Inhibition of Synthesis of α-Fetoprotein by Glucocorticoids in Cultured Hepatoma Cells

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ABSTRACT α-Fetoprotein (AFP) synthesis was studied in the presence and absence of glucocorticoids in rat hepatoma Mc-A-RH-7777 cells. Radioimmunoassay of media from cell cultures grown in the presence of glucocorticoid (dexamethasone or cortisol) showed a reduction in AFP, an increase in albumin, and no significant change in transferrin accumulation, as compared to controls. Labeling experiments with L-[35S]methionine indicated that in both cells and media of dexamethasone-treated cultures there was a 50–80% reduction in polypeptide precipitated by anti-AFP serum, as compared with controls; no change was seen in polypeptide precipitated by anti-transferrin serum. Pulse and pulse-chase experiments demonstrated that dexamethasone inhibited the synthesis of AFP but not its secretion. The half-time for secretion of AFP in the presence and absence of dexamethasone was 43 min.

α-Fetoprotein (AFP) is a major plasma glycoprotein synthesized by mammalian embryonic liver and yolk sac (1, 8–10, 18). The concentrations of AFP are higher in fetal or neonatal sera and in amniotic fluid but fall as the liver develops and matures. Nonpregnant adult mammals have extremely low circulating AFP levels (12, 16). Elevated serum concentrations of AFP have been associated with developmental, regenerative, and carcinogenic processes (1, 19). As a noncodevelopmental protein, AFP can serve as a marker for the detection of tumors, prenatal neural tube defects, or other fetal abnormalities (1, 4, 19, 21).

During pregnancy, the levels of glucocorticoids increase with gestation and increase rapidly during spontaneous labor (7, 14). Glucocorticoids induce the development and maturation of enzyme systems, promote differentiation, and play a role in the initiation of labor at term (13). Administration of glucocorticoids to newborn mice has been shown to cause an early fall in serum AFP levels (6). The inverse relationship between levels of glucocorticoids and AFP and the role of glucocorticoids in hormone-mediated differentiation made these hormones good candidates for direct regulators of AFP synthesis. The effects of glucocorticoids on AFP synthesis in rat hepatoma cells may serve as a model of gene regulation during development and neoplasia.

MATERIALS AND METHODS
Cells and Culture Conditions
Mc-A-RH-7777 rat hepatoma cells (2) were obtained from Dr. V. R. Potter and were grown in a-modified minimal essential medium supplemented with 4% fetal bovine serum (αMEM-4). Cells were maintained at 37°C in 95% air-5% CO2. We used in this study hepatoma cells in logarithmic phase of growth. Glucocorticoids were added 2 d after subculturing (day 0) and the medium was changed every day.

Radioimmunoassays
AFP, albumin, and transferrin were determined in culture media by double-antibody radioimmunoassays as previously described (5). Purified preparations of rat AFP, rat albumin, and rat transferrin were used as standards and also radioiodinated for use as tracers in the respective assays. Rat AFP was kindly provided by Dr. J. F. Chiu and antiserum against AFP were raised (in rabbits) to this highly purified rat AFP. Rat albumin, rat transferrin, rabbit antiserum against rat albumin, and rabbit antiserum against rat transferrin were obtained from N. L. Cappel Laboratories, Inc. (Cochran, PA). Sheep antiserum against rabbit γ-globulin was used as the precipitating antibody. The sensitivities of the assays were 0.2–10 ng for AFP, 0.5–25 ng for albumin, and 0.1–5 ng for transferrin. Less than 0.01% cross-reactivity exists between AFP, albumin, and transferrin in the respective radioimmunoassays. Complete medium not exposed to cells had no detectable AFP, albumin, or transferrin.

Labeling and Extraction of Cells
Cells were grown in 25-cm2 flasks in αMEM-4. Before labeling, the cultures were rinsed with 5 ml of αMEM-4 lacking methionine and incubated with 5 ml of this medium for 1 h. Then they were incubated for various times with L-[35S]methionine at 100 μCi/ml (1.370 Ci/mmol, Amersham Corp., Arlington Heights, IL) in methionine-free αMEM-4. Labeling was terminated by the addition of l-methionine (0.1 ml, 5 mg/ml) in αMEM-4. For pulse-chase experiments, the cells were washed with αMEM-4 containing l-methionine and incubated for various times in αMEM-4. After incubation, the medium was removed, and the cells were washed with phosphate-buffered saline (PBS) and lysed by the addition of 1 ml of PBS containing 1% Triton X-100. 0.5% sodium...
Effects of Glucocorticoids on the Production of AFP, Albumin, and Transferrin

Both cortisol and dexamethasone inhibited the accumulation of AFP in the culture media from rat hepatoma Mc-A-RH-7777 cells grown in either serum-containing or serum-free medium; the results with cells grown in serum-containing medium were shown in Fig. 1. The peak level of AFP in control as well as in cortisol- and dexamethasone-treated cultures was reached in 3 d. The production of AFP was coupled to cellular proliferation because these hepatoma cells were growing logarithmically from day 1 to day 3 (data not shown). When cell proliferation subsided after day 3, AFP production declined. This was expected, because it has been demonstrated that AFP production in primary fetal hepatocytes was proportional to the number of replicating cells (20). Both glucocorticoids increased the level of albumin (Fig. 1). Dexamethasone had a longer-lasting effect on albumin levels: after the peak, albumin levels dropped precipitously in the cortisol-treated cultures but not in the dexamethasone-treated cultures. Neither glucocorticoid affected the levels of transferrin significantly (Fig. 1). Although we used cortisol and dexamethasone at 10^{-6} M and 10^{-7} M, respectively, in this study, the two glucocorticoids, at 10^{-6} M and 10^{-7} M respectively, were almost as effective. Furthermore, progesterone did not block the ability of dexamethasone to inhibit AFP synthesis (data not shown).

Effects of Glucocorticoids on the Incorporation of L-[35S]Methionine into AFP

A labeling experiment was performed to determine whether the observed inhibition of AFP accumulation in culture media by glucocorticoids resulted from inhibiting AFP biosynthesis. Each day, cells grown in the absence and presence of dexamethasone or cortisol were labeled for 30 min and 180 min with L-[35S]methionine, and then cell lysates and medium samples were examined for anti-AFP-precipitable polypeptides. The 30-min labeling time was chosen because pulsing
hepatoma cells with L-[35S]methionine for 30 min measured mainly intracellular AFP biosynthesis (Fig. 2). Secretion of AFP occurred only after a lag of 30 min. Pulse labeling of these cells with L-[35S]methionine for 180 min, however, measured both intracellular synthesis and extracellular accumulation of AFP (Fig. 2). Parallel experiments were carried out with cortisol and dexamethasone; the results with cortisol (data not shown) were similar to those with dexamethasone.

Pulse labeling of hepatoma cells with L-[35S]methionine in the presence of dexamethasone showed inhibition of intracellular AFP synthesis after 30-min and 180-min labeling (Fig. 2). There was no appreciable secretion of anti-AFP-precipitable polypeptides into medium in either control or dexamethasone-treated cultures after 30-min labeling (Fig. 2). However, after labeling for 180 min, significant amounts of radioactive anti-AFP-precipitable polypeptides had accumulated in the medium; dexamethasone inhibited also the extracellular accumulation of newly synthesized AFP. Gel electrophoresis showed inhibition in all anti-AFP-precipitable bands. Dexamethasone did not affect the processing of AFP in hepatoma cells. The molecular weights of the fully processed AFP chains in medium of both control and dexamethasone-treated cultures were 69,000 and 73,000 daltons (Fig. 2).

The glucocorticoid effect was specific for AFP. The incorporation of L-[35S]methionine into total trichloroacetic acid-precipitable radioactivity was not affected by dexamethasone or cortisol (data not shown). Furthermore, the synthesis and accumulation of L-[35S]methionine-labeled anti-transferrin-precipitable polypeptides were not altered by dexamethasone (data not shown).

**Kinetics of AFP Biosynthesis in the Absence and Presence of Glucocorticoid**

Pulse and pulse-chase experiments were conducted to ascertain whether dexamethasone inhibited AFP synthesis or only its secretion. Dexamethasone decreased the rate of intracellular AFP biosynthesis and the rate of AFP accumulation in medium (Fig. 3). Dexamethasone did not affect AFP secretion by hepatoma cells. The kinetics of disappearance of intracellular anti-AFP-precipitable polypeptides and the kinetics of their appearance in medium were similar in dexamethasone-treated cultures and control cultures (Fig. 4). The half-time of secretion of AFP in cultures grown in the presence or absence of dexamethasone was 43 min (Fig. 5).

**DISCUSSION**

Glucocorticoids are seen to inhibit AFP biosynthesis in the rat hepatoma Mc-A-RH-7777 cells in vitro. Radioimmunoassays of media of cultures grown in the presence and absence of glucocorticoids indicate that the effect of glucocorticoids is a specific inhibition, as no such effect is seen in the levels of transferrin, and albumin levels are stimulated. This inverse relationship between albumin synthesis and AFP synthesis has
been documented in vitro and in vivo as concomitant with maturation and cellular differentiation (1,17).

Labeling experiments demonstrate the inhibitory effect of glucocorticoids on AFP levels on both intracellular biosynthesis and extracellular accumulation. The inhibitory effect of glucocorticoids is seen in all anti-AFP-precipitable bands. Pulse-chase experiments demonstrate that glucocorticoid does not affect the kinetics of AFP secretion. The half-time of secretion of AFP in both control and dexamethasone-treated cultures was 43 min.

The inhibition of AFP accumulation in media in response to glucocorticoids in this cell line has been reported previously (2). We have shown this effect to be initiated at the level of synthesis of AFP. Inhibition of AFP synthesis by glucocorticoids has been demonstrated in vivo in newborn mice and has been shown to take place at the transcriptional level (6). Our data support the view that the disappearance of mRNA coding for AFP seen in vivo is due to the direct regulatory effects of the glucocorticoid on the hepatocytes and is not a secondary result of the systemic effects of the hormone.

The effect of glucocorticoids on AFP in this system may serve as a model for inhibitory regulation. Further investigation of this system may elucidate the processes involved in hormone effects on maturation and cellular differentiation in both normal and neoplastic development.

REFERENCES