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INHIBITORS OF STEROL SYNTHESIS. 14α-ETHYL-5α-CHOLEST-7-ENE-3β,15α-DIOL INDUCES CHANGES IN THE STEROL COMPOSITION AND THE MORPHOLOGY OF CHO-K1 CELLS*

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Summary: Incubation of CHO-K1 cells in lipid-deficient medium containing 14α-ethyl-5α-cholest-7-ene-3β,15α-diol (0.1 μM) for 4 days was associated with a profound change in cellular sterol composition as reflected by a marked accumulation of lanosterol and 24,25-dihydrolanosterol. A striking elongation of the cells was also observed. Incubation of CHO-K1 cells in lipid-deficient medium containing lanosterol (10 μM) also caused a significant accumulation of lanosterol which was also associated with a marked elongation of the cells.

14α-Ethyl-5α-cholest-7-ene-3β,15α-diol (hereafter designated as ethyl diol) is a potent inhibitor of cholesterol biosynthesis. The combined results of studies in rat liver homogenate preparations (1) and in cultured mammalian cells (2-4) indicate that this 15-oxygenated sterol has two major actions: a lowering of the level of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity (3,4) and an inhibition of the metabolism of C_{30} sterols (1,4). For example, incubation of the ethyl diol (1 μM) with the 10,000 x g supernatant fraction of a rat liver homogenate (1) or incubation of the ethyl diol (0.5 μM) with CHO-K1 cells (4) caused an almost complete inhibition of the incorporation of [^{3}H]acetate into C_{27} sterols which was associated with a striking accumulation of labeled lanosterol and 24,25-dihydrolanosterol.

We now report that incubation of CHO-K1 cells in a lipid-deficient medium in the presence of the ethyl diol (0.1 μM) caused a profound alteration in cellular sterol composition which was associated with a marked change in the morphology of the cells.

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EXPERIMENTAL PROCEDURES

Thin layer chromatography (TLC) was carried out on plates of silica gel G (Analtech; Newark, DE). Compounds on the plates were detected after spraying with molybdic acid (5). Gas-liquid chromatography (GLC) was performed using Hewlett-Packard systems (Model 402 or Model 5730A) equipped with silanized glass columns (6 ft x 4 mm) packed 3% OV-17 on Gas Chrom Q (60-80 mesh) at 280° C. Capillary GLC analyses were carried out on a Perkin Elmer Model Sigma 2000 unit equipped with a 0.1 μm DB-5 column (30 m x 0.25 mm) with temperature programming from 50° C to 280° C at a rate of 30° C per min followed by isothermal operation (280° C). 5α-Cholestane was used as an internal standard. GLC-mass spectrometry (GLC-MS) was carried out using a Finnigan 3300 GLC-MS unit equipped with a Finnigan 6100 data system. Trimethylsilyl (TMS) ether derivatives were prepared using hexamethyl-disilazane and trimethylchlorosilane in pyridine (6).

14α-Ethyl-5α-cholest-7-ene-3β,15α-diol (single component on TLC; solvent system, 35% ethyl acetate in $CHCl_3$) was prepared as described previously (2,7). Cholesterol was purified by way of its dibromide derivative (8) and recrystallization (single component on TLC; solvent system, 20% ethyl acetate in toluene). Lanosterol was prepared as described previously (4) and showed a single component on TLC (solvent system, 20% ether in toluene). Powdered Ham's F12 medium (9), Dulbecco's modified phosphate buffered saline (PBS), and newborn calf serum (NCS) were obtained from M. A. Bioproducts (Walkersville, MD) or from Irvine Scientific (Santa Ana, CA). Chinese hamster ovary cells (CHO-K1) were obtained from the American Type Collection (Rockville, MD) and they were subcloned to obtain populations of morphological homogeneity.

CHO-K1 cells were maintained in Ham's F12 medium (9) supplemented with 10% NCS and using Lux tissue culture dishes (Flow Laboratories, MacLean, VA) at 37° C in a 5% CO_2-95% air environment. The cells (2.5×10^5 cells) were inoculated into 150 mm plates containing Ham's F12 medium (20 ml) supplemented with 8% NCS and incubated for 48 h. The medium was aspirated, the plates were rinsed with PBS (30 ml) and fresh Ham's F12 medium supplemented with 8% delipidated NCS (10) was added. Media containing sterols (added in ethanol) were prepared as described previously (4). Control cells (no added sterol) were incubated in medium containing the same amount of ethanol (final concentration, 0.004%) as cells treated with the ethyl diol (0.1 μM). Cells treated with lanosterol (10 μM) or cholesterol (10 μM) were incubated in medium containing the same amount of ethanol (final concentration, 0.2%). In each of the experiments, the medium was changed every 2 days. At harvest, plates were rinsed twice with PBS (10 ml portions) and the cells were collected by scraping into PBS (4 ml). Following centrifugation at 600 x g, the cell pellets were heated with 15% ethanolic KOH for 3 h at 80° C and the nonsaponifiable lipids (NSL) were extracted with petroleum ether.

In an attempt to quantitate changes in cell morphology (*vide infra*), the approach introduced by Hsie and Puck (11) to follow the morphological changes induced by dibutyryl cAMP in CHO-K1 cells was employed. This method involves determination of the ratio of the longest to shortest cellular dimensions (L/S ratio). Results are presented as mean ± S.E.M. and differences between mean values were evaluated using Student's t test.

RESULTS

Treatment of CHO-K1 cells with the ethyl diol (0.1 μM) induced a marked changes in cellular sterol composition as indicated by the results of GLC analyses of the NSL (Table 1). While control cells contained cholesterol as the major sterol (~96%), experimental cells showed a marked accumulation (~75%) of C_{30} sterols (lanosterol and 24,25-dihydrolanosterol). The identities of the major sterols from control and experimental cells were further confirmed by the results of GLC-MS analyses of the TMS derivatives of the NSL.

Table 1. Effect of 14α-ethyl-5α-cholest-7-ene-3β,15α-diol (0.1 μM) on the sterol composition of CHO-K1 cells incubated for 4 days in lipid-deficient medium

Cellular Sterol	% of Total Sterols	
	Control	Experimental
Cholesterol	96	25
Lanosterol	0	50
24,25-Dihydrolanosterol	0	25
Other	4	0

The cells incubated with the ethyl diol (0.1 μM) for 4 days showed a marked change in cellular morphology with a striking elongation of the cells which was similar, if not identical, to that reported previously for CHO-K1 cells incubated in the presence of dibutyryl cAMP (1 mM) (11). Figure 1 shows a comparison of the mean values for cells after 4 days of incubation in the absence and presence of the ethyl diol. The mean value of the L/S ratio of the cells treated with the 15-oxygenated sterol was increased by 115% ($p<0.001$) relative to that for control cells. While control cells showed 9.2% and 0.0% of cells with L/S ratios in excess of 4.0 and 8.0, respectively, experimental cells showed 66.4% and 11.2% of cells with ratios in excess of 4.0 and 8.0, respectively. The effect of ethyl diol on cell morphology was highly reproducible, with increases in the mean L/S ratio of 115% and 125% ($p<0.001$) in separate experiments in which the cells were treated with the 15-oxygenated sterol for 4 and 4.5 days, respectively.

Cells incubated with lanosterol (10 μM) showed changes in morphology similar to those induced by the ethyl diol but those cells incubated with cholesterol (10 μM) did not. Figure 2 shows the mean values of the L/S ratios observed in the cells treated with lanosterol and with cholesterol. The mean value for the L/S ratio of the lanosterol-treated cells was 118% higher ($p<0.0001$) than that of the cholesterol-treated cells. The sterol composition of the lanosterol-treated cells (cholesterol, 24%; 24,25-dihydrolanosterol, 9%; and lanosterol, 68%) differed markedly from that of cells incubated for 3 days in the presence of cholesterol (cholesterol, 89%; 24,25-dihydrolanosterol, 8%; and lanosterol, 3%).

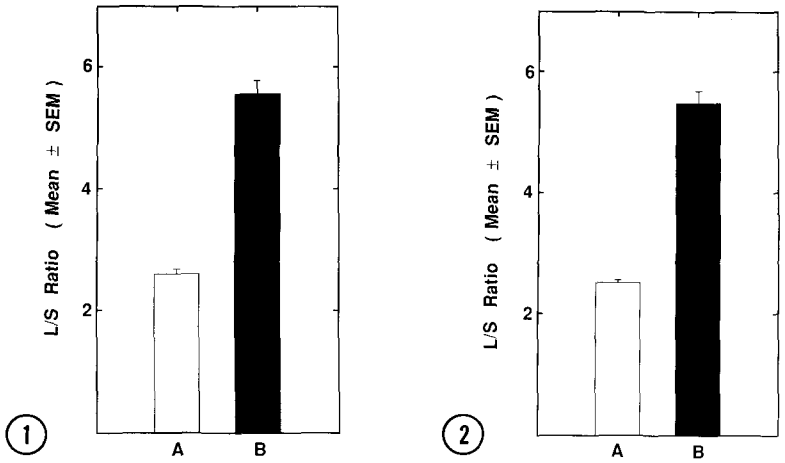

Figure 1. Effect of 14α-ethyl-5α-cholest-7-ene-3β,15α-diol (0.1 μM) on the morphology of CHO-K1 cells as reflected by determination of the ratio of longest to shortest dimensions (L/S ratio). A (open bar), control cells (no added sterol); B (shaded bar), experimental cells.

Figure 2. Effect of lanosterol (10 μM) on the morphology of CHO-K1 cells as reflected by determination by the ratio of longest to shortest dimensions (L/S ratio). A (open bar), cholesterol-treated cells; B (shaded bar), lanosterol-treated cells.

DISCUSSION

The results presented herein demonstrate that incubation of CHO-K1 cells with 14α-ethyl-5α-cholest-7-ene-3β,15α-diol, at a concentration of 0.1 μM in a lipid-deficient medium, causes a marked accumulation of lanosterol and 24,25-dihydrolanosterol. Thus, the inhibition of the metabolism of lanosterol and 24,25-dihydrolanosterol by the ethyl diol, demonstrated previously by the inhibition of the incorporation of [^{3}H]acetate into C_{27} sterols, and accompanied by the accumulation of labeled lanosterol and 24,25-dihydrolanosterol (4), can be used to profoundly modify the sterol composition of CHO-K1 cells in culture. Perhaps of equal importance is the demonstration that these cells showed a very marked change in morphology, with a striking elongation of the cells which appears to be similar, if not identical, to that observed previously for CHO-K1 cells incubated with dibutyryl cAMP (11). These findings prompted study of the effect of incubation of the cells with lanosterol (10 μM). A marked cellular accumulation of lanosterol was observed which was associated with the same elongation of the cells as observed upon incubation in the presence of the ethyl diol.

Modification of the sterol composition of mammalian cells, as described herein, should provide a very powerful approach for investigation of the role of cholesterol

and of other sterols in various aspects of cellular function. Moreover, the results presented here strongly suggest an important role of sterols in the control of cell morphology.

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