Expression of low density lipoprotein receptor in cultured human granulosa cells: regulation by human chorionic gonadotropin, cyclic AMP, and sterol

Thaddeus G. Golos, Allison M. August, and Jerome F. Strauss III

Departments of Obstetrics and Gynecology and Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104

Abstract Steroidogenic cells utilize lipoprotein-delivered cholesterol as a primary substrate for hormone synthesis. We studied low density lipoprotein (LDL) receptors in cultured human granulosa cells to determine what factors regulate receptor expression. Granulosa cells cultured under serum-free conditions were treated with human chorionic gonadotropin (hCG) for 1.5 to 14 hr. The LDL receptor content of cells increased by approximately twofold within 6 hr of hCG treatment, and the content continued to increase for at least 14 hr, as determined by immunoblotting. The rate of LDL receptor synthesis was also demonstrated to increase within 2.5 to 3.5 hr of hCG treatment by immunoisolation of LDL receptor from cells metabolically labeled with a pulse of [^{35}S]methionine. The cyclic AMP analogue, 8-bromo-cAMP, was also found to increase LDL receptor synthesis. This increased rate of synthesis was shown to be dependent on ongoing RNA synthesis, since actinomycin D abolished hCG- or 8-bromo-cAMP-stimulated LDL receptor synthesis. We also demonstrated that hCG- and 8-bromo-cAMP-mediated regulation of LDL receptor synthesis in granulosa cells supersedes the classical cholesterol-mediated regulation of the receptor described in fibroblasts. Although 25-hydroxycholesterol induced a decrease in LDL receptor content and synthesis within 6 hr, this action was overridden by simultaneous exposure to hCG. Our findings demonstrate the existence of a novel cAMP-mediated mechanism for regulation of LDL receptor synthesis in steroidogenic cells.—**Golos, T. G., A. M. August, and J. F. Strauss III.** Expression of low density lipoprotein receptor in cultured human granulosa cells: regulation by human chorionic gonadotropin, cyclic AMP, and sterol. *J. Lipid Res.* 1986. **27:** 1089–1096.

Supplementary key words LDL receptor • granulosa cell • chorionic gonadotropin • cyclic AMP

It is well established that cells acquire cholesterol for membrane and organelle assembly by uptake of lipoproteins (1, 2). Lipoprotein-carried cholesterol is also a major precursor of steroid hormones in the adrenals, placenta, and ovaries of many species including man (3, 4). Thus, the availability of lipoprotein cholesterol is a potentially important point of the regulation of steroidogenesis, and lipoprotein uptake has, in fact, been demonstrated to be under tropic hormone regulation in both the adrenals and the ovaries (3, 4).

We have previously demonstrated that the uptake of LDL by cultured human granulosa cells is subject to regulation by gonadotropins. The uptake and metabolism of ^{125}I-labeled LDL by these cells, as well as their ability to metabolize LDL-delivered [^{3}H]cholesteryl ester to secreted progesterone is increased by human chorionic gonadotropin (hCG) or 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP) (5, 6). These observations have been attributed, at least in part, to increased synthesis of the LDL receptor, as determined by metabolic labeling of cells with [^{35}S]methionine and immunoisolation of newly synthesized receptor (7). In the present report, we extend our previous observations to demonstrate the cellular dynamics of regulation of receptor synthesis and receptor content by hCG, 8-bromo-cAMP, and cholesterol, and provide insight into the roles of receptor degradation and RNA synthesis in hCG and 8-bromo-cAMP-regulated LDL receptor expression in cultured human granulosa cells.

MATERIALS AND METHODS

Culture and treatment of human granulosa cells

Granulosa cells were aspirated from preovulatory follicles of women undergoing ovum retrieval for in vitro fer-

Abbreviations: LDL, low density lipoprotein; hCG, human chorionic gonadotropin; 8-bromo-cAMP, 8-bromoadenosine 3',5'-cyclic monophosphate; SDS, sodium dodecyl sulfate; 25-OHC, 25-hydroxycholesterol; AG, aminoglutethimide; ACTH, adrenocorticotropic hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

tilization and embryo transfer and were cultured as previously described (7). Briefly, granulosa cells in follicular aspirates were separated from red blood cells by centrifugation through Lymphocyte Separation Medium (Litton Bionetics, Kensington, MD), plated in 35-mm culture dishes in Dulbecco's Minimum Essential Medium containing 25 mM glucose, 4 mM L-glutamine, 50 μg/ml gentamicin, 25 mM HEPES, and 20% (vol/vol) human male serum, and cultured at 37°C in an atmosphere of humidified air. After an initial period of 48 hr, serum-supplemented medium was replaced by serum-free medium for an additional 48-hr period. Subsequently, this medium was replaced with medium containing the treatments described in the Results Section.

After the prescribed treatment interval for a given experiment, cells were either harvested with a plastic spatula and solubilized for immunoblot analysis of LDL receptors as described below, or they were metabolically labeled with [^{35}S]methionine and LDL receptor was immunoisolated as previously described (7).

Immunoisolation of the [^{35}S]labeled LDL receptor

The procedure employed for immunoisolation of the LDL receptor is basically adapted from previous work (7). Briefly, cells labeled with [^{35}S]methionine were scraped from dishes, pelleted, and washed at 4°C after two cycles of freeze-thawing in 150 μl of a buffer previously described for solubilization of the LDL receptor (8).

All procedures were conducted at 4°C unless otherwise noted. Solubilizing buffer containing bovine serum albumin and Nonidet P-40 was added to each sample to bring the concentrations of these compounds to 5 mg/ml bovine serum albumin and 0.5% Nonidet P-40 to reduce nonspecific association (9). Equal amounts of trichloroacetic acid-precipitable radioactivity from each sample were subjected to a preclearing procedure (9) to reduce nonspecific association of radiolabeled proteins with the specific LDL receptor antibody IgG-C7 (10). Fifty μg of IgG-C7 in 25 μl was then added to each sample. Following a 15-hr incubation, 100 μg of Pansorbin (Calbiochem-Behring, La Jolla, CA) was added to each tube and incubations were continued for 2.5 hr. Preliminary studies determined that the LDL receptor was quantitatively immunoisolated from granulosa cell extracts using these procedures. The suspension was then centrifuged at 8000 *g* for 10 sec, and the supernatant was discarded. The pellet was then washed four times with phosphate-buffered saline containing 1.0 mM EDTA and 0.25% Nonidet P-40 and resuspended in 25 μl of final sample buffer (0.125 M Tris, 10% glycerol (vol/vol), 2% SDS (wt/vol), and 5% 2-mercaptoethanol (vol/vol)) and heated at 90°C for 10 min before SDS-polyacrylamide gel electrophoresis.

SDS-polyacrylamide gel electrophoresis of immunoisolated LDL receptor

Immunoisolated LDL receptor from ^{35}S-labeled cultured cells was subjected to electrophoresis on 12 × 17 × 0.15 cm 7.5% polyacrylamide gels at 10 mA for 16 hr at room temperature. Gels were calibrated with the following ^{14}C-labeled standards: carbonic anhydrase (29 kDa), ovalbumin (45 Kda), bovine serum albumin (66 kDa), phosphorylase B (97.4 kDa), β-galactosidase (116 kDa), and myosin (200 kDa). Following electrophoresis, gels were fixed for 1 hr, impregnated with 1.0 M sodium salicylate–1.0% glycerol for 45 min, dried, and stored in contact with Kodak-X-Omat X-ray film at −70°C for 8 hr–2 weeks. Densitometric data was obtained by scanning fluorograms with a Kontes densitometer (Kontes, Vineland, NJ).

Immunoblot analysis of the LDL receptor

Following harvesting of granulosa cells, cells were solubilized by two freeze–thaw cycles in 75 μl of a buffer previously described (11). Samples containing equal amounts of protein (50 to 200 μg) were subjected to electrophoresis as for ^{35}S-labeled samples, except that samples were not heated nor was reducing agent included in the loading buffer (10% (vol/vol) glycerol, 2% SDS, 0.005% bromophenol blue) since these procedures decrease the LDL receptor immunoreactivity (12). Following electrophoresis, proteins were immediately transferred from SDS gels onto nitrocellulose paper using a Hoefer Transphor apparatus (Hoefer Scientific Instruments, San Francisco, CA) and the buffer system of Towbin, Staehelin, and Gordon (12) (25 mM Tris, 192 mM glycine, 20% (v/v) methanol, pH 8.3). Following transfer, nitrocellulose sheets were first incubated for 2 hr at 37°C with blocking buffer (10 mM Tris-HCl at pH 7.4, 0.15 M NaCl, 5% (w/v) nonfat dry milk, and 0.2% (v/v) Nonidet P-40), followed by incubation for 2 hr at room temperature with 5 μg/ml IgG-C7 (10) in the same buffer. The paper was then washed briefly with 50 ml of a buffer consisting of 10 mM Tris-HCl and 0.15 M NaCl at pH 7.4, and then for two 15-min washes with this buffer containing 0.1% SDS, 0.2% Nonidet P-40, and 0.25% sodium deoxycholate. After another brief wash, filters were incubated again in the blocking buffer containing ^{125}I-labeled goat anti-mouse IgG at a concentration of 1–2 μg/ml (approximately 1×10^6 cpm/ml) in a volume of 10 ml for 2 hr. Nitrocellulose filters were washed again as before, air dried on filter paper, wrapped in plastic film, and placed in contact with X-ray film for autoradiography. The LDL receptor was not detected when immunoblotting was performed with a monoclonal IgG to a mouse testes antigen.

[^{35}S]Methionine and [^{3}H]leucine labeling of cultured granulosa cells

In some experiments, cells were labeled with both [^{3}H]leucine and [^{35}S]methionine to examine the possibility that hCG treatment alters the degradation rate of the LDL receptor. Following a 48-hr period of serum-free culture, cells were pulsed with 100 μCi of [^{3}H]leucine/ml for 1 hr. Radioactive medium was removed, and cells received either serum-free medium, or serum-free medium containing 500 mIU of hCG/ml for 4 hr. Cells were then pulsed with 100 μCi of [^{35}S]methionine/ml for 1 hr, exposed to medium containing only unlabeled methionine for an additional hour, and were harvested and solubilized as described earlier. Aliquots of both control and treated cell extracts containing equal amounts of trichloroacetic acid-precipitable ^{35}S radioactivity were immunoprecipitated with IgG-C7 or with an irrelevant monoclonal antibody of the same IgG-2_b subclass. The immunoprecipitated receptor pellet was resuspended in 3 ml of scintillation fluid (Biofluor, New England Nuclear, Boston, MA) and ^{3}H and ^{35}S radioactivity was determined for each sample. The values for nonspecific antibody precipitations were subtracted from those obtained with IgG-C7 to yield the amount of radioactivity incorporated into the LDL receptor.

Reagents and antibodies

Electrophoresis materials were obtained from Bio-Rad (Richmond, CA). hCG (CR-121) was provided by the NIADDK (Bethesda, MD). Dulbecco's Modified Eagle's Medium was purchased from Grand Island Biological Co. (Grand Island, NY). Nitrocellulose (BA 85, 0.45 μm pore size) was obtained from Schleicher and Schuell (Keene, NH). Aminoglutethimide (Cytadren) was obtained from Ciba-Geigy Corp. (Summit, NJ). 25-Hydroxycholesterol (5-cholesten-3β, 25-diol) was purchased from Steraloids (Wilton, NH). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). The monoclonal antibody to the LDL receptor (IgG-C7) was a generous gift from Drs. M. Brown, J. Goldstein, W. Schneider, and Y. K. Ho (University of Texas Health Science Center, Dallas, TX). Rabbit anti-mouse gamma globulin was purchased from Cappel Laboratories (Cochransville, PA) and was iodinated by the procedure of Greenwood and Hunter (13) to a specific activity of 500–1000 cpm/ng. Na ^{125}I was purchased from Amersham (Arlington Heights, IL), [^{35}S]methionine was obtained from New England Nuclear (Boston, MA), and [^{3}H]leucine was purchased from ICN Radiochemicals (Irvine, CA).

Statistical analysis

Where appropriate, data were analyzed by analysis of variance followed by Duncan's multiple-range test (14).

RESULTS

Immunoisolation and immunoblotting of the human granulosa cell LDL receptor

We previously demonstrated the specificity of the LDL receptor immunoisolation procedures employed in this report (7). As visualized in autoradiograms from 7.5% SDS-polyacrylamide gels, in the presence of β-mercaptoethanol, the LDL receptor migrates with an apparent molecular weight of approximately 160,000 (**Fig. 1**, lane 1). The LDL receptor precursor has an apparent molecular weight of approximately 120,000 (7, 8). The difference in mass between the precursor and the mature form of the receptor is attributable to glycosylation of the precursor (8, 15). In the studies presented below, only the mature form of the receptor was quantitated since labeling of the precursor form paralleled that of the mature form.

In the present study we also identified the granulosa cell LDL receptor by immunoblotting of electrophoretically separated proteins transferred to nitrocellulose. The immunoblotted receptor was revealed as a single band migrating with an apparent molecular weight of approximately 130,000 (Fig. 1, lane 2) as compared with unreduced standards run on the same gel (16). The apparent difference in mobility of the receptor under reducing and nonreducing conditions is thought to be due to changes in the three-dimensional configuration of the receptor upon reduction of numerous disulfide bands in the extracellular ligand-binding domain (17).

Effect of hCG treatment on LDL receptor levels

We had previously demonstrated that hCG increases the ability of cultured granulosa cells to bind and metabolize ^{125}I-labeled LDL (5). We hypothesized that this increase was due, at least in part, to an increased cellular LDL receptor content. This hypothesis was directly tested by immunoblot analysis of the LDL receptor content of cultured human granulosa cells employing a monoclonal antibody to the LDL receptor. We also wished to gain insight into the potential regulation of LDL receptor content by cholesterol. LDL receptor synthesis in nonsteroidogenic cells is regulated in a negative feedback fashion by cholesterol (1). Since hCG stimulates steroidogenesis, it could influence LDL receptor synthesis by depleting cellular sterol stores for use in steroid hormone synthesis. Therefore, granulosa cells were treated with hCG, 25-hydroxycholesterol (25-OHC) plus aminoglutethimide (AG), or a combination of hCG and 25-OHC plus AG. The soluble cholesterol derivative exerts negative feedback on LDL receptor synthesis, while the AG will prevent metabolism of cellular cholesterol and 25-OHC to progesterone by the cholesterol side-chain cleavage enzyme system. Since the only quantitatively significant metabolism of 25-OHC is via side-chain cleavage, the combination of 25-OHC plus AG

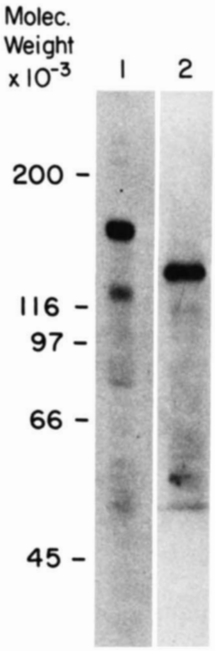

Fig. 1. Immunoisolation (lane 1) and immunoblotting (lane 2) of the LDL receptor in cultured human granulosa cells. Lane 1: Following 2 days of serum-free culture, granulosa cells were treated with hCG (500 mIU/ml) for 6 hr and preincubated with methionine-free medium for 1 hr prior to exposure to 100 μCi/ml [^{35}S]methionine for 2 hr. Cells were chilled to 4°C, scraped from dishes, solubilized, and subjected to immunoisolation with IgG-C7, the monoclonal antibody to the LDL receptor, as described in the text. The immunoisolate was then subjected to electrophoresis in 7.5% SDS-polyacrylamide gels, and the fluorogram was made of the dried gel. Molecular weight markers are indicated. Lane 2: Following 2 days of serum-free culture, granulosa cells were treated with hCG (500 mIU/ml for 6 hr and then chilled, scraped from dishes, and solubilized in a buffer lacking beta-mercaptoethanol. Extract containing 200 μg of granulosa cell protein was electrophoresed in a 7.5% SDS-polyacrylamide gel, and proteins were electrophoretically transferred to nitrocellulose which was then immunoblotted for the LDL receptor as described in the text. The fluorogram was prepared from the dried nitrocellulose paper.

should present high cellular sterol levels which exert a potent negative feedback signal to LDL receptor synthesis.

Fig. 2 presents results from a representative experiment in which immunoblots were performed on samples containing equal amounts of protein obtained from granulosa cells treated for 6 hr with hCG, 25-OHC plus AG, or hCG and 25-OHC plus AG. The LDL receptor bands appear above the histogram bars. In this experiment cellular LDL receptor content was reduced by 80% by treatment with 25-OHC plus AG, and increased 2-fold by hCG; hCG increased receptor levels by 1.4-fold in the presence of 25-OHC and AG, an attenuated response compared to that of hCG alone. **Table 1** presents data from four experiments conducted in exactly the same way. Within 6 hr of treatment, LDL receptor content of the cultured granulosa cells was increased an average of 3-fold by hCG and reduced to less than 50% by 25-OHC + AG. Additionally, hCG augmented LDL receptor levels in the presence of 25-OHC + AG by 2.3-fold, indicating that at least part of the effect of hCG was via a mechanism not dependent on hCG-induced depletion of cellular cholesterol stores. The attenuation of the response in cells treated with hCG, 25-OHC + AG as compared to hCG alone presumably reflects some contribution of cholesterol negative feedback to the control of LDL receptor expression in these cells.

Fig. 3 presents the results of three experiments in which the time course of the effect of hCG on LDL receptor levels was examined by immunoblotting. Within 6 hr of exposure to hCG, there was an appreciable increase in LDL receptor as noted previously, and receptor content was further augmented after hCG treatment for 14 to 24 hr.

Mechanisms of regulation of LDL receptor expression by hCG

To determine whether the increase in LDL receptor content of stimulated granulosa cells was due to an increase in the rate of receptor synthesis or to a dramatic decrease in the rate of receptor degradation, cells were pulse-labeled for 1 hr with [^{3}H]leucine, exposed to hCG for 4 hr, then exposed to [^{35}S]methionine for 1 hr, scraped from culture dishes and solubilized, and the LDL receptor was immunoisolated. Immunoisolates were then ana-

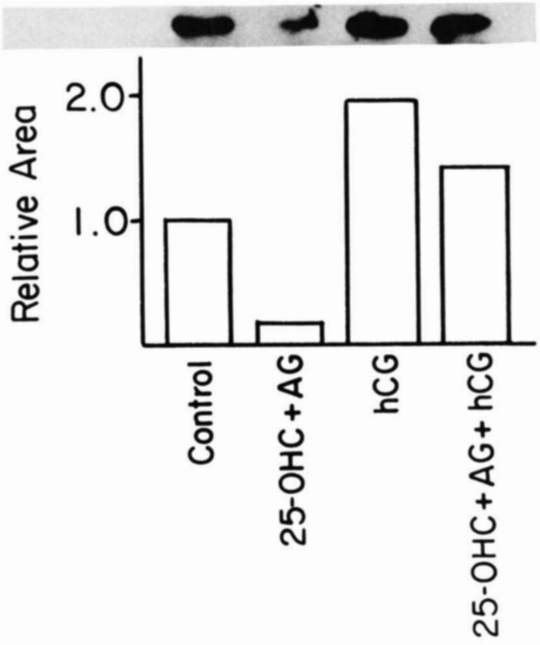

Fig. 2. Effect of hCG (500 mIU/ml) and 25-hydroxycholesterol (25-OHC) (10 μg/ml) plus aminoglutethimide (AG) (100 μg/ml) on LDL receptor content of cultured luteinized human granulosa cells. Cells were treated for 6 hr, after which cells were solubilized and aliquots of extract containing equal amounts of protein were subjected to SDS-polyacrylamide gel electrophoresis, electrotransfer, and immunoblotting as described in the text. The results presented in the histogram are the relative areas derived from densitometric scans of the autoradiogram from a representative experiment, with the area for the LDL receptor band in control cultures set to 1.0.

TABLE 1. Regulation of human granulosa cell LDL receptor content

	Control	Treatment		
		hCG	25-OHC + AG	hCG + 25-OHC + AG
Relative area	1.0[a]	3.04 ± 0.85[b]	0.43 ± 0.13[c]	2.34 ± 0.26[b]
Number of experiments	4	4	3	4

Data presented are the means ± SEM from the densitometric tracings of the immunoblotted LDL receptor from the indicated number of experiments conducted as described for Fig. 1. Values with different superscripts are significantly different ($P < 0.05$) as assessed by analysis of variance of the densitometric data. The abbreviations are: AG, aminoglutethimide; 25-OHC, 25-hydroxycholesterol.

lyzed by double-label liquid scintillation counting. The ^{35}S to ^{3}H ratio in immunoisolates was increased 2.9 ± 0.3-fold (n = 3) in hCG-treated cells over that in control cells, indicating increased synthesis of LDL receptor after hCG treatment, rather than a decrease in LDL receptor degradation rate, which would have decreased the ratio due to a conservation of the previously formed ^{3}H-labeled LDL receptor. SDS-polyacrylamide gel electrophoresis of aliquots of these immunoisolates confirmed that the radioactivity was indeed associated with the LDL receptor. These results strongly suggest that changes in LDL receptor degradation following hormone treatment are unlikely to be of major significance in the hCG-mediated increase in LDL receptor expression.

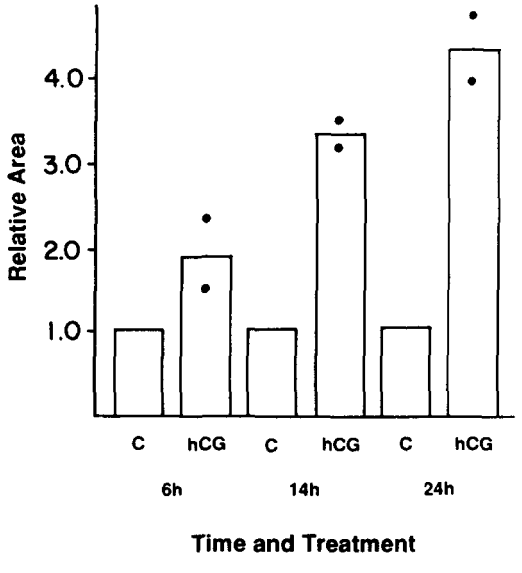

Fig. 3. Time course of the effect of hCG on LDL receptor content of cultured luteinized human granulosa cells. Cells received control or hCG (500 mIU/ml)-containing medium for the indicated period of time, and were then treated and analyzed by protein immunoblotting of the LDL receptor as described in Fig. 2. Results presented are the means of two experiments. Closed circles indicate the individual results.

Effect of hCG concentration on LDL receptor synthesis

Having verified that the LDL receptor content of human granulosa cells is increased by hCG, we sought to further investigate LDL receptor expression by examining the synthesis of the LDL receptor in cells metabolically labeled with [^{35}S]methionine. Cultured granulosa cells were incubated with 0–500 mIU of hCG/ml of culture medium, exposed to [^{35}S]methionine, and ^{35}S-labeled LDL receptor was immunoisolated and quantitated by densitometric scanning of fluorograms prepared from SDS-polyacrylamide gels. The results of a representative experiment, shown in **Fig. 4**, indicate that LDL receptor synthesis is maximally stimulated by doses of 100–500 mIU hCG/ml, which are physiological levels (17). In this 6-hr exposure to hCG as well as in subsequent experiments with 8-bromo-cAMP, 25-OHC, and AG, the specific activities of total cellular proteins differed by less than 10% from that of the control (untreated) cells.

Time course of LDL receptor synthesis in response to hCG

We next sought to examine the time course of LDL receptor synthesis in response to hCG. Cells were labeled with [^{35}S]methionine for 1 hr following 1.5, 2.5, 3.5, 5, and 7 hr of exposure to hCG or control medium (**Fig. 5A**). Results are presented as area of the densitometric tracing of the LDL receptor band in hCG-treated cells relative to the area of control cell tracings, which were set equal to 1.0. The mean rate of receptor synthesis was increased by 45% within 2.5 hr (n = 3), and an apparently maximal stimulation of LDL receptor synthesis of approximately 3-fold was attained within 5 hr of exposure to hCG. In several other experiments, it was found that this elevated rate of LDL receptor synthesis was maintained for at least 14 hr.

Effect of cholesterol on LDL receptor synthesis

The immunoblot analyses presented in Fig. 2 and Table 1 demonstrated that treatment with 25-OHC and AG can

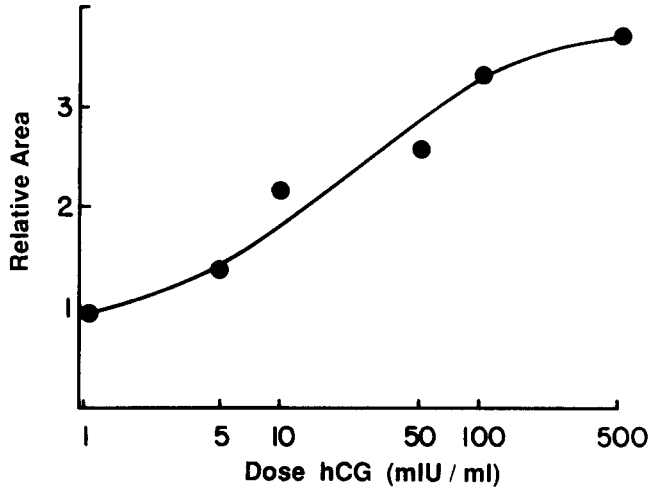

Fig. 4. LDL receptor synthesis by cultured luteinized human granulosa cells in response to increasing doses of hCG. Granulosa cells were exposed to [^{35}S]methionine-containing medium (100 μCi/ml for 2 hr) following 4 hr of exposure to the indicated dose of hCG. Equal amounts of trichloroacetic acid-precipitable radioactivity were subjected to immunoisolation of LDL receptor. Data presented represent the relative areas derived from densitometric scans of the immunoisolated LDL receptor bands on the autoradiogram from a representative experiment, with the area for the control LDL receptor band assigned a value of 1.0.

decrease LDL receptor content of granulosa cells within 6 hr. It was of interest to determine the time course of cholesterol down-regulation of LDL receptor synthesis in these cells and to compare the kinetics of that response with the cellular response to hCG. Therefore, cultured human granulosa cells were pulse-labeled with [^{35}S]methionine 1.5 to 7 hr after exposure to 25-OHC and AG or to a combination of hCG and 25-OHC plus AG.

Fig. 5B demonstrates that 25-OHC/AG treatment suppresses LDL receptor synthesis by approximately 40% within 5 hr of exposure to these agents; hCG, on the other hand, increases LDL receptor synthesis by nearly 50% within 2.5–3.5 hr despite the presence of 25-OHC and AG, indicating that hCG rapidly and specifically stimulates LDL receptor expression by a mechanism not dependent, and indeed, apparently superseding regulation by cellular cholesterol balance. Maximal LDL receptor synthesis rates are achieved within 5–7 hr after hCG treatment, regardless of the presence of 25-OHC and AG.

To further explore the effect of altered cholesterol metabolism on LDL receptor synthesis, we examined LDL receptor synthesis following treatment of cells with compactin, an inhibitor of HMG-CoA reductase (18). Under the serum-free culture conditions employed, de novo synthesis is the only means by which cells can acquire cholesterol. Compactin treatment might thus be expected to enhance LDL receptor synthesis by removing the only source of cellular cholesterol. Six hours of treatment with compactin (25 μM), previously shown to reduce incorporation of [^{14}C]acetate into cellular sterol by greater than 90% (19), did not significantly alter LDL receptor synthesis, but compactin potentiated the stimulatory effect of hCG on LDL receptor synthesis. While hCG alone increased LDL receptor synthesis 2.4-fold, the combination of hCG and compactin resulted in a 3.7-fold increase. These results are consistent with some modulation of LDL receptor synthesis in cultured human granulosa cells by negative feedback due to cholesterol.

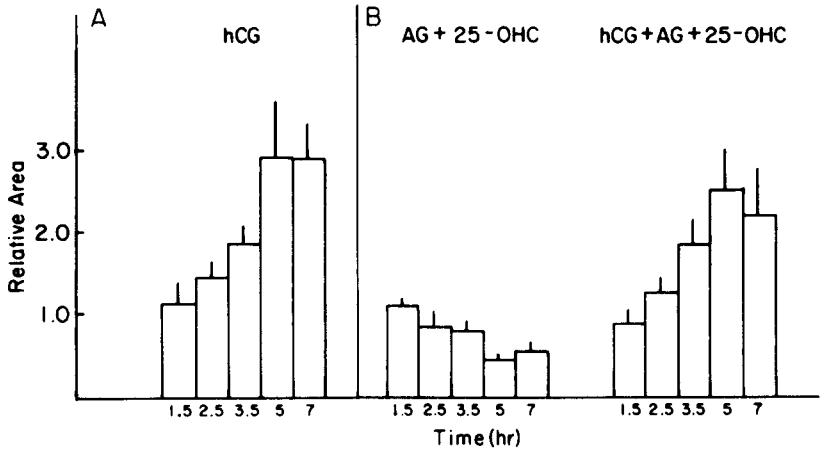

Fig. 5. Panel A: Time course of LDL receptor synthesis in response to hCG treatment. Cells were treated in three experiments with 500 mIU/ml hCG for the indicated period of time, which included 1 hr of exposure to [^{35}S]methionine. The LDL receptor was immunoisolated from samples as in Fig. 4. Values presented are means ± SEM. Panel B: Effect of 25-hydroxycholesterol (25-OHC, 10 μg/ml) and aminoglutethimide (AG, 100 μg/ml), or 25-OHC and AG plus hCG on LDL receptor synthesis. Experiments were conducted, analyzed, and presented as in Panel A.

Mechanism of hCG and 8-bromo-cAMP-induced LDL receptor synthesis

There are several mechanisms that could account for increased synthesis of LDL receptor following hCG or 8-bromo-cAMP treatment; one of these could be stimulation of transcription of the gene encoding the LDL receptor. To test this possibility indirectly, we examined whether active RNA synthesis was required for the stimulation of LDL receptor expression by hCG and 8-bromo-cAMP. **Fig. 6** illustrates the results of these experiments. Whereas LDL receptor synthesis was increased 2.8-fold by hCG (n = 3) or 3.2-fold by 8-bromo-cAMP (1.5 mM) (n = 1), the presence of actinomycin D (2 μg/ml) completely blocked the stimulatory effect of either of these agents, supporting the possibility that hCG and 8-bromo-cAMP stimulate LDL receptor synthesis, at least in part, at the level of mRNA synthesis.

DISCUSSION

The regulation of cholesterol metabolism in most cell types is primarily related to the needs of the cell for membrane and other organelle biogenesis. When sufficient exogenous cholesterol is available in the form of extracellular low density lipoproteins, lipoproteins are taken up by the cell through a receptor-mediated endocytotic pathway and the cholesterol is liberated for use by the cell (1, 2). The expression of the LDL receptor is regulated by cellular cholesterol balance so that the ability of the cells to bind and take up LDL is appropriate to cellular requirements.

Steroid-secreting cells are unique in that they require cholesterol both for the normal "housekeeping" functions as well as for metabolism to their steroidal products. Steroidogenic activity is regulated by tropic hormone, and the acquisition of cholesterol substrate is an important potential point of regulation of steroid synthesis. This possibility has been extensively addressed in the adrenal cortex and the ovary (4, 7). ACTH in the adrenal cortex and LH, hCG, or FSH in the ovary can enhance the ability of their respective target cells to acquire and metabolize lipoprotein-delivered cholesterol to steroid hormones. In the case of LDL, these cells utilize the classical LDL pathway as elucidated in human fibroblast (1, 5, 6), and the LDL pathway appears to be up-regulated by a cyclic AMP-dependent mechanism (5–7).

We have previously investigated the regulation of cholesterol acquisition in cultured human granulosa cells. hCG and 8-bromo-cAMP stimulate ^{125}I-labeled LDL binding and metabolism by these cells (6, 7) and stimulate the metabolism of the delivered cholesterol to progesterone (6). In the present study we have demonstrated that the increase in LDL metabolism is due, at least in part, to a gonadotropin-stimulated increase in the expression of the LDL receptor. In cultured granulosa cells, the cellular LDL receptor content as assessed by immunoblot analysis increases within 6 hr, an observation consistent with the results of previous studies in which the surface binding of ^{125}I-labeled LDL, which represents the biological activity of the LDL receptor, was quantitated (5). This increase in LDL receptor content follows a rapid (within 2.5 hr) increase in the apparent rate of synthesis of the LDL receptor following hCG treatment, an effect which appears to be maximal within 5 hr, and persists for at least 14 hr. The increase in the rate of synthesis and the cellular content of LDL receptor in cultured human granulosa cells following hCG treatment could be affected by a number of intracellular processes, and all the possible points at which hCG could influence LDL receptor expression have not yet been studied. However, the results presented in this manuscript demonstrate two important concepts. First, the increased cellular content of LDL receptor appears to be mainly, if not solely, due to increased receptor synthesis, since the ^{35}S/^{3}H ratio from the double-label experiments was very similar to the increase seen in receptor binding activity (5), in the receptor content (as demonstrated by LDL receptor immunoblotting), and in the synthetic rate (as determined by metabolic labeling with [^{35}S]methionine). Additionally, the experiments utilizing actinomycin D demonstrate that ongoing RNA synthesis is required for hCG or 8-bromo-cAMP to effect an increase in the rate of LDL receptor synthesis, suggesting that the major part of gonadotropic regulation of LDL receptor expression in cultured luteinized human

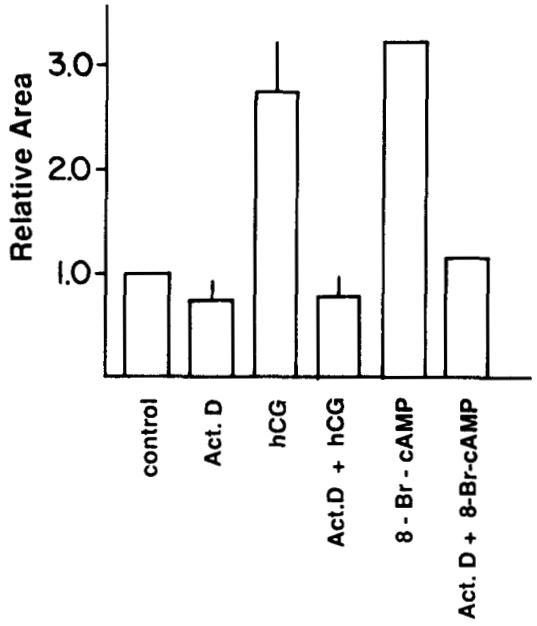

Fig. 6. Effect of actinomycin D (2 μg/ml) on hCG (500 mIU/ml) and 8-bromo-cAMP (1.5 mM)-stimulated LDL receptor synthesis. Cultured granulosa cells were exposed to the various treatments for 6 hr (which included a 2-hr labeling period with 100 μCi/ml [^{35}S]methionine), and LDL receptor synthesis was analyzed as described in Fig. 3. Results presented are the means ± SEM of three experiments conducted with hCG and one experiment conducted with 8-bromo-cAMP. The area of control LDL receptor band was assigned a value of 1.0.

granulosa cells is via increased transcription of the LDL receptor gene.

We have also provided several lines of evidence that support the concept that LDL receptor expression in human granulosa cells is regulated by two separated pathways: a stimulatory pathway, regulated by hCG, probably mediated via cAMP, and a suppressive pathway, mediated by cholesterol, as has been previously demonstrated for human fibroblast cells (1). First, the stimulatory effects of hCG on ^{125}I-labeled LDL binding and metabolism (5), cellular LDL receptor content, and LDL receptor synthesis rates are not dependent on alterations in cellular cholesterol content, and indeed, can be demonstrated in the face of a strong inhibitory cholesterol signal afforded by 25-OHC and AG treatment. Second, the enhancement of LDL receptor synthesis by compactin, which exerts its effect by suppressing intracellular cholesterol synthesis, is additive with the stimulatory effect of hCG, suggesting the the two agents act via separate mechanisms. Finally, the different time courses of hCG stimulation of LDL receptor synthesis and its suppression by 25-OHC + AG again indicate that separate mechanisms exist for gonadotropic stimulation and cholesterol inhibition of LDL receptor expression.

Two other points deserve mention. First, we noted a decrease in LDL receptor content by more than 50% following 6 hr of treatment with 25-OHC and AG. This was unexpected in view of the reported $t_{1/2}$ of 21–25 hr for the receptor in cultured fibroblasts (20). This may reflect a more rapid rate of receptor turnover in granulosa cells or an effect of sterol on receptor degradation. Also of interest is the observation that LDL receptor expression in fibroblasts, at least as manifested by ^{125}I-labeled LDL binding and metabolism, has been reported to be unaffected by cAMP treatment (20). Thus, the tropic hormone regulation of LDL receptor expression, which is independent of cellular sterol balance, may be unique to steroidogenic cells.

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