Proc. Natl. Acad. Sci. USA
Vol. 75, No. 4, pp. 1877–1881, April 1978
Cell Biology

Inhibition of cholesteryl ester formation in human fibroblasts by an analogue of 7-ketocholesterol and by progesterone

(acyl-coenzyme A:cholesterol acyltransferase/atherosclerosis/low density lipoprotein/3-hydroxy-3-methylglutaryl coenzyme A reductase)

JOSEPH L. GOLDSTEIN*, JERRY R. FAUST*, JOHN H. DYGOS†, ROBERT J. CHORVAT†, AND MICHAEL S. BROWN*

* Departments of Molecular Genetics and Internal Medicine, University of Texas Health Science Center at Dallas, Dallas, Texas 75235; † Searle Laboratories, Chicago, Illinois 60680

Communicated by E. R. Stadtman, January 25, 1978

ABSTRACT The synthesis of cholesteryl esters in cultured human fibroblasts is catalyzed by a microsomal acyl-coenzyme A:cholesterol acyltransferase (EC 2.3.1.26). The acyltransferase activity is enhanced when fibroblasts take up cholesterol contained in plasma low density lipoprotein. In the current studies two steroids, SC-31769 (an analogue of 7-ketocholesterol) and progesterone, were shown to inhibit acyltransferase activity in cell-free extracts of human fibroblasts. When added to intact cells, these steroids inhibited the incorporation of [^{14}C]oleate into cellular cholesteryl [^{14}C]oleate and reduced the accumulation of cholesteryl esters in fibroblasts exposed to low density lipoprotein. The inhibition of cholesteryl ester formation in intact cells by SC-31769 and progesterone was readily reversible. Neither compound inhibited the incorporation of [^{14}C]oleate into [^{14}C]triglycerides or [^{14}C]phospholipids. When incubated with fibroblast monolayers at a concentration of 1 μg/ml, SC-31769 suppressed the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase [mevalonate:NADP$^+$ oxidoreductase (CoA-acylating); EC 1.1.1.34], the rate-controlling enzyme in cholesterol synthesis. In contrast, progesterone had no effect on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity at concentrations as high as 25 μg/ml. The availability of two types of steroid compounds that inhibit the acyltransferase activity and cholesteryl ester synthesis in human fibroblasts should prove useful in further studies of the regulatory mechanisms responsible for cholesteryl ester accumulation in human cells under normal and pathologic conditions.

When cultured human fibroblasts are presented with an amount of cholesterol that exceeds that which can be incorporated into cellular membranes, the cells develop enhanced activity of an acyl-coenzyme A:cholesterol acyltransferase (acyltransferase; EC 2.3.1.26) (1). This microsomal enzyme esterifies the excess cholesterol with long-chain fatty acids, and the resultant cholesteryl esters are stored in the cytoplasm as neutral lipid droplets (1, 2). Acyltransferase can be stimulated in fibroblasts by incubation of the cells with cholesterol dissolved in ethanol or with cholesterol contained in low density lipoprotein (LDL), the major cholesterol transport protein in human plasma (1, 3). In fibroblasts, the cholesterol-mediated stimulation of acyltransferase occurs in concert with a suppression of the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase [HMG CoA reductase; mevalonate: NADP$^+$ oxidoreductase (CoA-acylating); EC 1.1.1.34] the rate-controlling enzyme in cholesterol biosynthesis (4–7). The reciprocal regulation of these two microsomal enzymes prevents an overaccumulation of free cholesterol and leads to an accumulation of cholesteryl esters when cells are exposed to LDL (1, 3).

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Interest in the acyltransferase reaction has been heightened by the awareness that this enzyme plays an important role in the accumulation of cholesteryl esters within smooth muscle cells of the artery wall during the development of atherosclerosis (1, 8, 9). Recently, a group of steroids has been described that mimics the actions of cholesterol in activating the acyltransferase (5) and suppressing HMG CoA reductase (10–12) in cultured cells. These steroids, which are polar derivatives of cholesterol, include 7-ketocholesterol, 25-hydroxycholesterol, and 6-ketocholestanol. Stimulation of the acyltransferase by these steroids causes the cells to esterify a fraction of their own endogenous cholesterol. As a result, these steroids produce an increase in the cholesteryl ester content of fibroblasts even though they suppress cholesterol synthesis within the cell (5).

In the current studies, we describe two steroids that differ from the previously described group of steroids in that both inhibit the synthesis of cholesteryl esters in intact fibroblasts by inhibiting the acyltransferase. One of these steroids, SC-31769, an analogue of 7-ketocholesterol, retains the ability to suppress HMG CoA reductase activity. The other steroid, progesterone, fails to suppress HMG CoA reductase activity. The inhibition of the acyltransferase by both steroids reduces the cellular accumulation of cholesteryl esters when fibroblasts are exposed to LDL.

METHODS

Steroids and Isotopes. SC-31769 (7-keto-20-oxacholesterol), SC-31448 (22-hydroxy-25-methylcholesterol), and SC-31082 (23,25-dihydroxy-23-methyl-21-norcholesterol) were synthesized at Searle Laboratories by procedures to be described elsewhere (unpublished data). The 3-oleate ester of SC-31769 was prepared by treatment of the steroid with oleoyl chloride in pyridine at room temperature for 18 hr, followed by purification on silica gel. Cholesterol was purchased from Applied Science, Inc. All other steroids were obtained from Steraloids, Inc. Each steroid was >98% pure, as judged by gas liquid and thin-layer chromatography. Solutions were prepared just prior to use by dissolving the steroid in ethanol or acetone. [^{14}C]Oleate (58 mCi/mmol) was purchased from Amersham/Searle. [*1*-^{14}C]Oleoyl CoA (55 mCi/mmol) and 3-hydroxy-3-methyl-[3-^{14}C]glutaryl CoA (26.2 mCi/mmol) were obtained from New England Nuclear Corp. Tissue culture supplies and reagents for assays were obtained from previously described sources (11).

Lipoproteins. LDL (density, 1.019–1.063 g/ml) and lipo-

Abbreviations: HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; acyltransferase, acyl-coenzyme A:cholesterol acyltransferase; LDL, low density lipoprotein; ^{125}I-LDL, ^{125}I-labeled LDL.

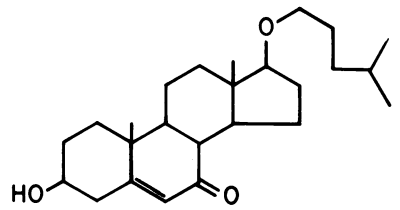

FIG. 1. Structural formula of SC-31769, a 20-oxa analogue of 7-ketocholesterol.

protein-deficient serum (density > 1.215 g/ml) were obtained from human plasma and prepared by differential ultracentrifugation (7). The concentration of LDL is expressed in terms of its protein content. ^{125}I-Labeled LDL (^{125}I-LDL) was prepared as described (13).

Cells. Fibroblasts from a healthy subject were grown in monolayer (11). All experiments were done in a similar format: confluent monolayers of cells from stock flasks were dissociated with 0.05% trypsin/0.02% EDTA solution and were seeded (day 0) at a concentration of 1×10^5 cells per dish into 60-mm petri dishes containing 3 ml of growth medium with 10% (vol/vol) fetal calf serum. On day 3 the medium was replaced with 3 ml of fresh growth medium containing 10% fetal calf serum. On day 5, each monolayer was washed with 3 ml of phosphate-buffered saline, after which 2 ml of fresh medium containing 5% human lipoprotein-deficient serum was added (final protein concentration, 2.5 mg/ml). All experiments were initiated on day 7 after the cells had been incubated for 48 hr in the presence of lipoprotein-deficient serum.

Incorporation of [*1*-^{14}C]Oleate into [^{14}C]Lipids by Intact Fibroblasts. Monolayers were incubated with the indicated additions in 2 ml of growth medium containing 5% lipoprotein-deficient serum and 0.1 mM [*1*-^{14}C]oleate bound to albumin (4). After incubation at 37° for the indicated interval, the cells were washed, harvested, and extracted with chloroform/methanol (2/1, vol/vol). The cholesteryl [^{14}C]oleate, [^{14}C]triglycerides, and [^{14}C]phospholipids were isolated by thin-layer chromatography (4). The data are expressed as the nmol or pmol of [^{14}C]lipid formed per mg of total cell protein.

Assay of Acyltransferase Activity of Cell-Free Extracts. The rate of transfer of [^{14}C]oleate from [^{14}C]oleoyl CoA to cholesterol was assayed as described (5). Cell-free extracts were prepared from fibroblasts that had been grown for 24 hr in growth medium containing 5% lipoprotein-deficient serum and 50 μg of LDL protein per ml (5, 6). Aliquots of the whole cell extracts (80–110 μg of protein) were incubated in 0.12 ml of solution containing 50 mM potassium phosphate, pH 7.4; 2 mM dithiothreitol; 1.2 mg of bovine serum albumin; 80 μM [*1*-^{14}C]oleoyl CoA (14,400–41,000 cpm/nmol); and varying amounts of the indicated steroids added in 2 μl of acetone. After 1 hr at 37°, the reactions were terminated by addition of 4 ml of chloroform/methanol (2/1), and the cholesteryl [^{14}C]oleate was isolated by thin-layer chromatography (4). Enzyme activity is expressed as the nmol of cholesteryl [^{14}C]oleate formed per mg of total extract protein.

Assay of HMG CoA Reductase Activity in Cell-Free Extracts. The rate of conversion of [^{14}C]HMG CoA (10,000 cpm/nmol) to [^{14}C]mevalonate was measured in extracts of detergent-solubilized cells as described (7). Enzyme activity is expressed as the pmol of [^{14}C]mevalonate formed per min per mg of soluble protein.

Other Assays. The proteolytic degradation of ^{125}I-LDL by intact fibroblasts was measured as described (14). The cellular content of free and esterified cholesterol was measured by gas-liquid chromatography (15). The content of protein was determined by the method of Lowry *et al.* (16), with bovine serum albumin as a standard.

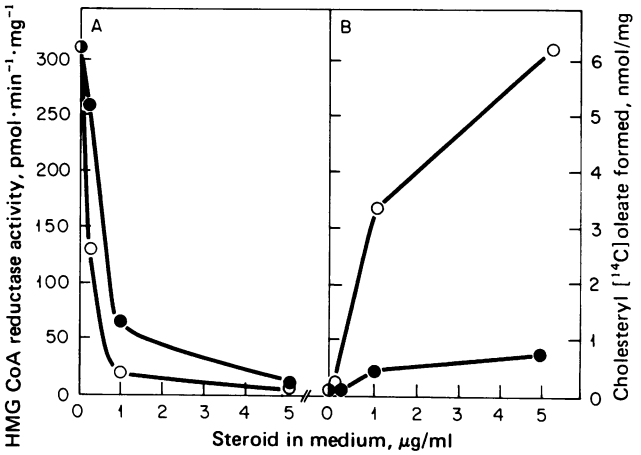

FIG. 2. Effect of 25-hydroxycholesterol (○) and SC-31769 (●) on HMG CoA reductase activity (*A*) and on cholesteryl ester formation (*B*) in fibroblast monolayers. (*A*) On day 7 of growth, each dish received 5 μl of ethanol containing the indicated amount of either 25-hydroxycholesterol (○) or SC-31769 (●). After incubation for 6 hr at 37°, cells were harvested for measurement of HMG CoA reductase activity. (*B*) On day 7 of growth, each dish received 0.1 mM [^{14}C]oleate–albumin (10,000 cpm/nmol) and 5 μl of ethanol containing the indicated amount of either 25-hydroxycholesterol (○) or SC-31769 (●). After incubation for 6 hr at 37°, cells were harvested for measurement of the cellular content of cholesteryl [^{14}C]oleate. Each value is the average of duplicate incubations.

RESULTS

Fig. 1 shows the structure of SC-31769, a 20-oxa analogue of 7-ketocholesterol. SC-31769 was nearly as potent as 25-hydroxycholesterol in suppressing HMG CoA reductase activity in human fibroblasts. At concentrations below 5 μg/ml, both steroids reduced this enzyme activity by more than 95% within 6 hr (Fig. 2*A*). However, whereas 25-hydroxycholesterol markedly stimulated the rate of [^{14}C]oleate incorporation into cholesteryl esters, SC-31769 had much less effect (Fig. 2*B*). We have previously shown that 7-ketocholesterol behaves similarly to 25-hydroxycholesterol in stimulating cholesteryl ester formation and in suppressing HMG CoA reductase activity in fibroblasts (5). The results in Fig. 2 therefore indicate that the modification of the 7-ketocholesterol side-chain in SC-31769 reduced the ability of this sterol to stimulate cholesteryl ester formation, but did not affect its ability to suppress HMG CoA reductase activity.

The failure of SC-31769 to maximally stimulate cholesteryl ester formation raised the possibility that the compound was actually an inhibitor of cholesterol esterification. Accordingly, we tested SC-31769 for its ability to inhibit the enhanced cholesteryl [^{14}C]oleate formation that occurs in intact fibroblasts incubated with 25-hydroxycholesterol (4, 5). At a concentration of 5 μg/ml, SC-31769 inhibited [^{14}C]oleate incorporation into cholesteryl [^{14}C]oleate by 90% when this incorporation had been stimulated by the addition of 25-hydroxycholesterol (Fig. 3).

In addition to inhibiting cholesteryl ester formation in intact cells, SC-31769 inhibited the activity of the acyltransferase in cell-free homogenates of fibroblasts (Fig. 4*A*). Neither cholesterol nor 25-hydroxycholesterol inhibited this enzyme (Fig. 4*A*). The addition of large amounts of exogenous cholesterol to the *in vitro* enzyme assay system did not reverse the inhibitory effect of SC-31769 on the acyltransferase (Fig. 4*B*).

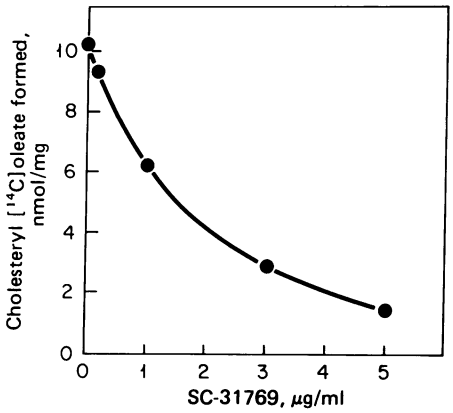

FIG. 3. Inhibition of the 25-hydroxycholesterol-mediated stimulation of cellular cholesteryl ester formation by SC-31769 in fibroblast monolayers. On day 7 the medium was replaced with 2 ml of growth medium containing 5% lipoprotein-deficient serum, 0.1 mM [^{14}C]oleate–albumin (9700 cpm/nmol), 2 μg of 25-hydroxycholesterol per ml, the indicated amount of SC-31769, and 7 μl of ethanol. After incubation for 18 hr at 37°, the cells were harvested for measurement of the cellular content of cholesteryl [^{14}C]oleate. Each value is the average of duplicate incubations.

To rule out the possibility that SC-31769 itself was being esterified during these reactions, we prepared the 3-oleate ester of SC-31769 and used it as a standard for thin-layer chromatography. No radioactivity from [^{14}C]oleate was incorporated into the 3-oleate ester of SC-31769 when SC-31769 was incubated with intact cells.

In addition to inhibiting cholesterol esterification when it had been stimulated by 25-hydroxycholesterol, SC-31769 also inhibited the enhanced activity that followed the addition of LDL to the culture medium (Table 1). This inhibition was reversed within 4 hr after removal of SC-31769 from the culture medium (data not shown). SC-31769 did not inhibit the incorporation of [^{14}C]oleate into [^{14}C]triglycerides or [^{14}C]phospholipids (Table 1).

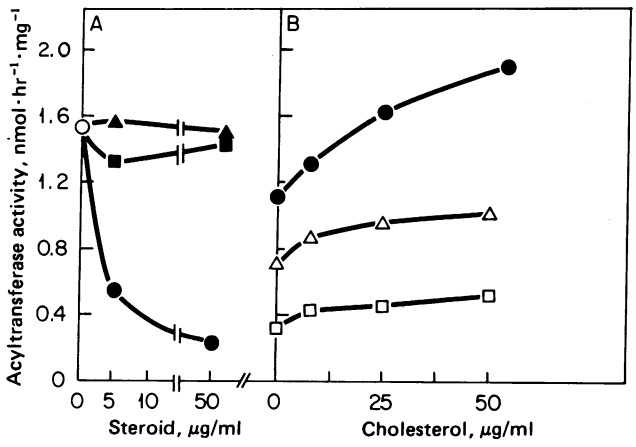

FIG. 4. Inhibition of acyltransferase activity in fibroblast extracts by SC-31769 and lack of inhibition by cholesterol and 25-hydroxycholesterol. (*A*) Fibroblasts were grown and cell-free extracts (80 μg of protein) were incubated for 1 hr at 37° with 80 μM [^{14}C]oleoyl CoA (14,000 cpm/nmol) in the presence of the indicated amount of one of the following steroids: ●, SC-31769; ■, cholesterol; or ▲, 25-hydroxycholesterol. (*B*) Extracts of fibroblasts (110 μg of protein) were incubated for 1 hr at 37° with 80 μM [^{14}C]oleoyl CoA (41,000 cpm/nmol) and the indicated amount of cholesterol in the presence of one of the following amounts of SC-31769: ●, none; △, 5 μg/ml; or □, 15 μg/ml. Assays were performed as described in *Methods*. Each value is the average of duplicate assays.

Table 1. Inhibition of LDL-mediated stimulation of cholesteryl [^{14}C]oleate formation in fibroblast monolayers by SC-31769

Addition to medium	Cholesteryl [^{14}C]oleate, nmol/mg		[^{14}C]Triglycerides, nmol/mg		[^{14}C]Phospholipids, nmol/mg	
	−SC-31769	+SC-31769	−SC-31769	+SC-31769	−SC-31769	+SC-31769
None	0.5	1.2	119	140	67	57
LDL	25.0	3.9	63	79	59	66

On day 7 the medium was replaced with 2 ml of growth medium containing 5% lipoprotein-deficient serum, 0.1 mM [^{14}C]oleate–albumin (9700 cpm/nmol), 5 μl of ethanol, 5 μg of SC-31769 per ml as indicated, and 21 μg of LDL protein per ml as indicated. After incubation for 18 hr at 37°, the cells were harvested for measurement of their content of cholesteryl [^{14}C]oleate, [^{14}C]triglycerides, and [^{14}C]phospholipids. Each value is the average of duplicate incubations.

When LDL is added to fibroblasts, the cellular content of free and esterified cholesterol increases (15). The latter increase results from a lysosomal hydrolysis of the cholesteryl esters contained within LDL followed by a reesterification of the liberated cholesterol by the microsomal acyltransferase (3). Inasmuch as SC-31769 inhibited the acyltransferase, the steroid reduced the accumulation of esterified cholesterol that otherwise occurred when fibroblasts were exposed to LDL (Table 2). The reduction in the cellular content of cholesteryl esters was balanced by an increase in the content of free cholesterol in the cells exposed to LDL in the presence of SC-31769. These data indicate that the total uptake and hydrolysis of LDL-cholesteryl esters was unaffected by the compound and that the inhibition of cholesterol reesterification produced a disproportionate increase in the free cholesterol content of the cell. This conclusion was further supported by the finding that SC-31769 did not inhibit the cellular uptake and lysosomal degradation of ^{125}I-LDL (Table 2).

In view of the observation by Flint *et al.* that progesterone inhibited acyltransferase activity in extracts of interstitial tissue of the rabbit ovary (17), it was of interest to study the effect of progesterone in the fibroblast system. Fig. 5 shows that at

Table 2. Inhibition of LDL-mediated increase in cholesteryl ester content in fibroblast monolayers by SC-31769

Addition to medium	Cellular cholesterol content, μg sterol/mg protein			^{125}I-LDL degraded, μg/mg protein
	Free (a)	Esterified (b)	Total (a) + (b)	
None	23	0.81	24	–
SC-31769 (5 μg/ml)	19	1.2	20	–
LDL (25 μg of protein/ml)	34	14	48	28
LDL + SC-31769	44	3.2	47	27

On day 7 the cells were divided into two groups. In one group, the medium was replaced with 2 ml of growth medium containing 5 μl of ethanol and the indicated amount of either SC-31769 or LDL, or both. After incubation for 15 hr at 37°, the cells were harvested for measurement of their content of free and esterified cholesterol. Each value is the average of duplicate samples (three dishes per sample). In the second group of cells, the medium was replaced with 2 ml of growth medium containing 5% lipoprotein-deficient serum, 5 μl of ethanol, 25 μg of ^{125}I-LDL per ml (39,000 cpm/μg), and either no SC-31769 or 5 μg of SC-31769 per ml. After incubation for 15 hr at 37°, the amount of ^{125}I-labeled trichloroacetic acid-soluble (non-iodide) material formed by the cells and released into the medium was measured as described in *Methods*. Each value is the average of duplicate incubations.

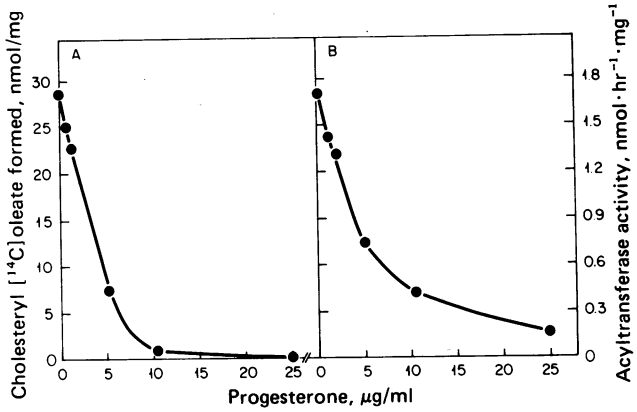

FIG. 5. Inhibition of LDL-mediated stimulation of cellular cholesteryl ester formation in fibroblast monolayers (*A*) and inhibition of acyltransferase activity in fibroblast extracts (*B*) by progesterone. (*A*) On day 7 each dish received 2 ml of growth medium containing 5% lipoprotein-deficient serum, 0.1 mM [^{14}C]oleate (10,000 cpm/nmol), 25 μg of LDL protein per ml, 5 μl of ethanol, and the indicated amount of progesterone. After incubation for 18 hr at 37°, the cellular content of cholesteryl [^{14}C]oleate was determined. Each value represents a single incubation except for the control value, which is the mean of triplicate incubations. (*B*) Extracts of fibroblasts (80 μg of protein) were incubated for 1 hr at 37° with 80 μM [^{14}C]oleoyl CoA (28,000 cpm/nmol) in the presence of the indicated amount of progesterone. Each value is the average of duplicate assays.

concentrations in the range of 10 μg/ml, progesterone caused a marked inhibition of LDL-mediated cholesteryl ester formation in intact cells and of acyltransferase activity in cell-free extracts. In striking contrast to SC-31769, progesterone at concentrations up to 25 μg/ml caused no suppression of HMG CoA reductase activity in intact cells (Fig. 6).

The action of progesterone in inhibiting the LDL-mediated increase in cholesteryl [^{14}C]oleate formation was reversed within 4 hr after progesterone was removed from the culture medium. Like SC-31769, progesterone inhibited the cellular accumulation of cholesteryl esters that occurred in fibroblasts incubated with LDL. Moreover, progesterone did not inhibit the incorporation of [^{14}C]oleate into cellular [^{14}C]triglycerides (data not shown).

Table 3 shows the results of an experiment in which we compared various steroids for their ability to stimulate the rate

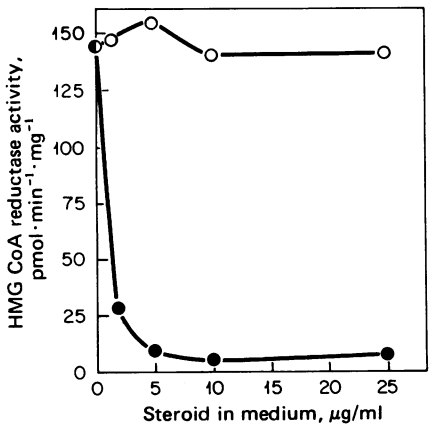

FIG. 6. Effect of progesterone (○) and SC-31769 (●) on HMG CoA reductase activity in fibroblast monolayers. On day 7 the medium was replaced with 2 ml of growth medium containing 5% lipoprotein-deficient serum, 10 μl of ethanol, and the indicated amount of either progesterone (○) or SC-31769 (●). After incubation for 17 hr at 37°, cells were harvested for measurement of HMG CoA reductase activity. Each value represents a single incubation except for the control value (◐), which is the mean of triplicate incubations.

Table 3. Effect of steroids on cholesteryl [^{14}C]oleate formation in fibroblasts incubated in the absence and presence of LDL

Steroid added	Cholesteryl [^{14}C]oleate formed, pmol·hr^{-1}·mg protein^{-1}	
	−LDL	+LDL
None	21 (1.0)†	1915 (1.0)†
Progesterone	22 (1.0)	192 (0.10)
20-α-Dihydroprogesterone	27 (1.2)	956 (0.50)
SC-31448*	30 (1.4)	235 (0.12)
Corticosterone	33 (1.6)	1787 (0.93)
SC-31769*	34 (1.6)	322 (0.17)
Cortisol	36 (1.7)	2225 (1.16)
Testosterone	37 (1.8)	1800 (0.93)
Estradiol	46 (2.2)	1945 (1.02)
SC-31082*	55 (2.6)	291 (0.15)
7-Ketocholesterol	212 (10)	1655 (0.86)
25-Hydroxycholesterol	856 (41)	2255 (1.18)

On day 7 the medium was replaced with 2 ml of growth medium containing 5% lipoprotein-deficient serum, 5 μl of ethanol and 10 μg of the indicated steroid per ml in the absence or presence of 25 μg of LDL protein per ml. After incubation for 20 hr at 37°, each monolayer was pulse-labeled for 2 hr at 37° with 0.1 mM [^{14}C]oleate–albumin (11 cpm/pmol), after which the cells were harvested for measurement of their content of cholesteryl [^{14}C]oleate. Each value is the average of duplicate incubations.

* SC-31448, 22-hydroxy-25-methylcholesterol; SC-31769, 7-keto-20-oxacholesterol; SC-31082, 23,25-dihydroxy-23-methyl-21-norcholesterol.

† The numbers in parentheses show the relative rate of cholesteryl ester formation as compared to the control cells to which no steroid was added.

of cholesteryl ester formation in the absence of LDL and to inhibit the rate of cholesteryl ester formation in the presence of LDL. 7-Ketocholesterol and 25-hydroxycholesterol markedly stimulated cholesteryl ester formation in the absence of LDL and did not significantly affect this process in the presence of LDL. Four steroids, including corticosterone, cortisol, testosterone, and estradiol, had no effect on cholesteryl ester formation either in the presence or absence of LDL. Four of the steroids—progesterone, SC-31082 (23,25-dihydroxy-23-methyl-21-norcholesterol), SC-31448 (22-hydroxy-25-methylcholesterol), and SC-31769 (7-keto-20-oxacholesterol)—were potent inhibitors of cholesteryl ester formation in the presence of LDL. Like SC-31769 and progesterone, SC-31082 and SC-31448 inhibited acyltransferase activity in cell-free extracts. When compared in the same experiment at a steroid concentration of 1 μg/ml, acyltransferase activity was inhibited 56, 74, and 75% by SC-31082, SC-31448, and SC-31769, respectively.

DISCUSSION

Steroids that influence cholesterol metabolism in cultured human fibroblasts can now be divided into three classes. (*i*) *Steroids that suppress HMG CoA reductase and raise the activity of the acyltransferase.* This class includes cholesterol, 25-hydroxycholesterol, 7-ketocholesterol, and 6-ketocholestanol. (*ii*) *Steroids that suppress HMG CoA reductase and directly inhibit the acyltransferase.* This class includes SC-31769, SC-31448, and SC-31082. (*iii*) *Steroids that do not suppress HMG CoA reductase but directly inhibit the acyltransferase.* This class includes progesterone and 20-α-dihydroprogesterone. (See Table 3, refs. 4–7, and unpublished data.)

The inhibition of the acyltransferase by SC-31769 and progesterone led to a block in the incorporation of [^{14}C]oleate into cholesteryl [^{14}C]esters by intact fibroblasts and inhibited the

increase in cholesteryl ester content of fibroblasts incubated with LDL. These observations provide further evidence that the LDL-mediated accumulation of cholesteryl esters in human fibroblasts requires the action of the acyltransferase (3).

SC-31769, which inhibits the acyltransferase, differs structurally from 7-ketocholesterol, which increases acyltransferase activity in intact cells, in two respects: (*i*) it lacks the methyl group at position 21 and (*ii*) it contains an oxygen in place of a carbon atom at position 20 (Fig. 1). Inasmuch as 22-hydroxy-25-methylcholesterol, which contains a methyl group at position 21, behaves similarly to SC-31769, the absence of a methyl group at position 21 may not be critical for inhibitory activity.

The mechanism by which certain steroids either enhance or reduce the activity of the acyltransferase remains to be elucidated. Those steroids that activate the acyltransferase do so only when added to intact cells; they have no effect on the enzyme when added to cell-free extracts. Inasmuch as this activation is not inhibited by cycloheximide (3), it would appear to involve a true activation of preformed enzyme molecules and not the induction of new enzyme synthesis. In contrast to the steroid activators of the acyltransferase, the steroids that inhibit the acyltransferase can be shown to have a direct effect on the enzyme in cell-free extracts.

Considered together, the observations on the activators and inhibitors of the acyltransferase suggest that this enzyme activity may be under the influence of one or more regulatory sites that can interact with certain steroid molecules. Determination of whether such a regulatory site is a part of the enzyme itself or whether it involves some other microsomal component will require solubilization and purification of the acyltransferase. The knowledge that the acyltransferase is susceptible to both negative and positive regulation suggests that it may be possible to reduce the pathologic accumulation of cholesteryl esters in human cells by administration of agents that modulate the activity of this enzyme.

We thank Gloria Y. Brunschede and Lynda Letzig for excellent technical assistance. Carol Mansen and Jean Helgeson provided excellent help with the cell culture. This work was supported by Grant PO-1-HL-20948 from the National Institutes of Health. M.S.B. is an Established Investigator of the American Heart Association.

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