^{3}H-adenosine were purchased from Schwarz-Mann; collagenase from Worthington.

Adrenal cells were prepared by digestion of decapsulated adrenal glands obtained from 15 male Sprague-Dawley rats (300–350 g) with collagenase by modification of published procedures (5, 6). Following incubation at 37° for 30 min with collagenase (4 mg/ml) in 7.5 ml Krebs–Ringer bicarbonate buffer (KRB), the cells were dispersed by mechanical agitation within a section of Tygon tubing. The cells were filtered through 2 layers of cheesecloth, collected by centrifugation at 100*g* for 10 min, resuspended in 10 ml KRB containing bovine serum albumin (BSA, 2 mg/ml) and ^{3}H-adenosine (10 μCi/ml, 27.4 Ci/mmole) or ^{3}H-adenine (25 μCi/ml, 19.6 Ci/mmole) and incubated at 37° for 1 hr. The cells were collected by centrifugation as before and resuspended in 60 ml KRB-BSA containing 0.1 mg/ml lima bean trypsin inhibitor. One milliliter aliquots of the cell suspension (4–5 $\times$ 10^5 cells) were incubated with the hormone (0.05 ml) in plastic culture tubes under 95% O_2:5% CO_2, with gentle shaking in a Dubnoff metabolic incubator. At the end of the incubation 1 ml of 20 m*M* Tris–HCl buffer (pH 7.4) containing 5 m*M* theophylline was added. The contents of each tube were immediately transferred to glass tubes and immersed in boiling water for 20 min. The heat-treated cells and medium were then applied on a column of neutral alumina (6 $\times$ 0.5 cm) (7) and washed with 5 ml 20 m*M* Tris–HCl, pH 7.4. The elutate was lyophilized and redissolved in 1 ml 0.1 *M* acetate buffer, pH 4.0. The tritiated cyclic AMP was measured by incubating 0.05 ml aliquots of the reconstituted eluate from alumina columns with 100 μg of cyclic AMP binding protein[2] prepared according to Gilman (8) in a total volume of 0.2 ml 0.1 *M* acetate buffer, pH 4.0. The ^{3}H-cyclic AMP bound to the protein was adsorbed on a Millipore filter and counted as described by Gilman (8). The aliquots added to the cyclic AMP binding protein were suitably diluted such that the amount bound was below the half saturation level. Under these conditions the amount of radioactivity bound to the protein showed a linear relationship to the amount of

[1] Work supported in part by U.S. Public Health Service Grant GM-2907.

[2] This protein as isolated from bovine diaphragm elevator muscle had a K_m of 40 n*M*. This protein (100 μg) was sufficient to bind 10 pmoles of cyclic AMP.

TABLE I. Effect of ACTH and NPS-ACTH on Cyclic AMP Synthesis in Isolated Adrenal Cells.[a]

Hormone	Concn ($\times 10^{-9}$ M)	Cyclic AMP/ flask (cpm)
None	—	116 ± 60[b]
ACTH	0.5	1480 ± 125
	5.0	9270 ± 1626
NPS-ACTH	50	251 ± 28
	500	472 ± 2

[a] Cells prelabeled with ^{3}H-adenosine were incubated for 10 min at 37° and ^{3}H-cyclic AMP was measured as described under "Materials and Methods."

[b] Values are the mean ± standard deviation.

radioactive cyclic AMP present. This was checked by comparing the binding observed with a 0.025 ml aliquot. Under conditions of half maximal binding and below, 55–60% of the total ^{3}H-cyclic AMP in the sample was bound to the protein. The newly formed cyclic AMP was also identified by chromatography on DEAE paper. The results obtained from protein binding agreed closely with that obtained by the chromatographic method.

All incubations were performed in triplicate. Results are expressed as total radioactivity due to cyclic AMP in each sample.

Results and Discussion. ACTH produces an 80-fold stimulation of cyclic AMP synthesis at a concentration of 5×10^{-9} *M* whereas NPS-ACTH increases cyclic AMP synthesis only 4-fold even at a concentration of 5×10^{-7} *M* (Table I). The maximum stimulation of cyclic AMP synthesis due to NPS-ACTH was always less than 10% of the maximal stimulation due to ACTH. The time course of cyclic AMP accumulation in isolated rat adrenal cells due to ACTH and NPS-ACTH is depicted in Fig. 1. Both peptides produce a rapid increase in cyclic AMP level which reaches a maximum at 30 min.

The effect of NPS-ACTH on the ACTH induced stimulation of cyclic AMP synthesis is shown in Fig. 2. ACTH produces maximal stimulation of cyclic AMP formation at a concentration of 1×10^{-8} *M*. Half-maximal stimulation is produced by ACTH at a concentration of 1.6×10^{-9} *M*. NPS-ACTH (2×10^{-6} *M*) increased cyclic AMP levels 9-fold. In the presence of 2×10^{-6} *M* NPS-ACTH the large stimulation observed with ACTH alone was almost completely inhibited. ACTH was able to partially overcome the inhibition due to NPS-ACTH at an ACTH:NPS-ACTH ratio of 1:100.

These results indicate that chemical modification of the tryptophan residue of ACTH leads to a significant reduction in the ability of the hormone to stimulate cyclic AMP ac-

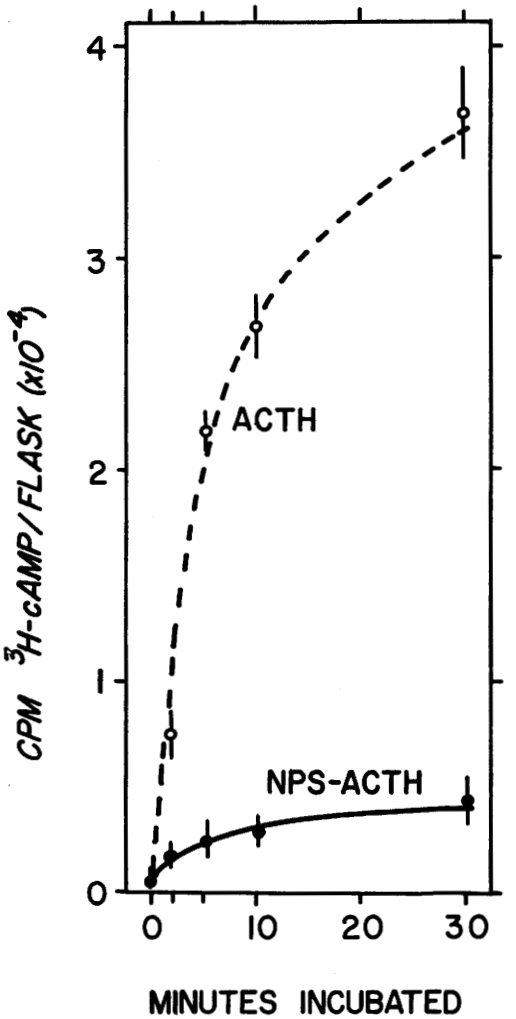

FIG. 1. Time course for the accumulation of ^{3}H-cyclic AMP in isolated adrenal cells preincubated with ^{3}H-adenosine. ACTH (1.7×10^{-8} *M*) or NPS-ACTH (6.3×10^{-7} *M*) were added at 0 min. Incubations were terminated at the times indicated.

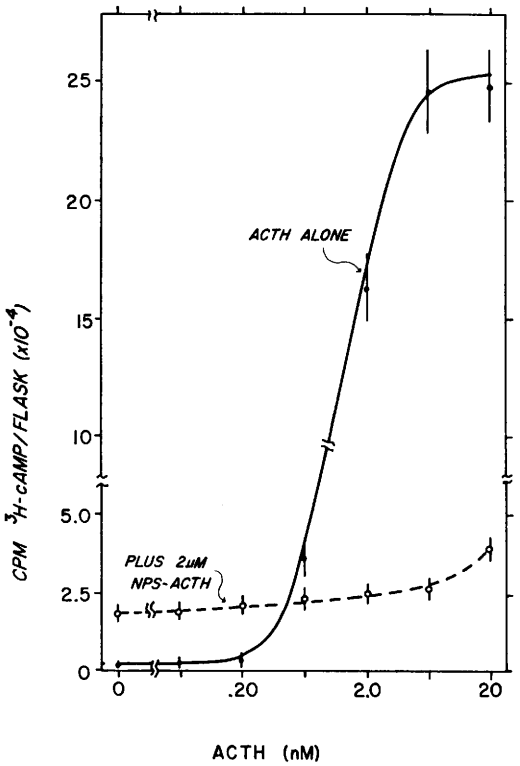

FIG. 2. Stimulation of ^{3}H-cyclic AMP synthesis as a function of ACTH concentration in the absence and presence of 2×10^{-6} *M* NPS-ACTH in isolated adrenal cells preincubated with ^{3}H-adenine. The cells were incubated for 20 min.

cumulation in isolated adrenal cells. The maximal stimulation is reduced by an order of magnitude and the concentration required for half-maximal stimulation is increased by two orders of magnitude. As in the case of the rat epididymal fat cell, the single tryptophan residue in ACTH appears to be essential for at least one of the actions of the hormone on the rat adrenal cell.

The ability of NPS-ACTH to act as a partial agonist as well as an inhibitor of the action of ACTH may reflect the heterogeneity of the isolated adrenal cell preparation. On the basis of binding studies with a mouse adrenal tumor preparation using ^{125}I-labeled hormone. Lefkowitz, Roth and Pastan (9) have suggested that there are two types of receptors in the adrenal, one with high affinity for the hormone and the other, more abundant type with lower affinity for ACTH. NPS-ACTH has already enabled us to distinguish between MSH receptors present in the rabbit fat cell and ACTH receptors present in the rat fat cell (2). It is now possible to investigate the adrenal receptors and distinguish between them in terms of structural requirements for favorable interaction. The effect of NPS-ACTH on steroidogenesis *in vivo* (10) and *in vitro* (11) have also been investigated and will be presented elsewhere.

Summary. The stimulation of cyclic AMP synthesis in isolated rat adrenal cells prelabeled with ^{3}H-adenine has been studied. Whereas adrenocorticotropin (ACTH) produces a very large increase in cyclic AMP levels, the *o*-nitrophenyl sulfenyl derivative (NPS-ACTH) causes only a small increase in cyclic AMP formation. NPS-ACTH also inhibits the ACTH induced cyclic AMP synthesis effectively.

We thank Professor C. H. Li for his continued interest and encouragement.

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Received May 24, 1972. P.S.E.B.M., 1972, Vol. 141.