INHIBITION OF CHOLESTEROL BIOSYNTHESIS IN HeLa CELLS BY GLUCOCORTICOIDS

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SUMMARY: Dexamethasone, at concentrations ranging from 10^{-6} M to 10^{-8} M inhibited the incorporation of acetate into sterol fraction of S3G strain of HeLa cells. The effect was abolished by the presence of serum supplement in the culture medium. Dexamethasone had no effect on the incorporation of mevalonate or cholesterol itself, indicating possible involvement of HMG-CoA reductase. A survey of representative steroids of the C_{21} series demonstrated a relationship between the structure required for glucocorticoid activity and the effect on cholesterol biosynthesis.

INTRODUCTION

Microviscosity of the plasma membrane in eucaryotic cells appears to be controlled by the cholesterol/phospholipid ratio. The biological importance of this aspect of membrane composition has been discussed recently by Inbar and Shinitzky (1) who proposed a model for the regulatory function of cholesterol which has wide implications for a variety of cell surface related phenomena, including regulation of activity of several membrane-associated enzymes.

Recently, we have reported (2) that alkaline phosphatase activity associated with the membranes of HeLa cells is stimulated by lysolecithin when this phospholipid is added to particulate cell fractions in the form of microdispersions. When such microdispersions are composed of both lysolecithin and cholesterol, the stimulatory effect of lysolecithin is reversed in proportion to cholesterol content. Therefore it appears that alkaline phosphatase can be added to the growing list of enzymes whose activity may be modulated by changes in fluidity of the lipid components of the plasma membrane.

The HeLa alkaline phosphatase is also stimulated very specifically by C_{21} steroids of the glucocorticoid series (3,4). However, such effects require

presence of steroids during cell growth for at least 12 to 24 hours. In spite of this, steroids do not affect new synthesis of enzyme protein but instead appear to alter its catalytic efficiency (5). It may be argued that the elevation of alkaline phosphatase activity in steroid-treated cultures is an eventual consequence of the smaller cholesterol/phospholipid ratio resulting from early effects of glucocorticoids on lipid metabolism; such small shifts have been previously reported from our laboratory (6). However, the experiments were done after long incubation in serum-containing media where both lipid uptake and efflux of lipids could have obscured changes in endogenous lipid biosynthesis. The results shown in this paper demonstrate that in the absence of serum, dexamethasone, as well as other glucocorticoids, markedly inhibit incorporation of acetate into cellular cholesterol, this effect occurring much earlier than the steroid induced elevation of alkaline phosphatase.

MATERIALS AND METHODS

The origin and methods of cultivation of the HeLa S3G ($HeLa_{65}$) strain have been reported (7). In the experiments described here the cells were grown for 18 hours in Eagle's minimal essential medium (MEM) supplemented with 2% calf serum. They were then incubated at 37°C in the same medium without serum to which 1.0 μCi/ml [^{3}H]-acetate (S.A. 685 mCi/mmole) was added. The representative samples were removed at different time intervals and the cell monolayers were washed with 0.85% NaCl containing 10 mM sodium acetate. Total sterols were extracted according to the method of Sperry and Webb (8) using acetone/ethanol (1:1, v/v) for lipid extraction followed by saponification with ethanolic KOH and precipitation of sterols with digitonin. The radioactivity was determined by dissolving the precipitate in an acetic acid/ethyl acetate mixture (1:2, v/v) and counting the samples in a Packard Tricarb scintillation spectrometer using toluene based scintillation fluid (0.6% PPO and 0.02% dimethyl POPOP in toluene). The determination of total cellular cholesterol was carried out as described by Bates and Rothblat (9) in a Varian Model 2100 gas/liquid chromatograph. The glass column was 6 ft x 1/8 inch packed with 100/120 mesh Gas Chrom Q coated with 3% OV-17 (Supelco, Inc.), and the flow rate was 30-35 ml/min. The cholesterol peak was evident at 290° and was quantitated by using coprostanol as an internal standard. No major steroids other than cholesterol were detected.

RESULTS AND DISCUSSION

When the incorporation of acetate into whole cells and into the total lipid fraction prepared according to the method of Folch *et al.* (10) was followed in the presence and in the absence of dexamethasone, no differences could be de-

tected. However, when the cellular sterol fraction was separated and analyzed for radioactivity, a significant decrease in labeling became evident two to six hours after addition of 10^{-6} M dexamethasone to the cells (Fig. 1). After 24 hours of incubation, the radioactivity of the sterol fraction decreased to about 50% of the control. The total amount of cholesterol was 14.3 µg/mg protein in the dexamethasone-treated cultures and 16.6 µg/mg protein in the controls. Since HeLa cells could not be maintained without serum for more than 24 hours, this relatively small decrease in total cholesterol could reflect the short incubation time. The absence of serum from the medium is essential to assure activation of the biosynthesis pathway for the *de novo* synthesis of cholesterol (11).

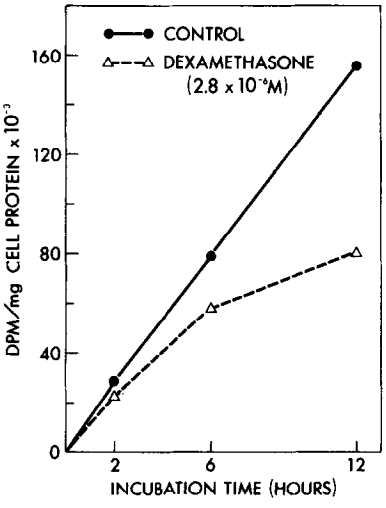

Figure 1. Inhibition of [H^3] acetate incorporation into cholesterol fraction of HeLa cells. The cells were grown in 10% serum-supplemented Eagle's medium for 18 hours and then changed to a serum-free medium that contained 1.0 µCi of [H^3]-acetate/ml. The incubation was continued either in the presence or in the absence of 2.8 x 10^{-6} M dexamethasone. Individual points represent means of three separate cultures.

The control function of both serum and dexamethasone is also evident from the results of another experiment in which cell cholesterol was labeled during growth in media containing either no serum, partly delipidized serum or whole

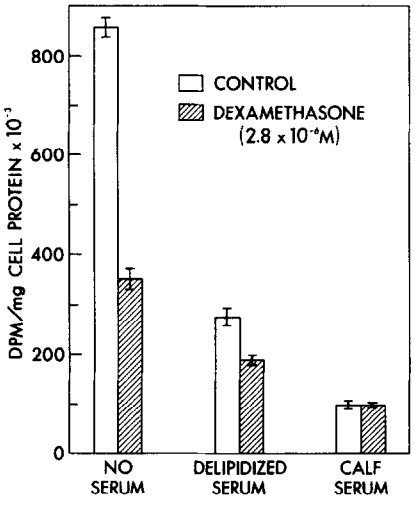

Figure 2. Comparison of the inhibitory effects of 2.8 x 10^{-6} M dexamethasone on cholesterol biosynthesis in HeLa cells incubated for 24 hours in Eagle's medium supplemented with either 10% calf serum, equivalent amount of partly delipidized calf serum protein or medium alone. The bars represent means of three cultures including ± S.D. (vertical line).

serum (Fig. 2). The presence of serum in the medium markedly reduced the uptake of acetate into cholesterol and abolished the difference between steroid-treated and control cultures. In cultures containing partly delipidized serum, cholesterol synthesis (as indicated by the acetate uptake) was considerably reduced, but dexamethasone still inhibited cholesterol synthesis by about 25%. No suppression of total cholesterol content by dexamethasone was observed in cells grown in the presence of serum.

In order to determine whether dexamethasone could have caused an increased efflux of cholesterol, cells were incubated with [^{3}H] acetate for 72 hours to label cellular cholesterol. The medium was then replaced by unlabeled medium either with or without serum. At different times the cellular sterol fraction was analyzed for the loss of label. As expected (9), cells grown in the presence of serum showed a much greater loss of cholesterol label than cells grown in medium without serum (data not shown). However, in both cases dexamethasone was without effect on the cholesterol efflux into the medium.

TABLE 1

Survey of Inhibitory Effects of C_{21} Steroids on Cholesterol Biosynthesis in HeLa Cells (S3G Strain)

Steroid	Percent Incorporation of [^{3}H] Acetate into Cholesterol Fraction				
	Steroid Concentration (M)				
	0	10^{-6}	10^{-7}	10^{-8}	10^{-9}
None	100	-	-	-	-
Dexamethasone		45	46	66	94
Betamethasone		47	51	76	105
Prednisolone		54	59	93	96
Triamcinolone		56	61	98	102
Cortisol		53	65	80	101
Cortisone		105	129	110	102
Progesterone		99	115	109	104
Deoxycorticosterone		88	106	101	87

Culture conditions the same as described for Fig. 1. The values are means from three flasks all from the same experiment.

TABLE 2

Precursor		µg protein / culture	Radioactivity Incorporated (dpm x 10^{-3}/mg protein)	
			"Pool"	Acid Insoluble Fraction
[^{3}H] uridine	Control	394 ± 76	308 ± 46	708 ± 41
	Dex	416 ± 49	384 ± 54	839 ± 81
[^{14}C] leucine	Control	410 ± 93	4.5 ± 1.0	38.3 ± 3.8
	Dex	437 ± 48	4.1 ± 0.9	36.0 ± 5.6

Lack of general inhibitory effect of dexamethasone on incorporation of label from [^{3}H] uridine and [^{14}C] leucine.

HeLa cells pregrown for 18 hours in MEM containing 2% calf serum were changed to the serum-free MEM and were labeled for 24 hours with [^{3}H] uridine (0.4 µCi/ml) and [^{14}C] leucine (0.04 µCi/ml). The radioactivity was determined in the perchloric acid-soluble and in the perchloric acid insoluble fractions. The values are means from three separate cultures ± S.D.

We have compared several C_{21} steroids in their effects on incorporation of acetate into cellular cholesterol. The results (Table 1) indicated that only steroids with known glucocorticoid activity were inhibitory. Moreover, the inhibition reflected activity of the individual steroids tested, dexamethasone being most active. The lack of inhibitory effects by cortisone can be explained by the inability of cell cultures to convert it to cortisol (13).

That the effect was not a result of a nonspecific suppression of macromolecular synthesis by the steroids was shown by following uptake of leucine and uridine into perchloric acid-soluble and perchloric acid-insoluble cell fractions (Table 2). Under conditions of short incubation (up to 24 hours) in serum-free media, dexamethasone was without effect on incorporation of either of these two macromolecular precursors. Moreover, the incorporation of acetate into cholesterol appeared to be independent of the amount of acetate present in the medium, and could not be shown if mevalonate or cholesterol were substituted for acetate as precursors. Although the exact mechanism of the inhibitory effect of glucocorticoids on cholesterol biosynthesis remains to be elucidated, it has

TABLE 3

Radiolabeled Precursor (1 μCi/ml)	Concentration (μg/ml)	DPM/mg Protein x 10^{-3}	
		Control	Dex (1.28×10^{-6} M)
[H^3] Acetate	0.40	503.5 ± 34.2	204.9 ± 4.4
	2.20	503.8 ± 29.0	191.8 ± 5.8
	10.20	343.7 ± 21.9	203.4 ± 8.6
[H^3] Mevalonate	0.040	16.4 ± 0.9	16.5 ± 1.1
[H^3] Cholesterol	0.006	130.9 ± 11.1	117.5 ± 10.5

Comparison of the effects of dexamethasone on the incorporation of acetate, mevalonate and cholesterol into cellular sterol fraction. Experimental conditions the same as described for Fig. 1. The values are means from three separate fractions ± S.D.

some similarities with the results obtained by Kandutsch and Chen (13) who used cholesterol derivatives oxygenated in the side chain to inhibit cholesterol biosynthesis in L cells. In their case the effect was mediated through the repression of the HMG-CoA reductase (E.C. 1.1.1.34). Until the present time, no specific effects of C_{21} steroids on cholesterol formation have been demonstrated. The inhibition of mevalonate incorporation into cholesterol by steroids in liver microsomes reported by Ohno and Imai (15) required very high concentrations of steroids (up to 10^{-3} M) and was not related to any specific steroid structure. It remains to be shown whether other surface changes that take place in steroid-treated epithelioid cells are related to the suppression of cholesterol biosynthesis. Even more important should be the evidence relating such effects to lympholytic properties of steroids as well as to the emergence of steroid resistance among neoplastic cells of lymphatic origin.

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