SITE OF ACTION OF ADRENOCORTICOTROPIC HORMONE (ACTH) IN ADRENAL CELL CULTURES

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(Publication 589)

Received July 29, 1968

Taunton et al. (1967) have demonstrated that the first step in ACTH stimulation of adrenals is binding of the hormone to receptors on the outside surface of the target tissue. Haynes (1958) and Grahame-Smith et al. (1967) have shown that one of the early biochemical actions of ACTH is activation of the membrane bound enzyme, adenyl cyclase (Davoren and Sutherland, 1963). The results presented here provide some insight into the transition from binding of ACTH to the cell surface to ACTH activation of adenyl cyclase. These results indicate that ACTH exerts its stimulatory effect on the outside surface of adrenal cells without entering the target tissue.

MATERIALS AND METHODS

Adrenocorticotropic hormone activity was assayed by measuring stimulation of steroid production in adrenal cell cultures (Buonassisi et al., 1962). Culture adapted adrenal cortex tumors were plated in 15 x 60 mm tissue culture dishes (Falcon) and grown in F10 growth medium (Ham, 1963) supplemented with 15% horse serum and 2.5% fetal calf serum. Medium was changed twice weekly. Adrenal cells were incubated overnight with ACTH in 2 ml of growth medium supplemented with sera. Steroid production was determined by 242 mμ absorp-

tion in ethanol following extraction of the incubation medium with methylene chloride. The media from 2 plates were pooled for each point. Incubation medium extracted in the same way served as a blank.

ACTH was diazotized to p-amino-benzoyl-cellulose (Cellex PAB, Bio-Rad Laboratories) by a modification of the procedure of Campbell et al. (1951) as recommended by Bio-Rad Laboratories. Using this procedure, 0.5 mg of chromatographically isolated ACTH (150 I.U./mg; Mann Research Laboratories) or 0.5 mg of the eicosapeptide analog of ACTH (100 I.U./mg; Hofmann et al., 1962) was coupled to 1 gm of the resin. The resin was washed with two 500 ml portions of 1 M NaCl and two 500 ml portions of distilled water.

RESULTS

The response observed when adrenal cells in culture are treated with a maximally stimulating dose of ACTH (Buonassisi et al., 1962) is shown in Table I. Steroid production is proportional to the log of the ACTH concentration (Buonassisi et al., 1962). When ACTH is diazotized to cellulose, suspended in growth medium, and then added to the cells at a concentration of 2 mg/plate, the cells are stimulated to produce 52.8 mμmoles of steroid above the control (Table I). The cellulose fibers are visible under the light microscope and appear at least as long as a single adrenal cell. Filtration of the growth medium suspension of ACTH-cellulose through a medium-sized, fritted glass funnel results in a much lower level of stimulation (Table I). These results indicate that most of the ACTH activity is chemically linked to a large, insoluble, cellulose polymer, and that the ACTH-cellulose is biologically active. As shown (Table I), β-naphthol diazotized to cellulose does not markedly affect the level of steroid production.

The synthetic eicosapeptide analog of ACTH, when tested in this in vitro system at a concentration of 10 mU/plate, exhibits full biological activity (Table I). When linked to cellulose and suspended in growth medium, the eicosapeptide stimulates production of an average of 54.7 mμmoles of steroids (Table I). The filtrate from the eicosapeptide-cellulose suspended in medium

Table I. Effect of ACTH-Cellulose on Adrenal Cell Cultures

	Experiment I		Experiment II	
Additions to plate	Absorbance 242 mμ	Δ^4-3-keto-steroids mμmoles	Absorbance 242 mμ	Δ^4-3-keto-steroids mμmoles
None	0.08 0.12	7.2	0.00 0.00	0.0
ACTH (Armour) 10 mU	0.96 1.00	70.0	1.10 1.12	80.0
Eicosapeptide 10 mU	1.04 1.14	78.0	- -	-
β-Naphthol-cellulose 2 mg	0.16 0.20	12.9	0.00 0.00	0.0
ACTH-cellulose wash 2 mg	0.30 0.30	21.4	- -	-
ACTH-cellulose 2 mg	0.74 0.93	60.0	- -	-
Eicosapeptide-cellulose wash 10 mg	0.14 0.16	10.7	0.00 0.00	0.00
Eicosapeptide-cellulose 10 mg	0.80 0.84	59.5	0.70 0.90	57.0

is devoid of biological activity as compared with the activity exhibited by the resin without ACTH (Table I).

DISCUSSION

The observation that the large, insoluble ACTH-cellulose complex is biologically active in adrenal cell cultures strongly indicates that the hormone exerts its effect without entering the target cells. Similar results with the synthetic eicosapeptide analog of ACTH minimizes the possibility that ACTH is cleaved from the cellulose resin before stimulating the adrenal cells. Cleavage of this ACTH analog would be expected to result in a sharp decrease in biological activity (Hofmann et al., 1962).

The mechanism whereby ACTH activates the membrane bound enzyme adenyl

cyclase (Davoren and Sutherland, 1963) is as yet unknown. In view of the results presented here, it is possible that interaction of ACTH with surface receptors (Taunton et al., 1967) results in a conformational change in the cell membrane which triggers the activation of adenyl cyclase. The conformational change at the cell surface could result in a change in membrane permeability or in a conformational change in the enzyme directly. Adrenal cells in culture are known to round up shortly after administration of ACTH (Sato and Yasumura, 1966). This peculiar morphological change might be a reflection of the conformational change that takes place at the surface receptors of the target cells.

It is quite possible that other polypeptide hormones which stimulate their target tissues by activation of adenyl cyclase act in an analogous manner. Consistent with this suggestion are the observations of Fong et al. (1960) and Pastan et al. (1966). Fong et al. (1960) have demonstrated that binding of vasopressin to proteins in the membrane fraction of kidneys through sulfhydryl groups is a necessary step in its mechanism of action. Pastan et al. (1966) have observed that the first step in the action of thyroid stimulating hormone on thyroid slices is the binding of the hormone to receptors at the surface of the target tissue.

Studies with mutants of the adrenal cell line are presently under way to further elucidate the mechanism of transition from ACTH binding to adenyl cyclase activation in adrenal cell cultures (Schimmer and Sato, unpublished observations).

ACKNOWLEDGEMENTS

We are grateful to Miss Cathleen O'Shea for her technical assistance, and we wish to thank Dr. Klaus Hofmann for his generous gift of the eicosapeptide analog of ACTH. This work was supported in part by research grants from the National Institute of Health (CA 04123) and the National Science Foundation (GB 7104). One of us, Bernard P. Schimmer, received support from a National Institute of Health training grant (5T01 NB05241).

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