DEXAMETHASONE REGULATES THE PROGRAM OF SECRETORY GLYCOPROTEIN SYNTHESIS IN HEPATOMA TISSUE CULTURE CELLS

HEINZ BAUMANN, THOMAS D. GELEHRTER, and DARRELL DOYLE

From the Department of Molecular Biology, Roswell Park Memorial Institute, Buffalo, New York 14263, and the Department of Internal Medicine and Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan 48109

ABSTRACT

The secretory glycoproteins synthesized by hepatoma tissue culture (HTC) cells were resolved by two-dimensional polyacrylamide gel electrophoresis of media from cells that were grown in the presence of [^{3}H]fucose. These cells synthesize and secrete a complex set of fucose-containing glycoproteins. These secretory glycoproteins are distinct from those glycoproteins present in the plasma membrane of HTC cells. Incubation of HTC cells with dexamethasone has a pronounced effect on the quality and quantity (denoted here as the program) of secretory protein synthesis, as assayed by the short-term incorporation of labeled mannose, fucose, or methionine. The synthesis of two mannose- and fucose-containing glycoprotein series, one of 50,000 mol wt and a more heterogeneous series with mol wt of 35,000–50,000, is increased to a high level by the hormone; conversely, the synthesis of other secretory proteins, particularly one with mol wt of 70,000, is decreased or stopped completely. The synthesis of some major secretory proteins is not affected by the hormone. Dexamethasone has less of an effect on the composition of either total cell membrane glycoprotein or plasma membrane glycoprotein. But there is a decrease in the synthesis of a major membrane glycoprotein series with mol wt of 140,000. These effects of dexamethasone are relatively specific to HTC cells. Neither Reuber H-35 cells nor primary cultures of rat hepatocytes show the same response to the steroid. Two variant HTC cell lines, which were selected for their resistance to dexamethasone inhibition of extracellular plasminogen activator activity, respond only partially to the steroid-induced regulation of the secretory and membrane glycoproteins.

In recent years, much has been learned about the steps involved in the biosynthesis of secretory proteins in mammalian cells (18). These proteins are synthesized mainly on membrane-bound polysomes as precursor forms, with part of the additional amino acid sequences providing a recognition signal that directs free polysomes containing the nascent polypeptide to the endoplasmic reticulum (6, 8, 9). The secretory polypeptide is then vectorially discharged into the cisternae of the endoplasmic reticulum. At this time, the recognition sequences may (7) or may not be removed by a membrane-associated protease (20). If the secretory protein is to be glycosylated, the core sugars

are added to asparagine residues via dolichol-linked intermediates, while the polypeptide is still nascent on the membrane-bound polysomes (6–9, 14, 15, 21, 26, 29). The secretory protein is presumably then transferred through the smooth endoplasmic reticulum to the Golgi, or a Golgi-associated, apparatus (17) where glycosylation is completed. The completed polypeptide is then carried to the surface of the cell in the lumen of a vesicle which presumably pinches off from the Golgi apparatus or GERL, releasing the luminal contents as secretion (11).

In spite of this rather thorough characterization of the pathway of biogenesis of secretory proteins, little is known about the way by which the mammalian cell regulates the level of different proteins or glycoproteins in the secretion. In fact, in rat liver and in pancreas, the two most thoroughly studied mammalian secretory tissues, it has been difficult to modulate significantly the concentration of the different secretory proteins (10). In chick oviduct, steroid hormones are known to be involved in the regulation of egg protein biosynthesis (19, 22, 30). In the present paper, we show that the steroid dexamethasone regulates the pattern of glycoproteins synthesized by hepatoma tissue culture (HTC) cells, a cell line of rat origin. This steroid has a particularly significant effect on two secretory glycoproteins, inducing their rate of synthesis by at least 30-fold over basal levels.

MATERIALS AND METHODS

Cells

A cloned cell line of rat HTC cells (27) was grown in monolayer culture under an atmosphere of 95% air and 5% CO_2 in Eagle's minimal essential medium containing 50 mM tricine, 2 mM glutamine, and 10% fetal calf serum. This wild-type cell line was denoted SR. Two variant cell lines selected from the wild type for resistance to dexamethasone inhibition of plasminogen activator were denoted Variant A and Variant C (25). Plasminogen activator is a membrane-associated serum protease which is secreted into the medium by HTC cells. Reuber H-35 hepatoma cells, also of rat origin, were grown in monolayer in Dulbecco's modified Eagle's medium containing 2 mM glutamine, 5% fetal calf serum, 5% calf serum, and 25 mM HEPES buffer. Hormonal stimulation was carried out by culturing the cells in 1 μM dexamethasone (Sigma Chemical Co., St. Louis, Mo.) in normal growth medium for 24 h. Hepatocytes from adult rat liver were prepared according to the procedure of Seglen (23) and cultured in Leibowitz L-15 medium containing 10% fetal calf serum, 2 mM glutamine, and 1 μM dexamethasone.

Labeling and Gel Electrophoresis

Monolayers in 3.5-cm dishes were labeled by the addition of [35S]methionine (680 Ci/mmol, [5,6-3H]fucose (60 Ci/mmol), or [2-3H]mannose (18.2 Ci/mmol) to the culture medium (for details, see legends to tables and figures). As shown elsewhere (28), fucose is not metabolized to any other utilizable compound by HTC cells. Similarly, [2-3H]mannose was chosen as precursor because the tritium is lost when this sugar is metabolized. The cells were washed and then were extracted twice with 2 ml of 10% trichloroacetic acid. The acid-insoluble fraction was extracted with 2 ml of chloroform:methanol (2:1) followed by 2 ml of chloroform:methanol:water (1:1:0.3). Both organic solvent-soluble fractions were combined and considered as the lipid fraction. The acid- and organic solvent-insoluble material was used for protein determination. The purification of glycoproteins by concanavalin A-Sepharose chromatography was performed as outlined previously (4, 5).

For electrophoretic analyses of the membrane glycoproteins, the labeled cells were homogenized by ultrasonication in 50 mM Tris-Cl, pH 7.6, containing 0.1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged for 60 min at 150,000 g. The crude membrane pellet was washed once and then dissolved by boiling in 10 mM Tris-Cl, pH 6.8, containing 1% sodium dodecyl sulfate and 5% 2-mercaptoethanol. To recover secreted glycoproteins, confluent monolayers in 75-cm² flasks were washed three times with medium lacking serum. Then, the cells were cultured in 8 ml of serum-free medium containing radioactive precursor (for details, see legend to appropriate figures). After 6 h of incubation, the medium was removed and centrifuged for 5 min at 400 g and 60 min at 150,000 g. The supernatant fraction was dialyzed for 24 h against 50 mM NH₄HCO₂ containing 0.05 mM phenylmethylsulfonyl fluoride, and then lyophilized. The lyophilized residue was redissolved in 10 mM Tris-Cl, pH 6.8, containing 1% sodium dodecyl sulfate and 5% 2-mercaptoethanol. The detergent-solubilized membrane fractions or medium proteins were subjected to two-dimensional separation, as described by Ames and Nikaido (1), with minor modifications (5). The tritium pattern was visualized by fluorography (9).

RESULTS

When HTC cells were stimulated with 1 μM dexamethasone for 24 h and then cultured in the presence of either [35S]methionine or [3H]fucose, the extent of incorporation of isotope into glycoproteins released into the medium is about twice that of nontreated cells (Table I). The uptake of the precursors is not significantly influenced by the steroid because the radioactivity in the acid-soluble fraction and the extent of labeling of cellular glycoproteins is the same in both the hormone-stimulated cells and the control cells. Because the release of labeled glycoprotein into the medium is first detected 3 h after the addition of the isotope to the culture (3), the values shown for medium proteins in Table I taken at 6 h do not represent the true proportion of secreted proteins to total proteins synthesized. When [3H]mannose was used for the incorporation studies, dexamethasone did stimulate a threefold increase in uptake of label, as indicated by the threefold increase in incorporation into all cell proteins in the presence of steroid. Mannose also becomes extensively in-

TABLE I

Effect of Dexamethasone on Protein Synthesis and on Release of Proteins into the Medium

Fraction	[35S]Methionine incorporation		[3H]Fucose incorporation		[3H]Mannose incorporation	
	−Dex	+Dex	−Dex	+Dex	−Dex	+Dex
	*cpm/μg of cell protein**					
Cell protein	80,800	83,700	840	830	230	800
Con A-bound fraction‡	6,460	7,700				
Acid-soluble	20,600	14,200	400	350	370	690
Cell lipid	—	—	—	—	140	1,250
Medium protein	950	1,700	60	100	—	—
Con A-bound fraction‡	620	1,260				

* Confluent monolayers of the wild-type HTC cells, SR, were either incubated for 6 h in 1 ml of culture medium containing 115 μCi of [35S]methionine, or 50 μCi [3H]fucose, or were incubated for 1 h in 0.5 ml of medium containing 50 μCi of [3H]mannose. Values shown represent averages of two identically treated monolayers, and each measurement was performed in duplicate.

‡ Values represent the fraction bound to concanavalin-A Sepharose column and is corrected for 100% recovery.

corporated into lipid, presumably dolichol derivatives (Table I). Furthermore, [3H]mannose is not very suitable for quantitative incorporation studies because the precursors are rapidly depleted from the medium by the cells (3). Methionine and fucose, however, are incorporated linearly into the proteins of hepatoma and liver cells for at least 24 h.

Two subclones of the HTC-SR line, Variants A and C, do not show inhibition of intracellular plasminogen activator activity or its secretion by dexamethasone (25). These cells were labeled with [3H]fucose for 24 h, and the incorporation into total cellular and released glycoproteins was examined. As shown in Table II, both variants, A and C, in the absence of dexamethasone, incorporate somewhat more fucose into released glycoproteins relative to the cellular glycoproteins than do the wild-type SR cells. But, unlike SR cells, they do not show any significantly enhanced secretion of these glycoproteins in the presence of dexamethasone.

The glycoproteins of a total membrane fraction from HTC cells labeled for 24 h with [3H]fucose were analyzed by two-dimensional gel electrophoresis. Fucose was chosen as precursor because all glycoproteins associated with the plasma membrane contain this carbohydrate, thereby facilitating identification (5). As depicted in Fig. 1, HTC cells treated with dexamethasone synthesize almost the same set of membrane fucoglycoproteins as nontreated cells. However, cells treated with steroid show reduced amounts of a major fucose-labeled glycoprotein series with an apparent mol wt of 140,000 (gp 140) relative to cells not treated

TABLE II

Effect of Dexamethasone on Uptake and Incorporation of [3H]Fucose in Hormone-Resistant HTC Cell Lines

Fraction	Cell type	[3H]Fucose incorporation	
		−Dex	+Dex
		cpm/μg of cell protein	
Cell protein	SR	4,320	4,240
	A	2,790	2,680
	C	2,900	2,270
Acid-soluble	SR	700	700
	A	830	810
	C	700	800
Medium proteins	SR	740	1,850
	A	1,080	1,130
	C	810	800

Confluent monolayers of the wild-type HTC-SR cells and the two variants, A and C, were cultured for 24 h in 1 ml of culture medium containing 50 μCi of [3H]fucose. The values shown represent averages of two identically treated monolayer cultures, and each measurement was performed in duplicate.

with steroid. On the other hand, two fucose-labeled glycoprotein series with apparent mol wt of 50,000 and 35,000–50,000 (gp 50 and gp 35–50) are increased in steroid-treated cells. The gp 35–50 glycoprotein, which is characterized by a high degree of size heterogeneity, is not detectable in the membrane fraction from cells not treated with steroid. The same hormone-induced alterations of membrane glycoproteins were seen when HTC cells were labeled with [35S]methionine, followed by isolation of the membrane glycoproteins by
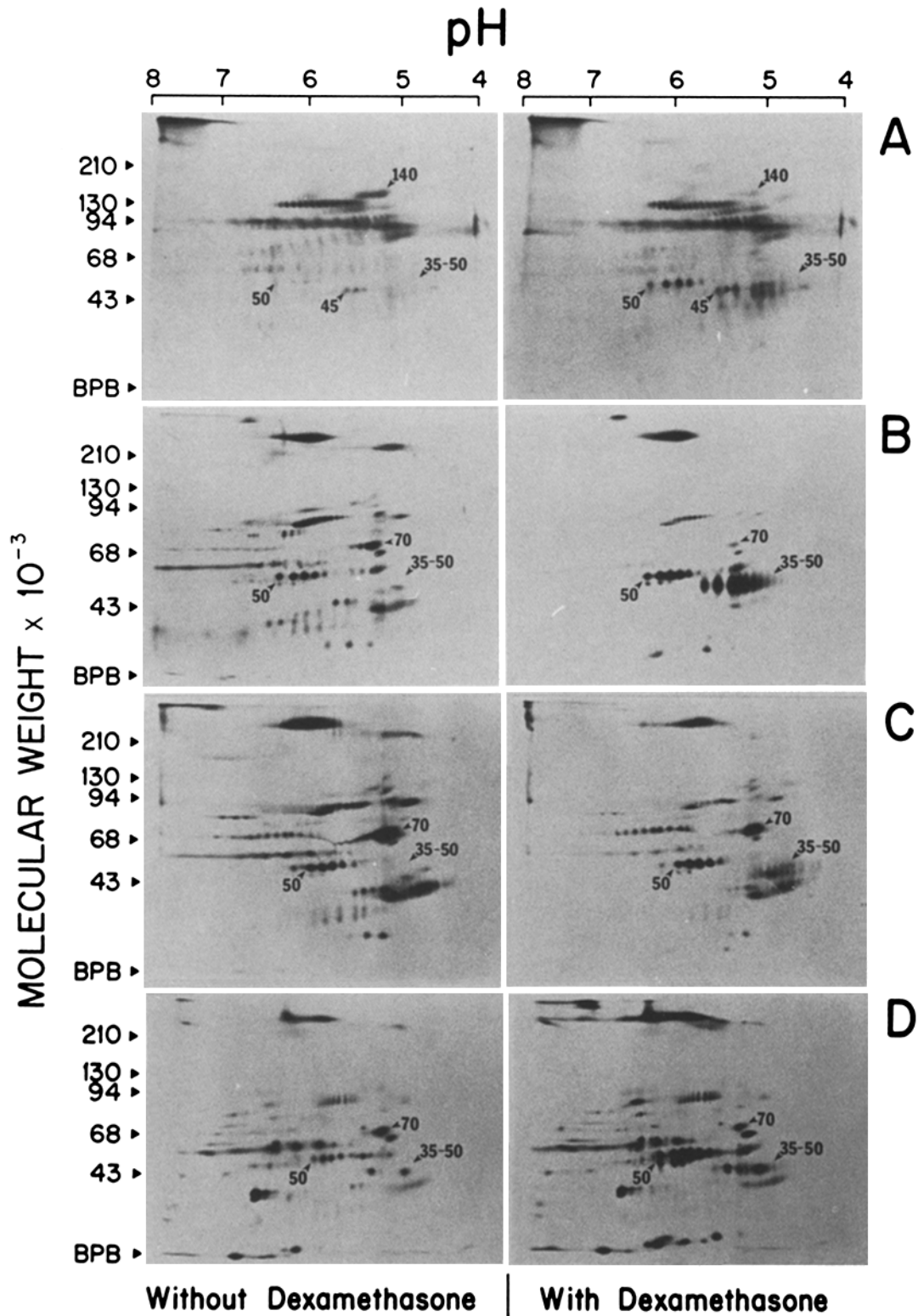
FIGURE 1 Effect of dexamethasone on the glycoprotein pattern of HTC cells. (A) Confluent monolayer cultures of wild-type HTC cells (SR) were cultured for 24 h in the presence of 50 μCi of [³H]fucose in normal growth medium. Aliquots of the membrane fractions each containing 50 μg of proteins were used for gel electrophoresis. (B) Proteins released by wild-type HTC cells (SR) labeled for 6 h with 60 μCi [³H]fucose/ml of serum-free medium. (C) Proteins released by HTC cells, Variant C, labeled in the same way as the cells used in B. In both cases, one-half of the recovered protein fractions were subjected to gel electrophoresis. (D) Proteins released by wild-type HTC cells (SR) labeled for 6 h with 115 μCi [³⁵S]-methionine/ml of serum-free medium. One-fifth of the recovered protein fractions were used. BPB, bromphenol blue.

concanavalin A-Sepharose chromatography (data not shown). Hence, the effects of the hormone are not only on sugar addition to these glycoproteins, but also on the synthesis of the protein backbone.

Electrophoretic analyses of the fucose-labeled proteins released into the medium show dramatic changes upon dexamethasone treatment of HTC cells (Fig. 1 B). The most striking feature is the appearance in the secretion of a glycoprotein which has an apparent mol wt ranging between 35,000 and 50,000 and which migrates the same as gp 35–50 found associated with the membrane fraction. Similarly, the fucose-labeled glycoprotein series, gp 50, is present in elevated amounts in the medium of dexamethasone-treated cells. Furthermore, the synthesis and secretion of many of the other fucose-labeled glycoproteins are apparently reduced in dexamethasone-treated HTC cells relative to nontreated cells. As pointed out in Table II, dexamethasone does not alter the rate of fucosylation of medium glycoproteins in the variant HTC cell lines, A and C. But dexamethasone does induce changes in the labeling pattern of these cell lines similar to those just described for the wild-type cell line, SR. The alterations in Variants A and C are, however, not so compelling as in SR (Fig. 1 C shows results for Variant C; Variant A showed a similar pattern). Similar results for both "wild type" and variant cells were obtained in

several different experiments.

To demonstrate whether this reduction in fucose incorporation is caused by reduced protein biosynthesis or to effects on fucosylation, we examined the pattern of proteins secreted from SR cells labeled with [^{35}S]methionine. With the exception of the increased amount of methionine incorporated into the two induced proteins (gp 50 and gp 35–50) and the decreased incorporation of methionine into the protein, gp 70, the overall pattern of methionine-labeled proteins was not significantly changed by dexamethasone treatment. The extent of overall incorporation of labeled methionine into the secreted proteins of steroid-treated cells is about twice that of control cells not treated with steroid (see Table I). Hence, some of the differences in the fucose-labeled pattern of Fig. 1 B are caused by depletion of the fucose precursor and its preferential incorporation into the steroid-"induced" proteins gp 50 and gp 35–50.

The changes in the secretion program brought about by dexamethasone can be detected at an early stage of biosynthesis of the glycoproteins (Fig. 2). As described previously (3), glycoproteins which will eventually be secreted into the medium can be identified as membrane-bound precursors during the 1st h of biosynthesis. Therefore, by analyzing the membrane fraction of cells labeled for a short time with [^{3}H]mannose, the effect of
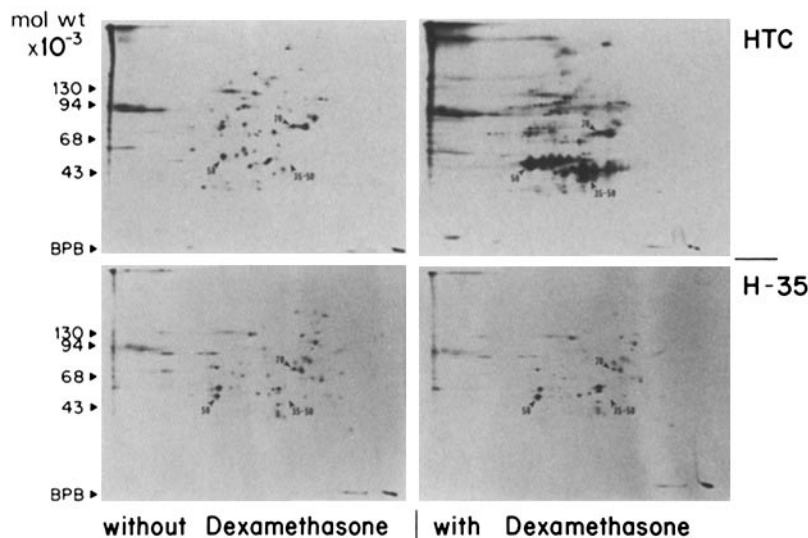

FIGURE 2 Effect of dexamethasone on [^{3}H]mannose-labeled membrane glycoproteins of HTC cells and H-35 cells. Confluent monolayers (10 cm^2) of wild-type HTC cells and H-35 cells were labeled for 1 h with [^{3}H]mannose, 500 μCi in 0.5 ml of medium. The whole membrane fractions were used for gel electrophoresis. BPB, bromphenol blue.

dexamethasone on the early steps in glycoprotein biosynthesis can be examined. Fig. 2 shows that the synthesis of the membrane-bound forms of the glycoproteins, gp 50 and gp 35–50, is significantly higher in the dexamethasone-treated HTC cells than in the control. A quantitative determination of the radioactivity present in individual spots of the two-dimensional pattern by scintillation spectrometry revealed that about two times more tritium was incorporated after dexamethasone stimulation relative to the same spots of nontreated cells. In contrast, glycoprotein gp 50 contained 30 times more radioactivity in steroid-treated cells than in control cells. The relative incorporation into glycoprotein gp 70, however, was reduced by
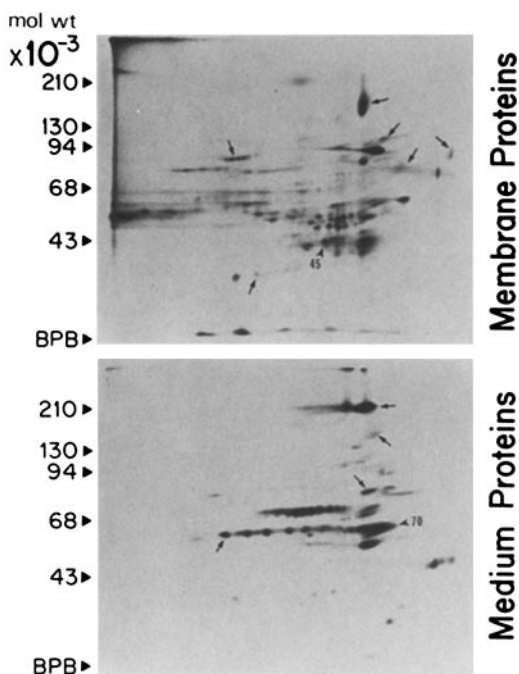

FIGURE 3 Fucose-containing glycoproteins synthesized by rat hepatocytes in the presence of dexamethasone. For labeling membrane glycoproteins, primary hepatocytes (2 × 10^5) were cultured immediately after preparation for 24 h in the presence of [^{3}H]fucose, 50 µCi in 2 ml of medium. For obtaining secreted glycoproteins, hepatocytes (4 × 10^5) were placed in culture for 24 h without added label, and then incubated for an additional 6 h with [^{3}H]fucose, 50 µCi in 1 ml of serum-free medium. Aliquots of the membrane fraction or concentrated medium, each containing 200,000 acid-insoluble cpm, were analyzed by two-dimensional gel electrophoresis. Arrows indicate glycoproteins that are also present in the membranes or the secretion of HTC cells. BPB, bromphenol blue.

twofold or more. Because of the absence of detectable levels of glycoprotein gp 35–50 in cells not treated with dexamethasone, a determination of relative incorporation into this protein was not possible. Reuber H-35 cells, another established rat hepatoma cell line, was also analyzed for similar changes in the program of glycoprotein biosynthesis and secretion induced by dexamethasone. No obvious qualitative or quantitative differences in glycoprotein patterns were detected (Fig. 2).

Does response of HTC cells to dexamethasone reflect that of a normal hepatocyte? As illustrated in Fig. 3, when primary cultures of rat liver hepatocytes were cultured in the presence of dexamethasone, the newly synthesized fucoglycoproteins present in the membrane fraction or released into the medium are very much different than those of HTC cells. Only a few spots are shared by the two types of cells. Furthermore, the dexamethasone-induced glycoproteins found in the secretion or membranes of HTC cells are completely missing in the rat hepatocytes.

DISCUSSION

In this paper, we show that the synthetic steroid hormone, dexamethasone, has multiple effects on glycoprotein metabolism in a subclone SR of rat HTC cells growing in monolayer culture. The most pronounced effect of the hormone is to double the rate of synthesis of secretory glycoproteins, as measured by the incorporation of methionine or fucose. The highest "induction" was found for two series of secretory glycoproteins of 50,000 and 35,000–50,000 mol wt, respectively. Elsewhere,[1] we will show that the mRNA for these secretory proteins is increased in steroid-treated cells. These glycoproteins exist in series because of different secondary carbohydrate modifications to the same primary protein with sialic acid residues supplying most of the charge heterogeneity (4, 5). Both of these glycoprotein series can be detected in the membrane fraction of cells early during the course of biogenesis. At least one of these, the glycoprotein series with mol wt of 50,000, can be labeled by lactoperoxidase-catalyzed iodination in intact cells and is probably identical to the protein denoted Belt I by Ivarie and O'Farrell (13). This glycoprotein is, however, probably not an authentic constituent of the plasma membrane but is a protein in transit to the secretion.

Dexamethasone also results in decreased incorporation of labeled methionine and fucose into a secretory protein of 70,000 mol wt. Thus, the hormone alters, by both a down-regulation and an up-regulation mechanism, the pattern of biosynthesis of several secretory glycoproteins in HTC cells. In contrast, the metabolism of only one membrane glycoprotein is significantly affected by the hormone. There is a down regulation in the biosynthesis of a membrane protein with mol wt of ~140,000.

Dexamethasone also enhances the extent of mannose uptake into HTC cells, with a significant portion of this sugar being diverted into cell lipids, probably in the form of dolichol-linked sugars. The steroid does not affect significantly the uptake of fucose, but the steroid does affect the pattern of fucose-labeled glycoproteins that are present in the secretion of wild-type SR cells. There are fewer fucose-labeled species in the secretion of steroid-treated cells relative to nontreated cells. However, with the exception of the induced proteins of mol wt 50,000 (Fig. 1A) and mol wt 35,000–50,000 and the down-regulated secretory protein of 70,000, the methionine-labeled pattern of the secretion is not different between steroid-treated and control cells, suggesting that some of the secretory proteins may be synthesized, but possibly they are under-fucosylated because much of the fucose precursor is diverted to the steroid-induced secretory proteins.

These effects of dexamethasone on secretory glycoprotein biogenesis are rather specific to HTC cells. In these cells, dexamethasone also decreases the transport of amino acids (16), enhances cell adhesiveness (2, 12), and decreases the intracellular activity and the secretion of plasminogen activator (24, 25). Hence, this cell line is a good one in which to analyze, at the molecular level, the program of diverse responses brought about by a single hormone. The availability of genetic variants (25) isolated on the basis of resistance to dexamethasone inhibition of secretion of plasminogen activator may aid in unraveling the complex response of this cell to steroids. In two such variant cell lines examined here, the production of secretory glycoproteins is already high and there is little or no additional effect of dexamethasone on the secretory program.

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