Glucocorticoids Increase Amylase mRNA Levels, Secretory Organelles, and Secretion in Pancreatic Acinar AR42J Cells

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ABSTRACT Previous studies have suggested a role for glucocorticoids in the differentiation of the acinar pancreas. We have now used the rat tumor cell line AR42J, derived from the acinar pancreas, to directly study this effect of glucocorticoids in vitro. The steroid hormones dexamethasone, corticosterone, aldosterone, and progesterone, but not estrogen, increased both the amylase content and the number of secretory granules of these cells. The potencies of the steroids were directly related to their effectiveness as glucocorticoids; dexamethasone was the most potent hormone and gave maximal effects at 100 nM. Morphometric analyses revealed that dexamethasone increased the volume density of granules 5.5-fold from 0.20 ± 0.08 to 1.10 ± 0.20% (n = 4) of the cytoplasmic volume. Dexamethasone treatment also increased the volume density of rough endoplasmic reticulum 2.4-fold from 1.20 ± 0.09 to 2.86 ± 0.30% (n = 5) of the cytoplasmic volume. After 48 h of dexamethasone treatment the cellular content of amylase increase eightfold from 2.8 ± 0.4 to 22.6 ± 3.8 U/mg protein (n = 6). This effect of dexamethasone was discernible after 12 h of incubation and approached maximal stimulation after 72 h of incubation. The increases in cellular amylase content were due to increased amylase synthesis as shown by specific immunoprecipitation of [^{35}S]methionine-labeled proteins. Moreover, in vitro translation of cellular mRNA indicated that dexamethasone treatment increased amylase mRNA. Dexamethasone treatment also led to increased secretion of amylase in response to the secretagogue cholecystokinin. These data indicate, therefore, that glucocorticoids induce a more highly differentiated phenotype in AR42J pancreatic cells, and they suggest that glucocorticoids act via the enhanced transcription of specific mRNAs for acinar cell proteins.

Steroid hormones exert many of their effects on target cells by modulating the expression of genes for specific proteins. Prior studies have suggested that glucocorticoids influence the morphological differentiation of the exocrine pancreas, and that they increase the expression of genes for amylase and possibly other digestive enzymes. In vivo, injections of glucocorticoids lead to increased amylase activity in the young rat (18) and the chick embryo (4). Also, adrenalectomy has been reported to decrease the pancreatic contents of amylase in the rat (8, 9). In vitro, glucocorticoids have been reported to enhance the accumulation of secretory enzymes in embryonic organ explants of the chicken (4) and the rat (11, 23–25, 29, 32).

To understand the direct interaction of glucocorticoids with the exocrine pancreas, the study of a cultured line of pancreatic acinar cells would be beneficial. Prior studies demonstrated the usefulness of a transplantable pancreatic acinar tumor in understanding normal pancreatic acinar physiology (13–15). In the present study, we used the AR42J cell line, which was derived from a chemically induced carcinoma of the acinar pancreas (16). These cells have been reported to contain digestive enzymes and, more importantly, can be grown in vitro. We found that these cells have measurable quantities of amylase and that they secrete this enzyme in response to the secretagogue cholecystokinin. Moreover, in AR42J cells, glucocorticoids were found to directly increase

THE JOURNAL OF CELL BIOLOGY · VOLUME 100 APRIL 1985 1200–1208

both the number of secretory granules and the levels of mRNA for amylase. These changes lead to an increase in the synthesis, content, and secretion of this enzyme.

MATERIALS AND METHODS

Materials: The following were purchased: bovine plasma albumin (fraction V) from Miles Laboratories Inc. (Elkhart, IN) and Reheis Co., Inc., (Chicago, IL); soybean trypsin inhibitor (type 1-S), bovine trypsin (type I), bovine ribonuclease-A (type IAS), bacitracin, benzamidine, HEPES, dexamethasone, aldosterone, corticosterone, progesterone, and estrogen from Sigma Chemical Co. (St. Louis, MO); IgG Sorb (protein A) from Enzyme Center Inc. (Boston, MA); [^{35}S]methionine and Enlightning from New England Nuclear (Boston, MA); bromophenol blue from J. T. Baker Chemical Co. (Phillipsburg, NJ); Dulbecco's modified Eagle's medium with and without methionine, penicillin, streptomycin, amphotericin B, fetal calf serum, and trypsin from the Cell Culture Facility at the University of California at San Francisco; 3,5-diaminobenzoic acid from Aldrich Chemical Co., Inc. (Milwaukee, WI); minimal Eagle's medium essential amino acid supplement from Gibco Laboratories (Grand Island, NY); dithiothreitol, TEMED (*N,N,N',N'*-tetramethylethylenediamine), molecular weight markers, ammonium persulfate, acrylamide, bis (*N, N'*-methylenebis[acrylamide]), and biorad protein reagent from Bio-Rad Laboratories (Richmond, CA); Oligo(dT)-cellulose from Collaborative Research, Inc. (Waltham, MA). Cholecystokinin octapeptide was a gift from Dr. Miguel Ondetti, E. R. Squibb & Sons (New Brunswick, NJ). All other chemicals and reagents were of analytical grade.

Cell Culture: AR42J cells (kindly provided by Dr. Y. Kim, VA Hospital, San Francisco, CA), were maintained as subconfluent monolayer cultures in Dulbecco's modified Eagle's medium containing penicillin, streptomycin, and 10% fetal bovine serum. Cells were routinely plated at 4×10^5 cells/ml into 16-mm wells (2 cm^2 surface area) in microcluster plates. The cells were cultured for 48 h in the same medium except during time-course studies when the media was changed daily. Steroid hormones were added at the beginning of the culture period except during time course studies when dexamethasone was added after various periods of culturing.

Amylase Secretion: To measure cholecystokinin-induced amylase release, we washed cultured cells twice with 1 ml HEPES-buffered Ringer solution containing 130 mM NaCl, 4.7 mM KCl, 1.0 mM NaH_2PO_4, 1.13 mM $MgCl_2$, 1.28 mM $CaCl_2$, 5.6 mM glucose, and 10 mM HEPES (pH 7.4) which was enriched with minimal Eagle's medium amino acid supplement, 0.1 mg/ml soybean trypsin inhibitor, 5 mg/ml bovine serum albumin (BSA), and gassed with 100% O_2. The cultures were then incubated at 37°C for 15 min and a sample of medium was taken from each well to determine the initial amylase concentration. This was followed immediately by the addition of various concentrations of cholecystokinin octapeptide diluted in HEPES-buffered Ringer solution. The cultures were then incubated for an additional 40 min at 37°C, followed by removal of the incubation media. Net amylase release was calculated as the amount of amylase present in the medium after 40 min minus the initial amount.

Biosynthetic Labeling of AR42J Cells and Immunoprecipitation: AR42J cells were plated into 35-mm dishes ($0.8–1.2 \times 10^6$ cells/dish). After 48 h the cells were washed once with methionine-free medium. The cells were then incubated for 3 h at 37°C in 1 ml of methionine-free medium containing 20 μCi of [^{35}S]methionine. The cells were then washed twice with 154 mM NaCl at 4°C and lysed by incubation at 24°C in 1 ml phosphate-buffered saline (PBS) containing 1% Triton X-100, bacitracin (1 mg/ml), and benzamidine (10 mM). After centrifugation at 10,000 *g* for 2 min at 4°C, the supernatants, referred to as cell lysates, were stored at −70°C or processed immediately.

Immunopurification of biosynthetically labeled pancreatic enzymes from cell lysates was carried out using antibodies bound to *Staphylococcus aureus* (IgG Sorb). Rabbit antibodies raised against purified bovine trypsin, ribonuclease-A, purified rat amylase, or a control nonimmune serum were incubated with a 10% suspension of *S. aureus* for 4 h at 4°C (20 μl serum/180 μl *S. aureus* suspension). Various amounts of cell lysate were added, and the incubation was continued overnight at 4°C. The immunoabsorbent was collected by centrifugation and washed twice with a buffer that contained 0.15 M NaCl, 0.01 M Na_2HPO_4/NaH_2PO_4, 0.02% NaN_3, 0.5% Nonidet P-40, 0.1% SDS, and 0.1% BSA (pH 8.6). After washing, the absorbed immune complexes were dissociated by boiling for 5 min in 2% SDS, 0.1 M dithiothreitol, 0.5 M 2-mercaptoethanol, 10 mM sodium phosphate, and 0.01% bromophenol blue (pH 7.0).

SDS PAGE: Electrophoresis in the presence of 0.1% SDS was performed by the method of Laemmli (21) in 10% polyacrylamide slab gels (12.5 mm × 16 cm × 1.5 mm). After electrophoresis, the gels were stained with 0.25% Coomassie Blue in 50% trichloroacetic acid for 30 min and destained in 5% methanol (vol/vol) and 7% acetic acid (vol/vol), treated with Enlightning (R), dried under vacuum and heat and autoradiographed with Kodak X-Omat film for 24 h at −70°C. Molecular weights were calculated by using as standards: phosphorylase B (92,500), BSA (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysosyme (14,400). After developing, autoradiograms of total lysates were scanned with a Zeineh Soft laser scanning densitometer (Biomed Instruments, Chicago IL). The incorporation of ^{35}S into the amylase band was quantitated by cutting out the peak from the densitometer tracing and weighing it on a balance. Incorporation into the amylase peak was expressed as the percentage of total labeling.

Assays: To determine cell numbers, we washed the cultures with PBS, then removed them from the dish by exposure to 100 μg/ml trypsin for 3 min. The cells were then washed again with PBS, resuspended in 1 ml PBS, and counted using a hemocytometer. When cell numbers were not to be determined, media from culture wells were removed and the cell monolayers were gently washed twice with 2 ml of 154 mM NaCl at 4°C. The cells were then scraped into 1 ml of buffer containing 0.05 M $NaPO_4$ and 0.05 M NaCl (pH 6.9) and sonicated with a probe-type sonicator for 30 s at 4°C. Amylase was assayed in aliquots of the sonicated homogenate by the method of Jung (17) which uses procion yellow coupled to starch as a substrate. For protein determination, an aliquot of cell lysate was diluted 1:1 (vol/vol) with 0.2 N NaOH, then boiled for 2 min; the protein content was then measured using Biorad reagent (2), bovine serum albumin diluted in 0.1 N NaOH was used as a standard. For DNA determination, a 200-μl aliquot of cell lysate was precipitated with sodium acetate at −70°C, washed twice with ice-cold ethanol, and air-dried. DNA content was determined by the fluorometric assay of Hinegardner (12), using diaminobenzoic acid and calf thymus DNA as a standard. Statistical analyses were carried out using Student's *t* test or, when appropriate, the paired *t* test.

Isolation of Polyadenylated RNA: Total cytoplasmic RNA was isolated according to the technique of Favaloro (7), with modifications. Cells grown in tissues culture flasks were washed twice with PBS at 4°C, scraped into 5 ml PBS using a rubber policeman, and then centrifuged at 300 × *g* for 5 min at 4°C. The cell pellet, containing ~10^7 cells, was resuspended in 1 ml of buffer containing 0.14 M NaCl, 10 mM $MgCl_2$, 10 mM Tris (pH 8.6), 0.5% Nonidet P-40, 1,000 U/ml RNAsin, and then vortexed for 20 s. To remove nuclei, we centrifuged the suspension at 10,000 × *g* for 1 min at 4°C. The supernatants were mixed with an equal volume of Proteinase K solution which contained 200 μg/ml Proteinase K, 25 mM EDTA, 0.3 M NaCl, 2% wt/vol SDS, and 0.2 M Tris-HCl (pH 7.0), and were incubated at 37°C for 30 min. The material was then shaken for 5 min at 4°C with phenol/chloroform (1:1 vol/vol) with isoamyl alcohol (1:24 vol/vol), and centrifuged at 10,000 × *g* for 5 min. The aqueous phase was extracted again with 2 ml chloroform and isoamyl alcohol, then mixed with 2.5 vol of ice-cold ethanol; the nucleic acids were precipitated overnight at −20°C.

Polyadenylated RNA was isolated from total RNA by oligo(dT)-cellulose chromatography (1). The purified mRNA was precipitated overnight in 2.5 vol of ethanol containing 0.2 M potassium acetate at −20°C.

In Vitro Translation Assay: Amylase mRNA activity was measured using a wheat germ system. Wheat germ extract was prepared by the procedure of Erickson and Blobel (6). The in vitro translation reaction was performed in a total volume of 100 μl consisting of: 40 μl wheat germ extract, 1.2 mM ATP, 0.08 mM GTP, 0.5 mM magnesium acetate, 9.6 mM creatine phosphate, 64 μg/ml creatine phosphokinase, 8 mM potassium hydroxide, 110 mM potassium acetate, 2 mM dithiothreitol, 0.08 mM spermine, 0.1 μg/ml pepstatin A, 0.1 μg/ml chymostatin, 0.1 μg/ml antipain, 0.1 μg/ml leupeptin, 10 U/ml Trasylol, 2.5 μg tRNA, 20 μM unlabeled amino acids excluding methionine, 45 μCi [^{35}S]-methionine, to which ~5 μg of RNA were added. The reaction was allowed to proceed for 2 h at 26°C and was terminated by freezing in liquid nitrogen. The translation products were analysed by SDS PAGE.

Electron Microscopy and Morphometric Analysis: Cells in 16-mm tissue culture wells were fixed overnight at 4°C in 2.7% glutaraldehyde and 0.8% paraformaldehyde in 80 mM sodium cacodylate (pH 7.5). The cultures were then postfixed with 2% OsO_4 for 1 h at room temperature in the above buffer, and then dehydrated in increasing concentrations of ethanol, passed through propylene oxide, and embedded in Polybed 812. Thick sections of 0.5 μm were stained with toluidine blue and viewed with a Zeiss photomicroscope (Carl Zeiss, Inc., Thornwood, NY). Thin sections were cut on a Sorvall MT1 ultramicrotome (Beckman Instruments, Inc., Palo Alto, CA) with a diamond knife. The sections were then collected on Parlodion-coated 300 mesh grids, stained with uranyl acetate and lead citrate, and examined with a Zeiss 10C electron microscope at 60 kV.

For morphometric analysis, we examined a total of five culture wells per condition from two separate experiments. Pictures were taken of the first ~20 cells in each well (~100 cells/condition) and a montage was constructed. The cells were further evaluated only if >90% of their cross-section was available in

the photographs. After printing, the final magnification was × 12,500. Membrane surface density (surface area of membrane per unit of cell volume) and organelle volume density were measured according to the stereological point-counting method of Weibel (33). A transparent test screen, with a double period square lattice (with a 1:5) ratio was used. To estimate the volume of the cell and the nucleus we used the coarse grid formed of 5-cm long lines. To estimate the volumes of secretory granules and rough endoplasmic reticulum, we used fine grid formed of 1-cm long lines. The profile area was estimated as the area derived by point-counting divided by the number of profiles. Volume densities (v) were estimated by determining the fraction of points falling on the organelles (P) compared to the total number falling within profiles of the cell or cytoplasm (P_t): $V = P/P_t$. When the fine grid was used, the values were divided by 25 to correct for differences in grid areas. The surface density (S_v) of the rough endoplasmic reticulum was derived from the number of intersection points (I) of the surface contour of membrane profiles. These variables were related by the following equation: $S_v = I/L_t$, in which L_t equals the number of points falling within the cytoplasm times the line length.

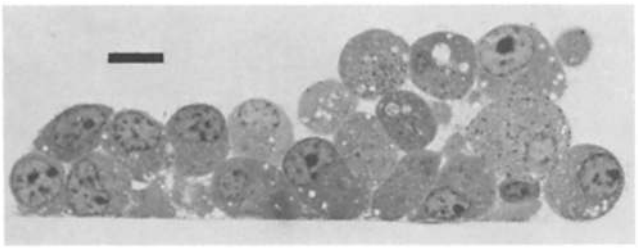

FIGURE 1 Light micrograph of a colony of AR42J cells cultured for 48 h in the presence of 10 nM dexamethasone. The cells grow on top of each other forming a pile. The nuclei and secretory granules are randomly organized, with no obvious polarity. Bar, 10 μm. × 680.

RESULTS

Glucocorticoid Effects on the Morphology of AR42J Cells

AR42J cells grew in colonies that form clumps rather than monolayers (Fig. 1). The cells did not have the polarity typical of normal secretory cells and secretory granules were scattered throughout the cytoplasm. At the ultrastructural level, under control conditions, the cells appeared relatively undifferentiated, with few secretory granules, numerous free polysomes, and sparse rough endoplasmic reticulum (Fig. 2). Dexamethasone treatment had little effect on cell size, as indicated by the lack of an effect on cell profile area (Table I). However, dexamethasone treatment led to a marked change in the content of cellular organelles of AR42J cells. In cells cultured for 48 h in the presence of 10 nM dexamethasone, there was a 4.8-fold increase in the volume density of secretory granules (Table I). This reflects both an increase in number and size

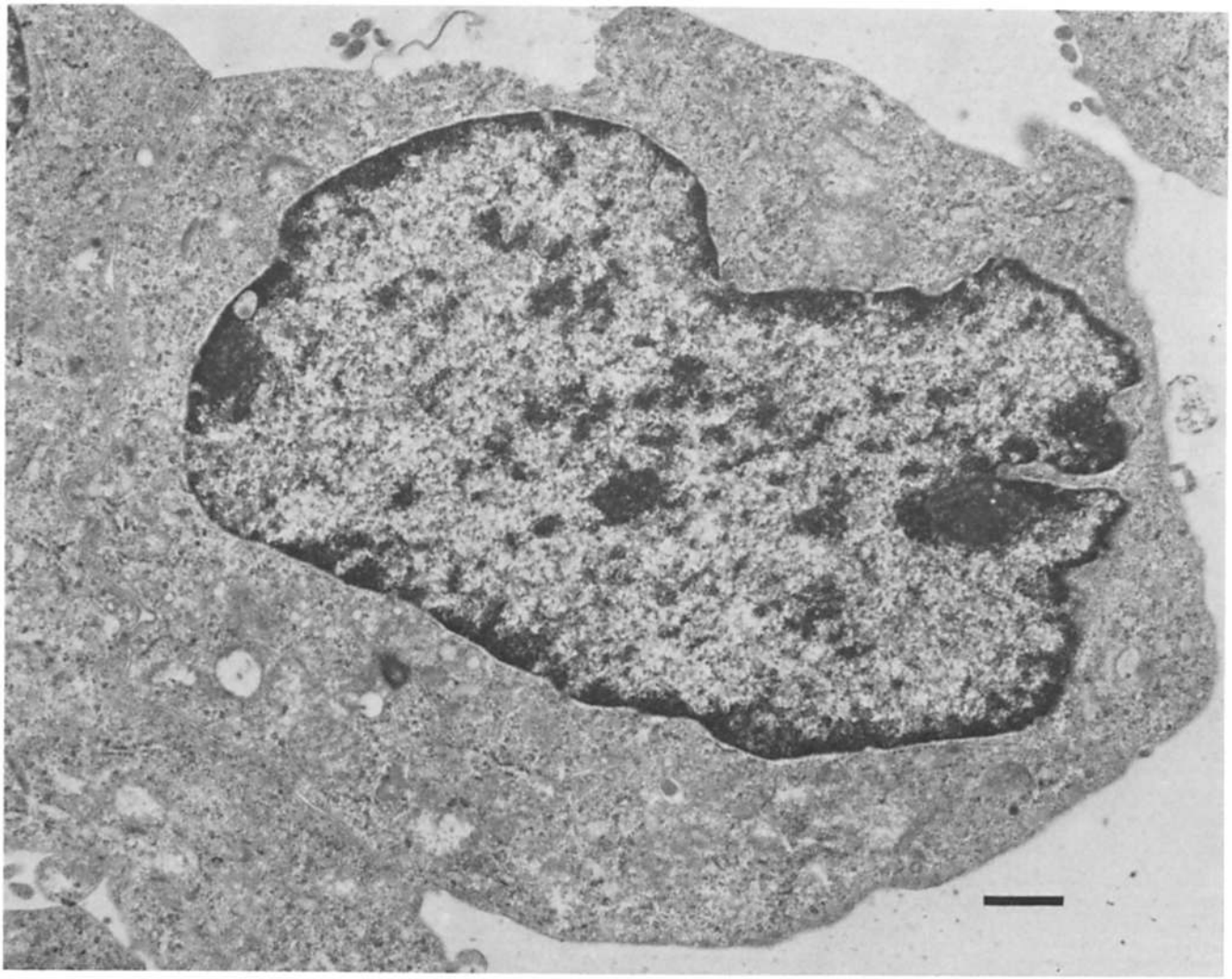

FIGURE 2 Electron micrograph of a typical AR42J cell cultured in the absence of dexamethasone. The cell displays an undifferentiated appearance. Structural specializations for secretion are not present. Bar, 10 μm. × 11,500.

TABLE I

*Morphometric Data on the Ultrastructure of AR42J Cells**

Structure	Control	Dexamethasone‡
Secretory granules (% cytoplasmic vol.)	0.20 ± 0.08	1.10 ± 0.20 ($p < 0.05$)§
RER‖ (% cytoplasmic vol.)	1.20 ± 0.09	2.86 ± 0.30 ($p < 0.001$)
RER‖ (cm^2/cm^3 cytoplasm)	184 ± 14	1017 ± 96 ($p < 0.001$)
Nucleus (% AR42J cell vol.)	31 ± 3	27 ± 3 (NS)
Cell profile (u^2)	104 ± 16	115 ± 5 (NS)

* Morphometric analyses were carried out as described in Material and Methods. The results are expressed as the mean ± SEM for five cultures (~100 cells/condition).
‡ Cultures were raised for 48 h in the presence of 10 nM dexamethasone.
§ Students't *t* test.
‖ *RER*, Rough endoplasmic reticulum.

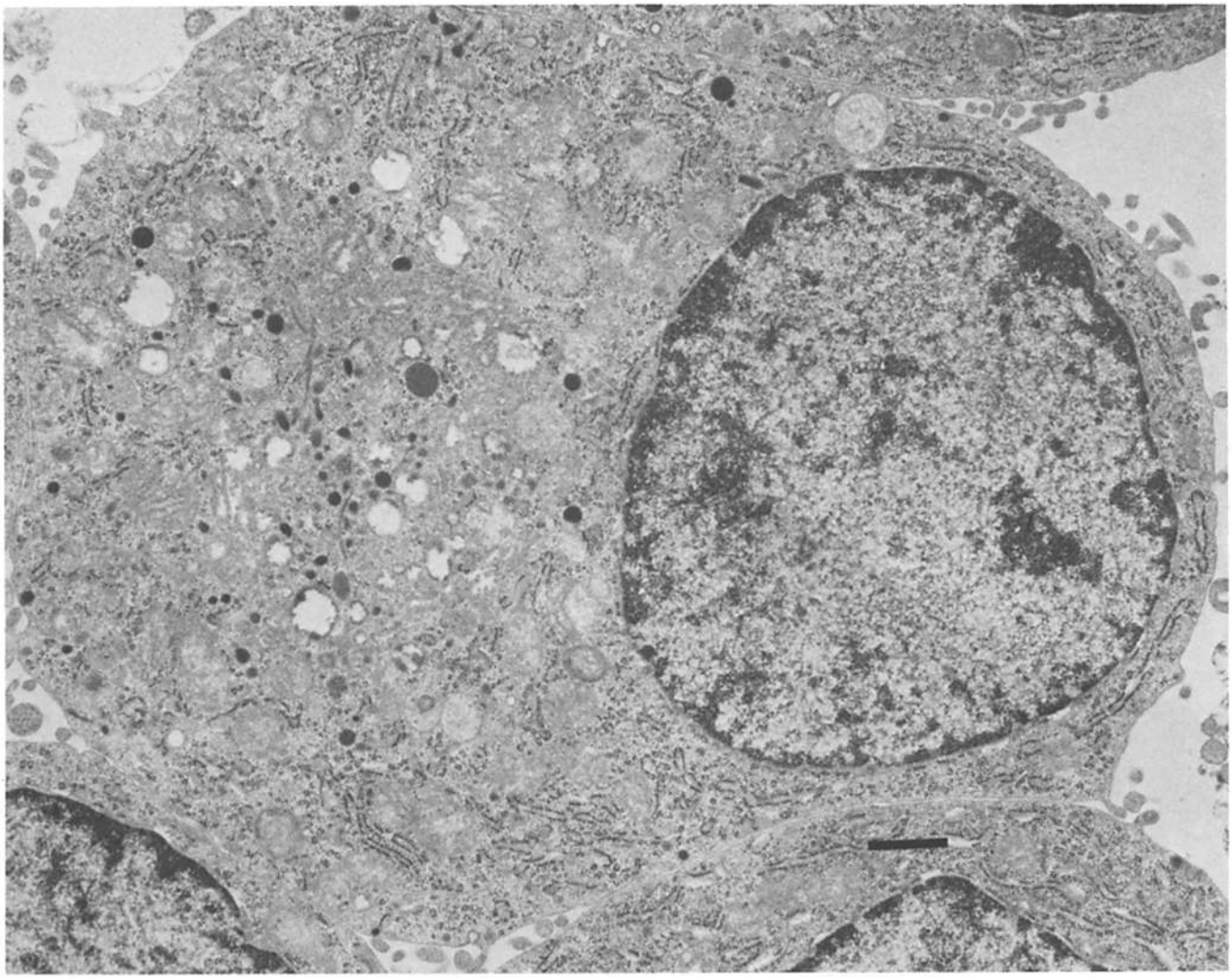

FIGURE 3 Electron micrograph of a typical AR42J cell cultured for 48 h in the presence of 10 nM dexamethasone. The cell has secretory granules and abundant rough endoplasmic reticulum. Bar, 10 μm. × 11,600.

of secretory granules (Fig. 3). The granules were small and often irregularly shaped in cross-section (Fig. 4).

Dexamethasone treatment also increased the amount of rough endoplasmic reticulum. Control cells had numerous free polysomes, but little rough endoplasmic reticulum (Fig. 5 [left]); however, dexamethasone-treated cells showed an increased amount of rough endoplasmic reticulum (Fig. 5 [right]). Morphometric analyses indicated that dexamethasone treatment had induced a 2.4-fold increase in the volume density and a 5.5-fold increase in the surface density of rough endoplasmic reticulum (Table I).

Glucocorticoids Effects on the Growth of AR42J Cells

Cells treated with 10 nM dexamethasone for 48 h grew more slowly than control cells. The inhibition of growth by dexamethasone was indicated by a reduction in cell number, and DNA and protein content of the cultures (Table II). The average amount of protein per cell was 341 ± 40 pg in control cultures and 441 ± 34 pg ($n = 3$) after treatment with 10 nM dexamethasone for 48 h. The amount of DNA per cell was 6.5 ± 1.0 pg in control cultures and 6.7 ± 0.7 pg ($n = 3$) after

treatment with dexamethasone. Thus, after treatment with dexamethasone the protein to DNA ratio increased from 58 ± 4 to 66 ± 5 (n = 5).

Glucocorticoid Effects on Amylase Concentration

Next, we investigated the effects of various steroids on the concentration of amylase in AR42J cells. Cells plated for 48 h in the presence of either dexamethasone, corticosterone, aldosterone, or progesterone showed dose-dependent increases in amylase content when compared with total protein (Fig. 6). Estrogen, in contrast, had no effect. Dexamethasone was the most potent steroid with one half-maximal stimulation of amylase content occurring at 1 nM and maximal stimulation occurring at 100 nM (Fig. 6). The potency of the other steroids paralleled their relative effectiveness as glucocorticoids. Dexamethasone at a concentration of 10 nM induced an eightfold increase in amylase concentration from 2.7 ± 0.3 in the control cultures to 22.6 ± 3.8 (n = 5) (U/mg).

This effect of dexamethasone on the cellular contents of amylase in AR42J cells required a prolonged incubation period. No effect was seen before 12 h and the increase in amylase content was still increasing slightly after 72 h (Fig. 7).

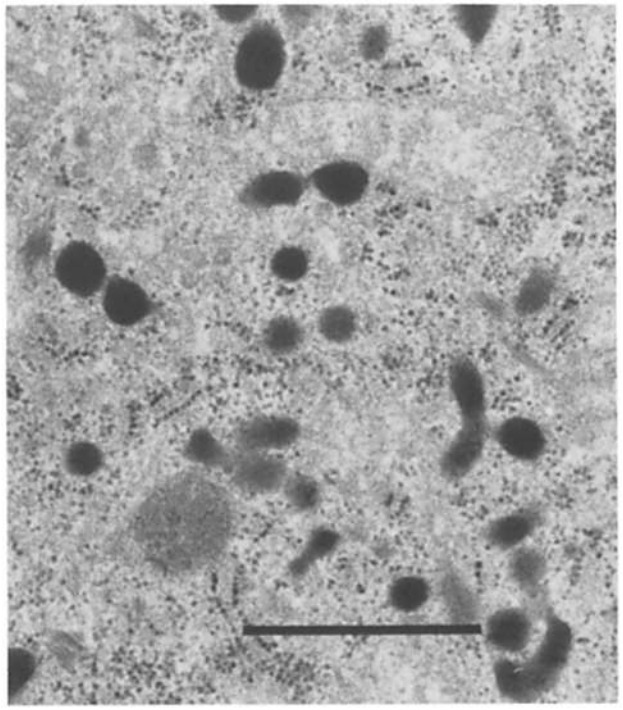

FIGURE 4 High magnification electron micrograph showing secretory granules in the cytoplasm of an AR42J cell treated with 10 nM dexamethasone for 48 h. The granules are small and somewhat elongated. Bar, 1 μm. × 31,250.

Effect of Glucocorticoids on Spontaneous and Cholecystokinin-stimulated Amylase Secretion by AR42J Cells

AR42J cells spontaneously secreted amylase into the incubation medium (Fig. 8). Furthermore, AR42J cells secreted amylase at a linear rate for at least 50 min either basally or after stimulation with cholecystokinin at 37°C (not shown). In control cultures, cholecystokinin induced a 2.6-fold increase amylase secretion in these cells, from 0.10 ± 0.01 to 0.26 ± 0.02 U/40 min (n = 5) (Fig. 8). Dexamethasone pretreatment led to enhanced secretion of amylase. In cells pretreated with 10 nM dexamethasone for 48 h, basal amylase secretion was increased 5.5-fold from 0.10 ± 0.01 to 0.55 ± 0.06 U/40 min (n = 5) (Fig. 8). Furthermore, in cells pretreated with dexamethasone, cholecystokinin-induced amylase secretion was increased 5.6-fold, from 0.26 ± 0.02 to 1.44 ± 0.14 mU/40 min (n = 5) (Fig. 8). These data indicate an

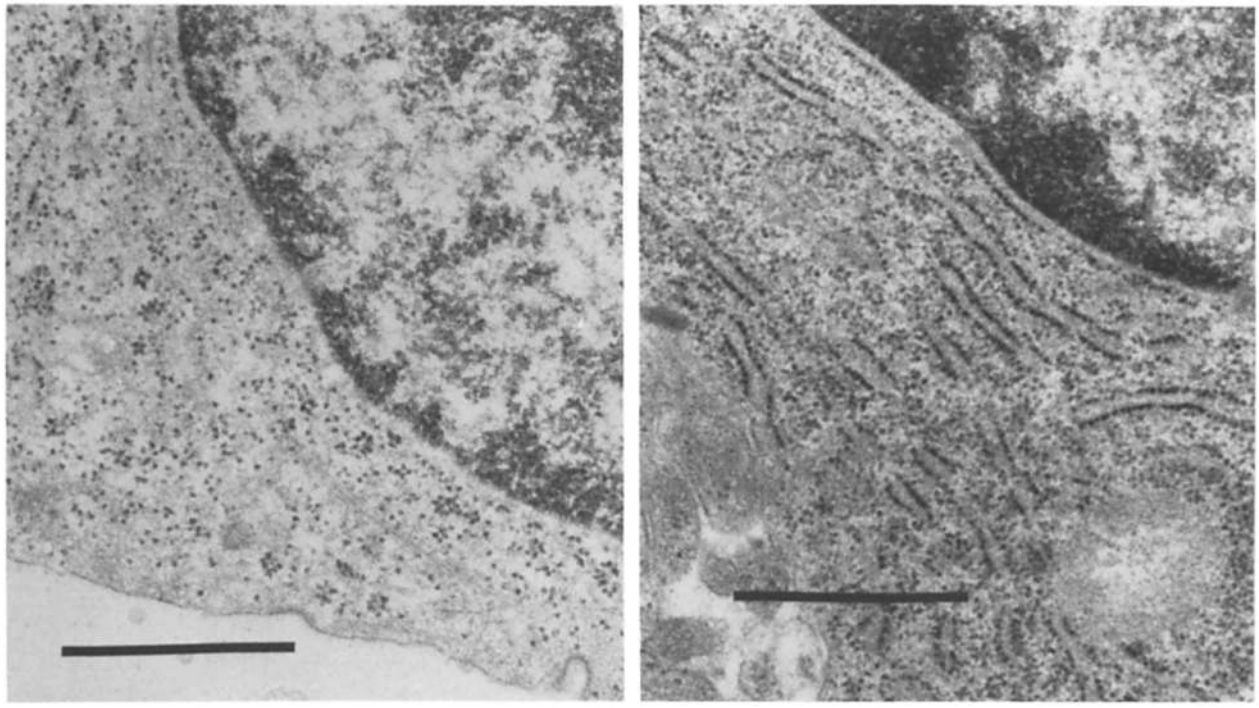

FIGURE 5 High magnification electron micrograph showing the nature of the protein synthetic apparatus in the cytoplasm of AR42J cells. (*Left*) Control cell. (*Right*) After treatment for 48 h with 10 nM dexamethasone. Bar, 1 μm. × 31,250.

increased secretory ability of the AR42J cells after treatment with dexamethasone.

Glucocorticoid Effects on Amylase Synthesis

Treatment for 48 h with 10 nM dexamethasone led to a specific increase in the synthesis of amylase. AR42J cells were pretreated for 48 h in the presence or absence of 10 nM dexamethasone and then pulsed for 3 h with [^{35}S]methionine. The labeled proteins were analysed by SDS PAGE either with or without immunoprecipitation with antisera to various digestive enzymes (Fig. 9). Dexamethasone treatment increased the labeling of the band corresponding to amylase in lysates extracted from equal numbers of cells. Densitometry of autoradiographs indicated that amylase synthesis accounted for 19% of the total incorporation of [^{35}S]methionine under control conditions and 47% after treatment with 10 nM dexamethasone for 48 h. Furthermore, dexamethasone increased the amount of labeled amylase immunoprecipitated from equal amounts of biosynthetically labeled proteins, indicating a specific increase in amylase synthesis (Fig. 9). In contrast to amylase, no immunoprecipitable trypsin was detected either in control cells or in those treated with dexamethasone. In other immunoprecipitation experiments, ribonuclease was also undetected (not shown).

Glucocorticoid Effects on Amylase mRNA

Cytoplasmic polyadenylated mRNA was isolated from AR42J cells grown in the presence and absence of 10 nM dexamethasone. This mRNA was translated in vitro using a wheat germ translation system and the proteins analysed by SDS PAGE either before or after immunoprecipitation with amylase antiserum. Analysis of the translated proteins indicated that amylase mRNA was one of the most abundant proteins translated (Fig. 10). Dexamethasone treatment resulted in an increase in the amount of amylase translated, indicating that the hormone increased the level of amylase mRNA (Fig. 10).

TABLE II

Effect of Dexamethasone on the Growth of AR42J Cells

	% Initial*	
	Control	Dexamethasone
Cell number	182 ± 14	136 ± 10 (n = 3) ($p < 0.05$)
DNA	217 ± 14	168 ± 12 (n = 4) ($p < 0.0025$)
Protein	209 ± 20	183 ± 19 (n = 5) ($p < 0.0025$)

* AR42J cells were plated at 8 × 10^5 cells per 35-mm petri dish for 24 h, and the initial number of cells, DNA, and protein contents were each assayed. Cultures were then grown for an additional 48 h in the presence or absence of 10 nM dexamethasone, the analyses of contents were repeated, and the results were expressed as the percent of initial content.

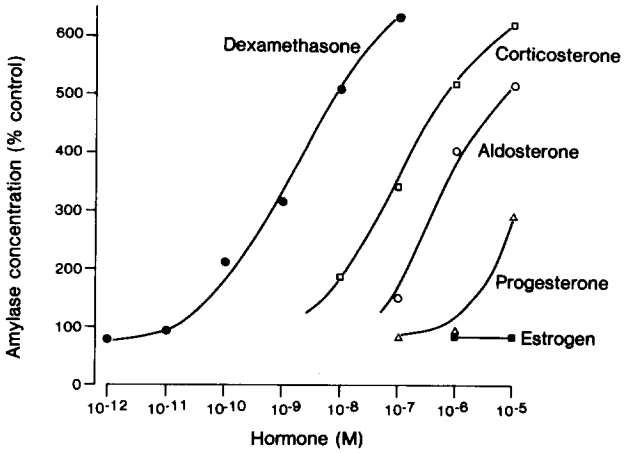

FIGURE 6 The effect of various steroid hormones on the concentration of amylase in AR42J cells. Cells were cultured in the presence of various concentrations of the different hormones for 48 h and then assayed for their contents of amylase and protein. Values are expressed as percent control and are the means of two experiments (SD [omitted for clarity] were 15–30% of the means).

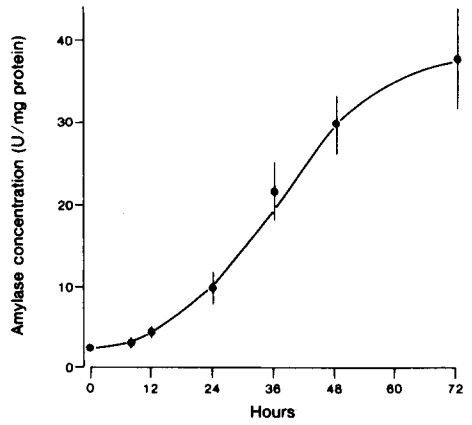

FIGURE 7 Time-course of the dexamethasone-induced increase in amylase concentration in AR42J cells. Dexamethasone (10-nM) was incubated with AR42J cells for up to 72 h. The cells were then analysed for contents of amylase and protein. Values are expressed as units of amylase per mg total protein and are means ± SD of triplicate determinations from a representative of three experiments.

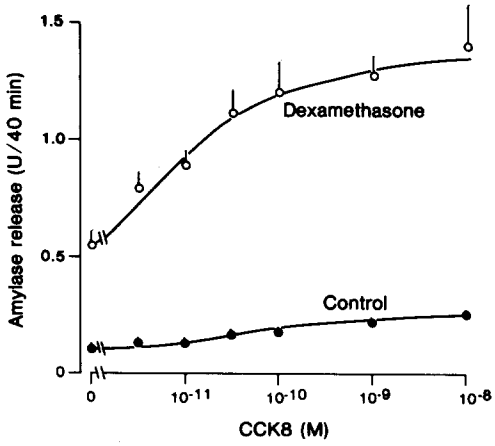

FIGURE 8 Cholecystokinin-stimulated amylase release from AR42J cells. Cells were grown for 48 h in the presence or the absence of 10 nM dexamethasone, and then stimulated for 40 min with various concentrations of cholecystokinin octapeptide (CCK8). Results are expressed as the units of amylase released per dish and are the means ± SEM of five experiments.

DISCUSSION

Several types of in vivo and in vitro studies have suggested that glucocorticoids have important effects on the exocrine pancreas. However, a direct effect of glucocorticoids on acinar cells has not been established. Islet cell hormones are important regulators of the acinar pancreas (19, 20, 30), and glucocorticoids are known to inhibit both the secretion of insulin and the growth of islet cells (23, 25, 29). Thus, in previous studies using whole animals and organ cultures of embryonic

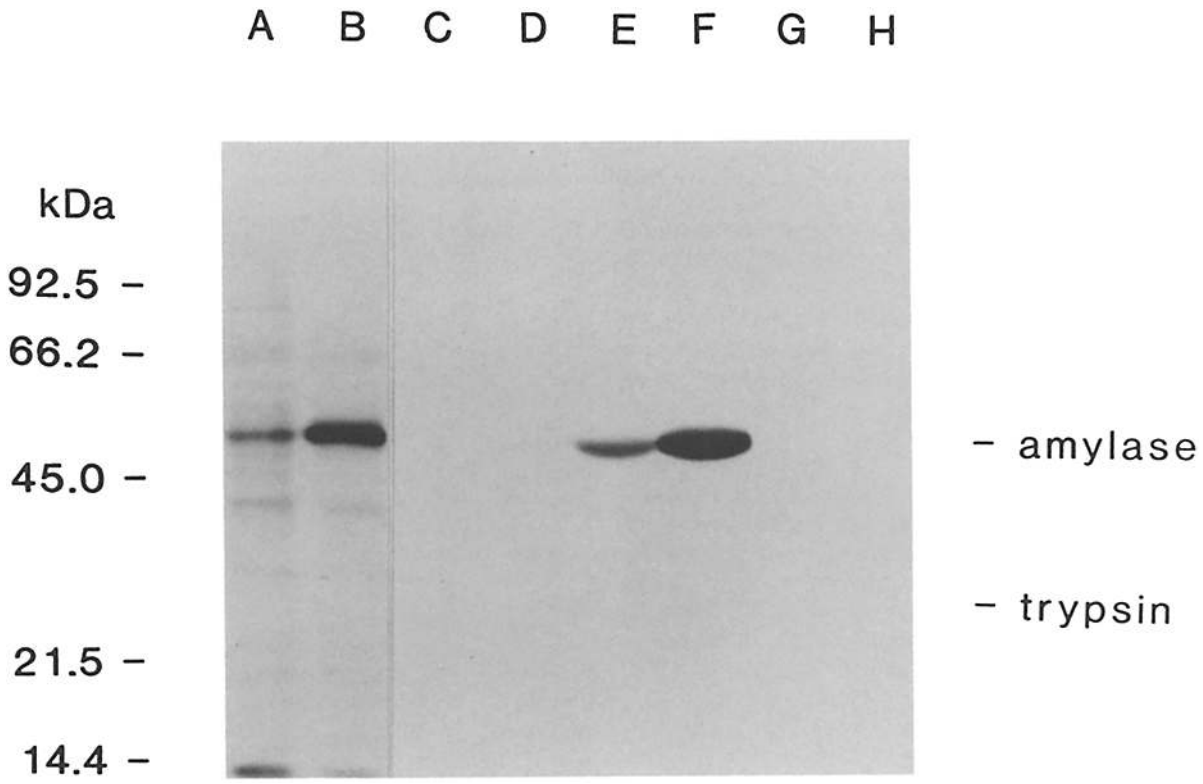

FIGURE 9 Analysis of the [^{35}S]proteins synthesised by AR42J cells. Cells were grown in the presence or absence on 10 nM dexamethasone for 48 h; they were then pulse-labeled for 3 h with [^{35}S]methionine. The cells were then lysed and the proteins were separated by PAGE on a 10% reducing gel either before or after immunoprecipitation. Lane *A*, Control cells, total lysate; lane *B*, dexamethasone-treated cells, total lysate; lane *C*, control cells, lysate precipitated with normal rabbit serum; *D*, dexamethasone-treated cells, lysate precipitated with normal rabbit serum; lane *E*, control cells, lysate immunoprecipitated with antiamylase antiserum; lane *F*, dexamethasone-treated cells, lysate immunoprecipitated with antiamylase antiserum; lane *G*, control cells, lysate immunoprecipitated with antitrypsin antiserum; lane *H*, dexamethasone-treated cells, lysate immunoprecipitated with antitrypsin antiserum. *kDa*, Kilodaltons.

pancreas, the effects of glucocorticoids noted on acinar cell function could have been due to their direct effects on endocrine cells. To examine this problem directly, we have used cultured AR42J cells which are derived from the acinar pancreas (16). The present study indicates that glucocorticoids do have direct effects on AR42J cells. Thus, this study supports the concept that glucocorticoids act directly on pancreatic acinar cells.

We found that glucocorticoids led to a more differentiated morphology of AR42J cells. Before treatment, the cells appeared relatively undifferentiated. Secretory granules were small and not abundant, and there were numerous free polysomes, but few strands of rough endoplasmic reticulum. After treatment with dexamethasone, there was a dramatic increase in the number and size of secretory granules, a morphological feature that reflects enhanced secretory function, and in the amount of rough endoplasmic reticulum, a morphological feature that reflects increased synthesis of secretory proteins. Similar effects of glucocorticoids on these morphological features have previously been noted both in organ cultures of embryonic rat pancreas (29) and in vivo chick pancreas (3).

Glucocorticoids also increased the cellular content of amylase. The following observations suggest that this increase was due to an effect on amylase synthesis. (*a*) The effect of dexamethasone occurred with a lag time of at least 12 h and reached a maximum after several days; such delayed effects are typical of steroid effects on the synthesis of proteins. (*b*) Studies of the immunoprecipitation of metabolically labeled proteins showed that after dexamethasone treatment there was an increase in the specific synthesis of amylase. Similar observations have been made in both chick pancreas in vivo (3) and organ culture of embryonic rat pancreas. In these earlier studies, the major effect of glucocorticoids was to increase amylase synthesis; however, glucocorticoids also increased to a lesser extent the synthesis of procarboxypeptidase A and chymotrypsinogen in the chick pancreas (3) and procarboxypeptidase A and B in organ cultures of embryonic rat pancreas (29). In the present study, dexamethasone induced a large increase in the synthesis of amylase in AR42J cells. Amylase was the major protein synthesized in these cells, and after dexamethasone treatment, amylase synthesis accounted for nearly 50% of the total protein synthesis. AR42J cells were also analysed for their synthesis of trypsin and ribonuclease, but these enzymes were not detected.

After dexamethasone treatment, in vitro translation studies of cytoplasmic mRNA indicated an increase in mature amylase mRNA. Although the measurement of an enhanced content of mature amylase mRNA does not precisely define the mechanism by which glucocorticoids act, the most likely explanation for the increased amylase synthesis is that these hormones influence gene transcription. In most circumstances where the early events in steroid action have been examined, the hormone increases specific mRNA content. Moreover, the transcription of several genes are known to be increased after glucocorticoid treatment (5, 10, 31). Alternatively, glucocorticoids could decrease the rate of mRNA deg-

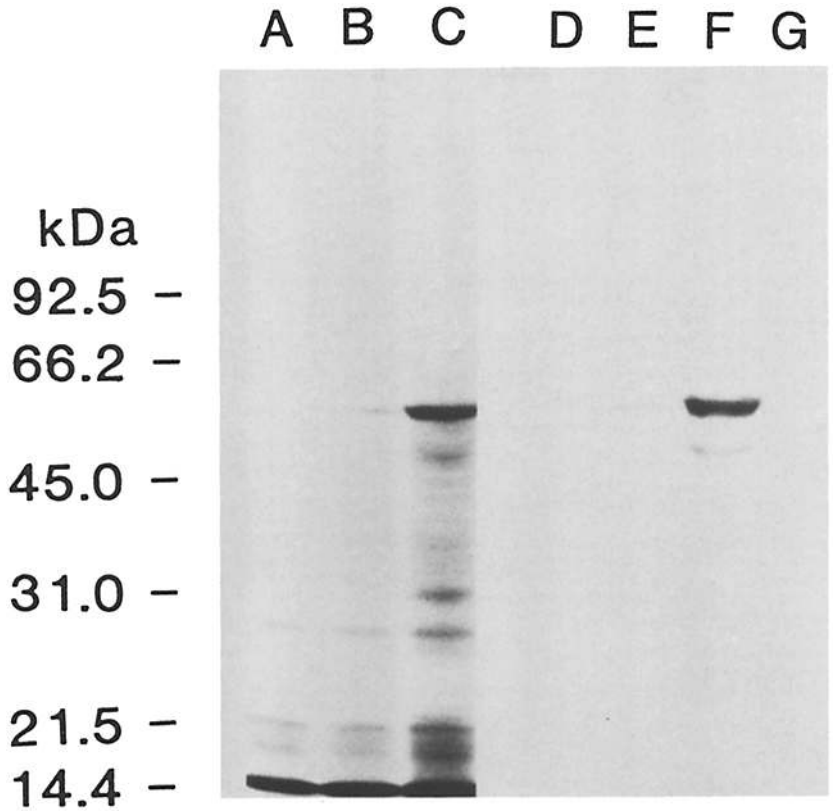

FIGURE 10 Analysis of proteins translated in vitro from mRNA isolated from AR42J cells. Cell were cultured in the presence or absence of 10 nM dexamethasone for 3 d and the cytoplasmic mRNA was then isolated and translated in vitro. Translation products were analysed by PAGE on a 10% gel either before or after immunoprecipitation of amylase. Lane *A*, No exogenous mRNA total translation product; lane *B*, control cells, total translation product; lane *C*, dexamethasone-treated cells, total translation product; lane *D*, no exogenous mRNA, translation product immunoprecipitated with antiamylase antiserum; lane *E*, control cells translation product, immunoprecipitated with antiamylase antiserum; lane *F*, dexamethasone-treated cells, translation product immunoprecipitated with antiamylase antiserum; lane *G*, dexamethasone-treated cells, translation product precipitated with normal rabbit serum. *kDa*, Kilodaltons.

radation (26). The present effects of glucocorticoids on AR42J cells are in agreement with previous studies showing the increased transcription of amylase mRNA by dexamethasone in embryonic rat pancreas in organ culture (11).

Pretreatment of AR42J cells with glucocorticoids increased the secretion of amylase. Basal release of amylase was increased fivefold after dexamethasone treatment. This finding indicates that the accumulation of amylase seen after dexamethasone treatment is not due to an inhibition of amylase release. Furthermore, dexamethasone also increased the secretion of amylase in response to cholecystokinin. Moreover, recent studies indicate that dexamethasone treatment of AR42J cells elicits an increase in the numbers of CCK receptors, and that these cells also display an increased sensitivity to CCK on the stimulation of secretion (Logsdon, C. D., I. D. Goldfine, and J. A. Williams, unpublished observations). In contrast, glucocorticoids have been reported to inhibit pancreatic amylase release in both whole animals (8) and in pancreatic acini in short-term suspension cultures (22). These differences may reflect alterations in the secretory mechanisms of the AR42J cell.

In the present study, glucocorticoids inhibited cell replication. Inhibition by glucocorticoids of pancreatic growth has been reported previously from studies with adult rats (27, 28) and in organ cultures of embryonic rat pancreas (29). In contrast, glucocorticoids enhanced pancreatic growth in fetal and suckling rats in vivo (27, 28). The mechanisms for these differences in the growth effects of glucocorticoids may reflect either the age of the animals or the type of cells studied in vitro.

Our findings demonstrate that glucocorticoids have pleiotropic effects on pancreatic acinar cells. These effects lead in turn to a more differentiated phenotype. The most likely explanation for these phenomena is that glucocorticoids regulate the expression of specific genes, the products of which are important for the differentiated function of the acinar pancreas.

We thank M. Mesirow and L. Ruslim for technical assistance, Dr. P. Walter for his help in setting up the in vitro translation assays, K. Y. Wong and B. Maddox for performing the in vitro translation, E. Roach for the electron microscopy, and A. Boulet for suggestions during the course of this study.

This work was supported by National Institutes of Health grant AM 32994, the Hedco Foundation, the Elise Stern Haas Research Fund, and other research funds of Mount Zion Hospital and Medical Center.

Received for publication 16 August 1984, and in revised form 14 December 1984.

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