SERUM ALBUMIN PRODUCTION BY HEPATOMA CELLS IN CULTURE: DIRECT EVIDENCE FOR STIMULATION BY HYDROCORTISONE

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SUMMARY

Addition of hydrocortisone to the medium of a clonal strain of rat hepatoma cells (MH_1C_1) stimulated their production of serum albumin. The stimulated rate of production reached a maximum by 50 hours, at which time it was about four times the rate in control cultures. Stimulation was observed at a hydrocortisone concentration as low as 10^{-7}M. Removal of hydrocortisone from the medium caused a return of the rate of albumin production to that in control cultures.

INTRODUCTION

Hydrocortisone has been found to increase the levels of specific enzymes in a number of cell and organ culture systems. For example, it causes increases of tyrosine aminotransferase in rat hepatoma cells (1), alkaline phosphatase in HeLa cells (2) and glutamine synthetase in embryonic chick retinas in organ culture (3,4). In addition, it has recently been shown that hydrocortisone stimulates the production of growth hormone both by a clonal strain of rat pituitary cells (5), and by primary cell cultures obtained from normal monkey pituitary glands (6).

The establishment of a clonal strain of rat hepatoma cells, designated MH_1C_1, and the production by these cells of serum albumin has been described (7). Most of the albumin synthesized by MH_1C_1 cells is secreted into the culture medium; very little albumin is stored inside the cells. These cells also

synthesize tyrosine aminotransferase, and they respond to hydrocortisone by increasing substantially the levels of this enzyme (7,8). We wish to describe here the finding that hydrocortisone causes a marked stimulation of the rate of albumin production by MH_1C_1 cells, and that the continued presence of the steroid in the medium is required for the effect to persist.

MATERIALS AND METHODS

Hydrocortisone hemisuccinate was obtained from the Upjohn Company, Kalamazoo, Michigan. The MH_1C_1 cells were grown at 37°C in a humidified atmosphere of 5% CO_2 and 95% air, in plastic tissue culture dishes (Falcon Plastics, 50 x 15 mm). The growth medium was Ham's F 10 (9), supplemented with 15% horse serum and 2.5% fetal calf serum (complete F 10) (7). Under these conditions the population-doubling time of MH_1C_1 cells is 8-12 days (8). Serum albumin secreted into the culture medium was measured by a specific microcomplement-fixation immunoassay method (7) using antiserum to purified rat albumin. The 95% confidence limits of the result of a single determination of albumin are ± 25%; the differences observed between stimulated and control cultures in these experiments, usually equal to or greater than 2-fold, are therefore highly significant ($p < 0.001$). Medium was changed every 24 hours during an experiment. Control experiments have shown that degradation of albumin in the culture medium is less than 4% in this time.

RESULTS

When medium containing hydrocortisone (3×10^{-5}M) was added to MH_1C_1 cells, the rate of albumin production* rose relative to that in control cultures (Fig. 1). By 50 hours, production in the hydrocortisone-treated cultures had reached a

*The quantity "rate of albumin production" as referred to in this report is equal to the rate of appearance of albumin in the medium of the MH_1C_1 cells. Since in these experiments the medium which was assayed for albumin had been in contact with the cells for 24 hours, and since the amounts of albumin detected were in each case divided by the total cell protein, each reported value for albumin production represents the average specific rate of albumin production by MH_1C_1 cells over a 24 hour period.

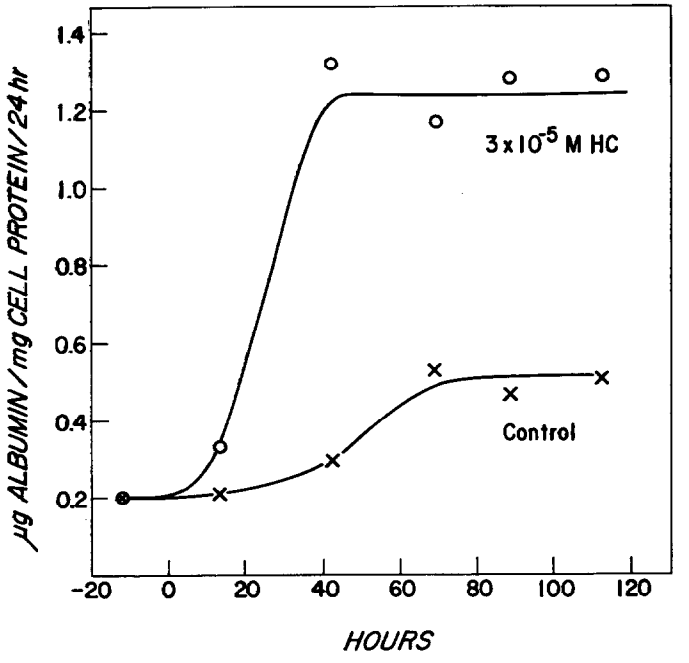

Figure 1. Stimulation by hydrocortisone of albumin production. Replicate dishes containing cells in the late exponential phase of cell growth were used. At zero time fresh medium (complete F 10) either containing hydrocortisone (3×10^{-5}M) or lacking hydrocortisone was added to each dish. Every 24 hr the medium was collected from each dish for albumin assay. Fresh medium either with hydrocortisone (HC) or without hydrocortisone (Control) was then added. At the same time, cultures which had been treated in an identical fashion were washed twice with isotonic NaCl and frozen for determination of cell protein (10). (X——X = Control; O——O = HC.)

maximum level about four times the rate in control cultures. The increased production rate in the stimulated cultures was maintained during the remainder of the experiment. Results similar to those shown in Fig. 1 have been observed in three independent experiments.

The maximum stimulation observed with various concentrations of hydrocortisone is shown in Table 1. A significant stimulation of albumin production was seen at 10^{-7}M hydrocortisone. Maximum stimulation was seen at a steroid concentration of 10^{-6}M. At concentrations higher than 10^{-6}M, stimulated rates of albumin production decreased gradually with increasing hydrocortisone concentration.

Table 1

EFFECT OF VARIOUS CONCENTRATIONS OF HYDROCORTISONE ON ALBUMIN PRODUCTION

Hydrocortisone concentration	Albumin production (μg albumin/mg cell protein/24 hr)
Control	0.42 0.54
10^{-9}M	0.54
10^{-7}M	0.84
10^{-6}M	1.92
10^{-5}M	1.60
10^{-4}M	1.28

Conditions were the same as described in Fig. 1, except that medium (complete F 10) containing hydrocortisone at final concentrations from 10^{-9}M to 10^{-4}M was added to experimental dishes, and medium lacking hydrocortisone was added to duplicate control dishes. Rates of albumin production in the interval 85-109 hours, when maximum stimulated rates were observed, are shown.

Removal of hydrocortisone from stimulated cultures at 46 or 97 hours caused an immediate decrease in the rate of albumin production compared to that in cultures receiving hydrocortisone throughout (Fig. 2). Thereafter, the rates of production by these cells continued to fall, eventually approaching the rates observed in control cultures.

Medium lacking serum (thus totally synthetic) is chemically better defined than medium supplemented with serum. It also lacks the corticosteroid-binding proteins known to be present in serum (11). Hence the effect of hydrocortisone on albumin production in serum-free medium was investigated. The results of such an experiment are shown in Fig. 3. There was a gradual decrease in albumin production by control cells maintained in serum-free medium, indicating that albumin production by MH_1C_1 cells is impaired in the absence of serum. Never-

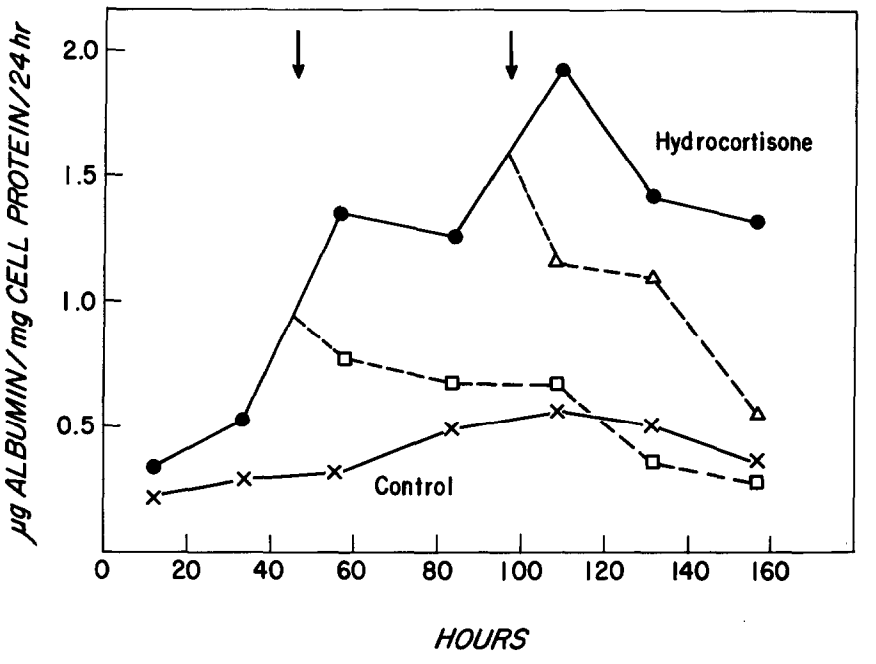

Figure 2. Effect on albumin production of the removal of hydrocortisone. Conditions were similar to those described in Fig. 1, except that the concentration of hydrocortisone was 1.0×10^{-5}M. At the times indicated by the arrows, medium was collected from a dish which had received hydrocortisone (+HC) until that time. The cells were then washed three times with isotonic NaCl, and medium lacking hydrocortisone (-HC) was added. These dishes were treated thereafter in the same fashion as the control cultures. (X——X = -HC throughout ●——● = +HC throughout; □-----□ = -HC at 46 hr; Δ-----Δ = -HC at 97 hr.)

theless, a large stimulation of albumin production by hydrocortisone was observed under these conditions. Due in part to the decrease in albumin production in control cultures, by 80 hours the rate of production in stimulated cultures had reached a level about 10 times the rate in the controls.

Results similar to those shown in Fig. 3 have been observed in three independent experiments carried out in serum-free medium. In experiments continued for a longer time interval, we have observed that the ratio of the production rate in stimulated cultures to that in control cultures did not remain at the high level attained at 80 hours in the experiment shown in Fig. 3. Instead, within 24-48 hours after this ratio had reached a maximum, it was observed to decline to a value of 2-4.

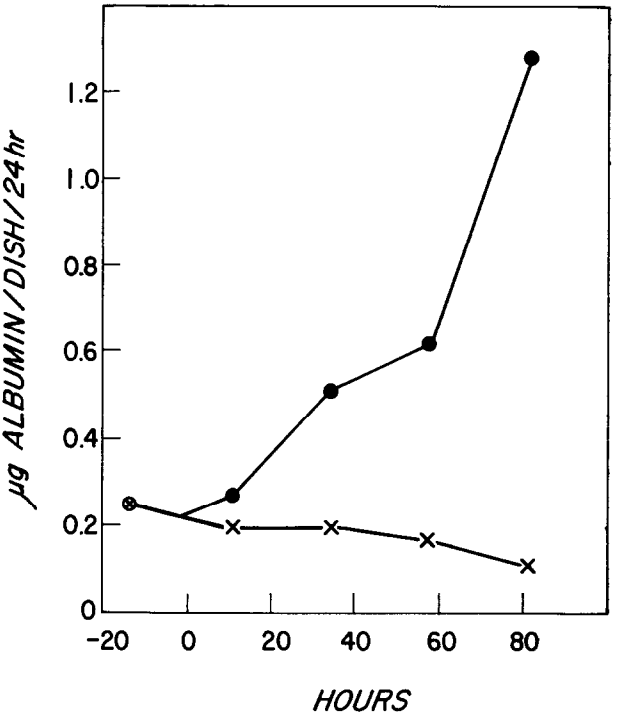

Figure 3. Effect of hydrocortisone on albumin production in F 10 medium lacking serum (F 10). Cells were grown in complete F 10 medium until the start of the experiment. At -24 hr, F 10 was added to each dish. At zero time this medium was collected, and F 10 either containing hydrocortisone (3×10^{-6}M) or lacking hydrocortisone was added. Every 24 hr thereafter medium was collected from each dish for albumin assay. Fresh F 10 (either -HC or +HC) was then added. Each point gives the mean result of assays performed on medium collected from duplicate dishes. (X——X = -HC throughout; ●——● = +HC throughout.)

In experiments described in detail elsewhere (8,12), MH_1C_1 cells have been shown to synthesize and secrete into the culture medium the ninth component of complement (C9) (13). To investigate the specificity of the observed stimulation by hydrocortisone of albumin production, assays for C9 were performed on each of the medium samples obtained in the experiment shown in Fig. 3. In the same samples in which there were clearly large effects of hydrocortisone on albumin production, there was no increase in C9 above the values measured in control cultures. Hence there appears to be a degree of specificity in the effect of hydrocortisone on MH_1C_1 cells, in that the steroid causes an increase

in albumin production and in tyrosine aminotransferase levels, but it does not increase the production of the ninth component of complement.

DISCUSSION

Previous studies of the effects of adrenocortical hormones on serum albumin production have been performed using human subjects and intact animals (14-16). The method used in these investigations involved the injection of ^{131}I-albumin, followed by measurements of the turnover of this material. Consequently, the effects of steroid hormones on albumin production could only be inferred from differences observed between ^{131}I-albumin turnover and changes in the albumin pool size. Results obtained using this technique have been inconclusive. One group reported increases of 13% and 53% in albumin production on injection of steroid hormones into humans and rabbits, respectively (14), while another group reported that hydrocortisone caused no increase in albumin production in human subjects (15).

We believe that the MH_1C_1 cells represent a unique model system in which to study the effects of steroid hormones, as well as other external stimuli, on albumin production. Thus, the ability to quantitate directly the amount of albumin which the cells produce has led to an unequivocal demonstration that hydrocortisone increases the rate at which serum albumin is produced by these cells.

Since the MH_1C_1 cells are a clonal strain, intracellular events accompanying the observed effects of hydrocortisone may be studied using a homogeneous population of the cells responsible for albumin synthesis. It is anticipated that further study of the stimulation by hydrocortisone of albumin production by these cells will yield useful information regarding the mechanisms whereby animal cells control the production of non-enzymic, secretory proteins.

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