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Regulation of Actin in Rat Adrenocortical Cells by Corticotropin*

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Corticotropin (ACTH) induces a characteristic retraction of rat adrenocortical cells in culture. Densitometric analysis of sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels of cell lysates showed that ACTH induced a 20% decrease in actin content. These results were fully confirmed by quantitation of actin by the DNase 1 inhibition assay. The decrease in actin is hormone-specific, concentration-dependent, and correlates temporally with the morphological change induced by ACTH. The change in actin content and the reorganization of microfilament ultrastructure may together mediate hormone regulation of cell shape at the cytoskeletal level.

Nonmuscle actin is a major structural protein in many cell types and is a principal component of the cytoskeleton. Actin has been localized in a wide spectrum of organelles including pseudopodia, microvilli, and the mitotic spindle. It is the major component of microfilaments, which comprise the so-called "stress cables" seen in cells by phase contrast microscopy (1). Due to the many physicochemical properties in common with muscle actin, nonmuscle actin has been implicated in the movement of these organelles as well as the movement of the entire cell and its attachment to the substratum in culture (1). Furthermore, the ability of nonmuscle actin to polymerize reversibly has led to the proposal that the organization of stress cables determines the cytoskeletal architecture of the cell to a large extent.

The shapes of many cell types are altered by specific external stimuli. Lutropin and dibutyryl-cAMP influence the morphology of bovine luteal cells in culture (2) and thrombin affects the shape of human platelets (3). Follitropin induces retraction of Sertoli cells in culture (4) and corticotropin produces a characteristic retraction of rat adrenocortical cells in culture (5, 6).

Immunofluorescent staining of the cytoskeleton has revealed that such shape changes are accompanied by dramatic alterations in microfilament organization. It has been suggested that the regulation of cell shape may be mediated by the organization of actin (7). We have examined the effects of ACTH[1] on rat adrenocortical cell actin. Quantitative measurements of the actin content of cells showed that ACTH causes a significant decrease in the amount of actin. In this communication we present biochemical evidence that ACTH regulates rat adrenocortical cell actin.

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[1] The abbreviation used is: ACTH, corticotropin.

MATERIALS AND METHODS

Highly purified porcine ACTH (8), synthetic corticotropin-inhibiting peptide (9), and synthetic endorphin (10) were prepared in this laboratory by the published procedures. Deoxyribonuclease I from bovine pancreas, calf thymus DNA, type I, lima bean trypsin inhibitor, and phenylmethylsulfonyl fluoride were obtained from Sigma. All gel electrophoresis reagents were obtained from Bio-Rad. Purified rabbit skeletal muscle actin was a gift of Dr. Roger Cooke.

Cell Culture—Adrenocortical cells were isolated from the decapsulated adrenals of adult male Sprague-Dawley rats as previously described (6) and plated in medium 199-D-valine containing 10% dialyzed fetal bovine serum and garamycin (80 μg/ml) at a density of 5×10^5 cells/well (11). The medium was changed every other day and cultures were used 7-14 days after plating.

DNase 1 Inhibition Assay for Actin—The assay was performed essentially as described by Blikstad *et al.* (12). Cells were treated with hormone or other agents and at specified times, the cells were washed 3 times with ice-cold phosphate-buffered saline and lysed mechanically by scraping in 150 μl of lysis buffer (5 mM phosphate, pH 7.6, containing 150 mM NaCl, 2 mM $MgCl_2$, 0.2 mM Na_2ATP, 0.2 mM dithiothreitol, 0.5% (v/v) Triton X-100, 10 μM phenylmethylsulfonyl fluoride, and 0.001% lima bean trypsin inhibitor. A 50-μl aliquot was immediately removed, mixed with 50 μl of dissociating buffer (20 mM Tris-HCl, pH 7.6, 1 M sodium acetate, 1 mM $CaCl_2$, 1 mM Na_2ATP, 1.5 M guanidine-HCl), and incubated on ice for 15 min. Then 20 μl of DNase 1 (0.1 mg/ml in 50 mM Tris-HCl, pH 7.5, 0.5 mM $CaCl_2$, 10 μM phenylmethylsulfonyl fluoride) were added. Sixty μl of this mixture were removed and added to 3 ml of DNA at a concentration of 50 μg/ml in 100 mM Tris-HCl, pH 7.5, 4 mM $MgSO_4$, 1.8 mM $CaCl_2$. The change in absorbance at 260 nm was recorded for at least 5 min to determine V_{max}. Standard curves were generated in exactly the same way using purified rabbit skeletal muscle actin.

DNA was determined by the method of Ceriotti (13) using the modifications of Short *et al.* (14).

Polyacrylamide Gel Electrophoresis—Cells were washed 5 times with ice-cold phosphate-buffered saline and then lysed in 58 mM Tris-HCl, pH 6.8, containing 2% sodium dodecyl sulfate, 10% glycerol, 0.4% dithiothreitol, and 0.1% bromphenol blue. The lysates were heated for 5 min at 90 °C, cooled to room temperature, and 25-50 μl were applied per lane of gel and subjected to electrophoresis according to Laemmli (15) on 10% polyacrylamide gels (1.5 mm thickness). Gels were stacked at 18 mA for 15 min and run at 35 mA for 4 h. The gels were stained with 0.025% Coomassie brilliant blue (R-250) overnight and destained first in 25% isopropyl alcohol, 10% acetic acid, 0.0025% Coomassie blue, and then in 10% acetic acid. The gels were air-dried between dialysis membranes and scanned in a GCA/McPherson Model EU 205-11 scanning densitometer at 550 nm at a rate of 5 cm/min at 1 mA sensitivity.

RESULTS

Rat adrenocortical cells in culture formed a confluent monolayer of flattened cells. A marked retraction of the cells was observed after 6 h in the presence of 0.22 nM ACTH. The morphological changes were identical with those described previously (6) except that they were produced by physiological concentrations of the hormone. Maximal retraction was observed in the presence of 2.2 nM ACTH. Other basic polypeptides such as insulin, endorphin, or corticotropin-inhibiting peptide (ACTH (7-38)) had no effect on rat adrenocortical cell morphology.

The sodium dodecyl sulfate gel patterns of cells incubated with or without 2.2 nM ACTH are shown in Fig. 1. The major protein band in control as well as ACTH-treated cells corresponded to M_r = 43,000. That this band is indeed actin was

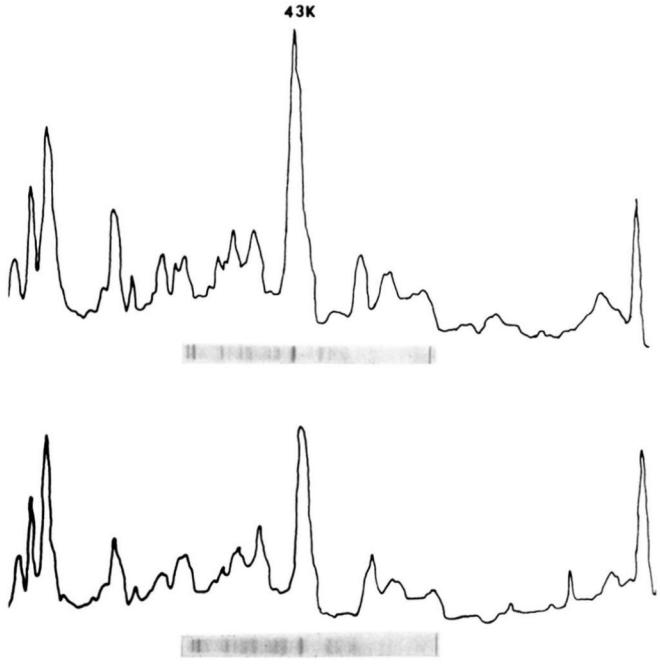

FIG. 1. **Densitometric analysis of sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels.** Cells were incubated with (*bottom*) or without (*top*) 2.2 nM ACTH for 24 h and processed as described under "Materials and Methods" for sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The actin band is denoted by the molecular weight (43K, 43,000).

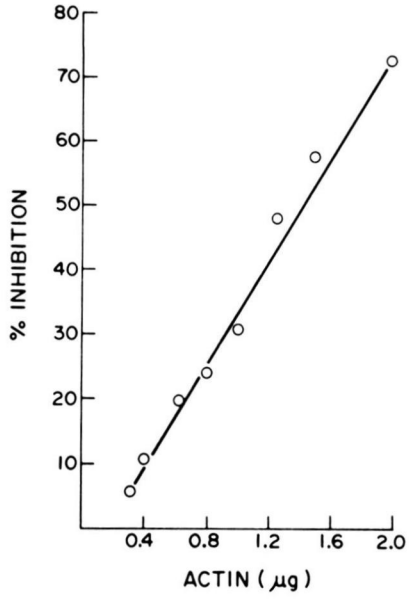

FIG. 2. **Standard curve for actin measurement by the DNase 1 inhibition assay.** Purified rabbit skeletal muscle actin was used to inhibit DNase 1. Details of the assay are outlined under "Materials and Methods." Each point represents the mean of three determinations. The correlation coefficient is 0.992.

confirmed by amino acid analysis of the protein eluted from the gels and DNase 1-Sepharose affinity chromatography (data not shown). By weighing the actin peak as well as the other protein peaks, it was found that actin amounted to 10–12% of the total cellular protein in control cells. The actin band was significantly decreased in ACTH-treated cells. The decrease was estimated to be 25% compared to the control cells.

In order to confirm and quantitate this change in actin content more accurately, we resorted to the DNase 1 inhibition assay for actin (12). This assay is based on the fact that monomeric actin (G-actin) binds tightly and irreversibly to DNase 1 and inhibits its enzymic activity. The presence of guanidine-HCl at a final concentration of 0.75 M ensures complete depolymerization of actin in cell lysates. The degree of inhibition is therefore a direct measure of actin content. A standard curve of inhibition using purified rabbit skeletal muscle actin is shown in Fig. 2. It is known that both muscle and nonmuscle actins inhibit DNase 1 in a comparable manner (12). It is evident from Fig. 2 that in the range 0.3 to 1.5 μg of actin the inhibition is linear. Cell lysates were diluted appropriately to produce inhibitions within this range.

Using this assay, the effects of ACTH on rat adrenocortical cell actin were investigated. The results in Table I show that ACTH caused a selective dose-dependent decrease in the actin content which cannot be attributed to changes in cell number. During the 12-h incubation with the hormone the DNA content of the cells was unchanged. The kinetics of the action of ACTH on actin content are shown in Fig. 3. It is apparent that a maximal decrease in actin content is produced within 12 h after the cells are exposed to the hormone.

The specificity of the change in actin content induced by ACTH are shown in Fig. 4. Other peptide hormones which do not stimulate adrenocortical function did not alter the actin content of these cells. *o*-Nitrophenylsulfenyl-Trp^9-ACTH, which is a weak ACTH agonist (16), also produced a concentration-dependent decrease in actin but only at higher concentrations. The effect of ACTH on the actin content of adreno-

TABLE I

Effect of ACTH on rat adrenocortical cell actin

Adrenocortical cells (5×10^5/well) were incubated with or without ACTH for 12 h, 7 days after plating. The cells were processed as described under "Materials and Methods" and actin content was determined by the DNase 1 inhibition assay and DNA content according to Short *et al.* (14). Values are mean ± S.E. of the number of separate determinations shown in parentheses.

Hormone	Actin	DNA	Actin/DNA	Decrease
	μg	μg		%
None	18.84 ± 0.64 (4)	4.05 ± 0.02 (3)	4.65 ± 0.16	
ACTH (0.22 nM)	16.70 ± 0.00 (8)	4.08 ± 0.07 (3)	4.09 ± 0.07	12
ACTH (2.2 nM)	14.85 ± 0.07 (6)	3.98 ± 0.04 (3)	3.67 ± 0.04	21

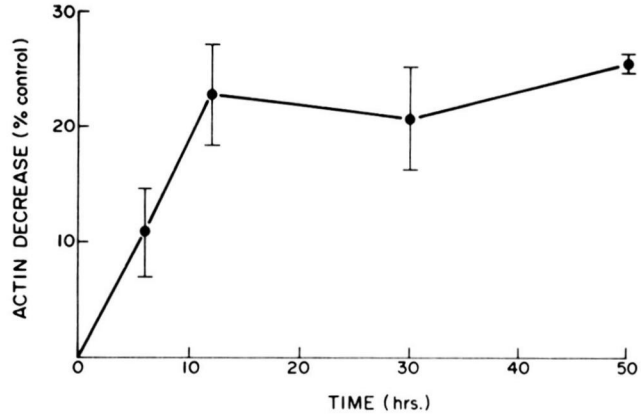

FIG. 3. **Time course of the decrease in actin induced by ACTH.** Cells were incubated with and without 2.2 nM ACTH and at specified times the cells were processed as described under "Materials and Methods" for the determination of actin by the DNase 1 inhibition assay. DNA was also measured at each time point. Results are from four experiments. Each point represents the mean ± S.E. of at least three experiments. In each experiment cells were incubated in triplicate with or without ACTH. Percentage of decrease was calculated from micrograms of actin/μg of DNA values at each time point.

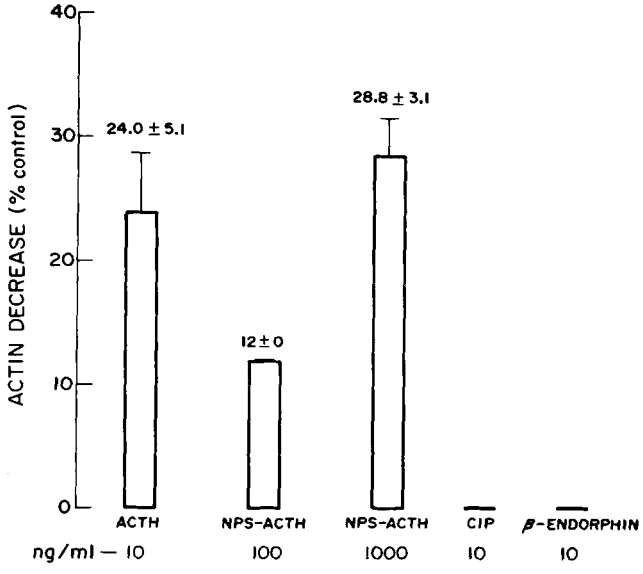

FIG. 4. **Specificity of ACTH-induced decrease in actin.** Cells were incubated with the various peptides for 24 h and then processed for actin determination by DNase 1 inhibition assay. Values are the means ± S.E. of triplicate incubations. *CIP*, corticotropin-inhibiting peptide; *NPS*, o-nitrophenyl sulfenyl.

cortical cells was reversed when the hormone was removed. Cells incubated in the absence of ACTH for 20 days had 5.38 ± 1.23 μg of actin/μg of DNA compared to a value of 2.45 ± 0.43 for cells incubated with ACTH. Cells treated with ACTH for 14 days and incubated for 6 more days in the absence of ACTH had 6.35 ± 1.12 μg of actin/μg of DNA.

DISCUSSION

Actin is a major protein in a variety of cell types. In rat adrenocortical cells, actin represents at least 10% of the total cellular protein. Both densitometric scans of sodium dodecyl sulfate gels and the DNase 1 inhibition assay show that ACTH induces a 20–25% decrease in actin. Therefore, at least 2% of the total cellular protein is lost in response to the hormone. Such a major change in protein must have tremendous consequences for cell function and shape.

Changes in actin content in response to hormonal stimuli have been reported (17, 18). Sidhu (19) found a fall in total actin to approximatelv 20% of unstimulated levels during the maturation of 3T3-L1 mouse preadipocytes into functional adipocytes by treatment with dexamethasone. This decrease was accompanied by a morphological transformation from flattened to spherical shape. Bragina *et al.* (20) used the DNase 1 inhibition assay to demonstrate a fall in actin content in mouse myeloma cells in response to dexamethasone.

Immunofluorescent techniques for visualizing specific components of the cytoskeleton have implicated microfilaments in defining cell shape. Weber *et al.* (21) have shown that agents such as cytochalasin B which disrupt microfilaments cause both retraction and rounding of cultured 3T3 cells and a dramatic depolymerization of actin stress cables.

Direct measurements of monomeric and polymeric actin pools in cells confirm the results obtained by immunofluorescent methods. Carlsson *et al.* (3) utilized the DNase 1 inhibition assay to demonstrate a rapid depolymerization of microfilaments in human platelets stimulated with thrombin to change shape from flattened discs to spheres. Swanston-Flatt *et al.* (22) used a similar approach to show microfilament formation in pancreatic β-cells during glucose stimulation of insulin release. These observations, taken together with the immunofluorescence data and measurements of actin content, strongly suggest that changes in both actin content and organization define and possibly regulate cell shape. Preliminary investigations of microfilament organization in cultured rat adrenocortical cells by indirect immunofluorescence show a depolymerization of actin stress cables in response to ACTH.

The results presented here demonstrate a hormonally mediated decrease in actin in cultured rat adrenocortical cells. The observation that both the loss of actin and the retraction of cells in response to ACTH are hormone-specific, concentration-dependent, and follow similar time courses is suggestive evidence that these effects are in some way related. The ACTH-induced changes in cell shape and actin content are both observable 6 h after the addition of the hormone. This lag period is very similar to that observed for other trophic actions of ACTH, namely, inhibition of DNA synthesis (23), induction of 3β-hydroxysteroid dehydrogenase-isomerase (11) and ectokinase activity (24). It is tempting to speculate that all of these effects are interrelated manifestations of the pleiotropic response of the adrenocortical cell to ACTH. Whether these effects are regulated via some common mediator and how reorganization of the cytoskeleton might be related to growth control are important questions that remain to be elucidated.

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