Oxysterol Regulation of Steroidogenic Acute Regulatory Protein Gene Expression

STRUCTURAL SPECIFICITY AND TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL ACTIONS*

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Oxysterols exert a major influence over cellular cholesterol homeostasis. We examined the effects of oxysterols on the expression of steroidogenic acute regulatory protein (StAR), which increases the delivery of cholesterol to steroid-metabolizing P450s in the mitochondria. \((22(R)-\text{hydroxycholesterol})\), \((22(R)-\text{OHC})\), \((25\text{-OHC})\), and \((27\text{-OHC})\) each increased steroidogenic factor-1 (SF-1)-mediated StAR gene transcription by 2-fold in CV-1 cells. In contrast, cholesterol, progesterone, and the \((27\text{-OHC})\) metabolites, \((27\text{-OHC}-5\text{a},3\text{b}-\text{one})\) and \((7\text{a},27\text{OHC})\), had no effect. Unlike our findings in CV-1 cells, SF-1-dependent StAR promoter activity was not augmented by \((27\text{-OHC})\) in COS-1 cells, Y-1 cells, BeWo choriocarcinoma cells, Chinese hamster ovary (CHO) cells, and human granulosa cells. Studies examining the metabolism of \((27\text{-OHC})\) indicated that CV-1 cells formed a single polar metabolite, \((3\text{b}-\text{OH}-5\text{-cholestenolic acid})\) from radiolabeled \((27\text{-OHC})\). However, this metabolite inhibited SF-1-dependent StAR promoter activity in CV-1, COS-1, and CHO cells. Because \((7\text{a},27\text{-OHC})\) was unable to increase SF-1-dependent StAR promoter activity, we examined \((27\text{-OHC})\) \((7\text{a}-\text{hydroxydihydrofarnesol})\) in COS-1 and CHO cells. COS-1 cells contained high \((7\text{a}-\text{hydroxydihydrofarnesol})\) activity, whereas the enzyme was undetectable in CHO cells. The hypothesis that oxysterols act in CV-1 cells to increase StAR promoter activity by reducing nuclear levels of sterol regulatory element binding protein was tested. This notion was refuted when it was discovered that sterol regulatory element binding protein-1a is a potent activator of the StAR promoter in CV-1, COS-1, and human granulosa cells. Human granulosa and theca cells, which express endogenous SF-1, contained more than 5-fold more StAR protein following addition of \((27\text{-OHC})\), whereas StAR mRNA levels remained unchanged. We conclude that 1) there are cell-specific effects of oxysterols on SF-1-dependent transactivation; 2) the ability to increase transactivation is limited to certain oxysterols; 3) there are cell-specific pathways of oxysterol metabolism; and 4) oxysterols elevate StAR protein levels through posttranscriptional actions.

Oxysterols regulate a variety of cellular processes involved in cholesterol metabolism (1). Certain oxysterols reduce nuclear levels of the key transcription factors controlling genes regulating cholesterol homeostasis by suppressing the proteolytic processing of sterol regulatory element binding proteins (SREBPs) (1, 2). They also activate cholesterol ester synthesis by allosterically modulating acyl-CoA cholesterol acyl transferase activity (3). Recently, oxysterols have been proposed to be activators of two members of the nuclear receptor family of transcription factors, LRX and steriodogenic factor-1 (SF-1), both of which were originally thought to be orphan receptors (4–6). SF-1 is a key transcription factor controlling the expression of steriodogenic enzymes (7). Thus, oxysterols regulate transcriptional as well as posttranscriptional events.

The steroidogenic acute regulatory protein (StAR) plays an essential role in steroid hormone synthesis by enhancing the delivery of cholesterol to the inner mitochondrial membrane, where the cholesterol side-chain cleavage enzyme (P450scC) system resides (8). This enzyme catalyzes the first committed step in steroid hormone synthesis. In addition to enhancing cholesterol delivery to P450scC, StAR also increases cholesterol metabolism by another mitochondrial cholesterol metabolizing enzyme, cytochrome P450c27, which generates the oxysterol, \((27\text{-hydroxycholesterol})\) \((27\text{-OHC})\) and \((3\text{b}-\text{hydroxy-5-cholestenolic acid})\) \((3\text{b}-\text{OH}-5\text{-CA})\) through further oxidation of the \((27\text{hydroxyl function})\) (9). SF-1 response elements in the StAR gene promoter are essential for basal as well as cAMP-stimulated gene transcription (10–12). Because of the pivotal role that StAR plays in controlling one arm of the cholesterol metabolic pathway, we wished to determine whether oxysterols influence StAR expression.

EXPERIMENTAL PROCEDURES

Plasmids—The cDNA for mouse SF-1, a generous gift from Dr. K. L. Parker (Southwestern Medical Center, Dallas, TX), was cloned into the pSV-SPORT-1 expression vector in the correct and reverse orientations using standard procedures. The pCMV-CSA and pCMV-CS2 expression plasmids that produce the cleaved transcriptionally active forms of SREBP-1a and SREBP-2 were previously described (13). The pGII2-basic vector (Promega) was the source of the luciferase reporter gene for the 1.3-kilobase human StAR promoter (11) and human LDL receptor promoter (13). The β-galactosidase expression vector (pCH110, Amer sham Pharmacia Biotech) was used for normalization of luciferase data. Plasmids for transfection were prepared using the Qiagen

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Maxiprep system.

Cell Culture and Transfection—BeWo choriocarcinoma cells, COS-1 cells, CHO cells, and Y-1 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum and 50 μg of gentamycin/ml in an atmosphere of 5% CO2, 95% air at 37°C for subculture and plating. CV-1 cells were propagated in the same basal medium plus 4 mM L-glutamine. BeWo (b30 clone) cells were a gift from Dr. Arnold Schwartz (Washington University, St. Louis, MO). The COS-1, CHO, and Y-1 cells were obtained from the American Type Culture Collection (Manassas, VA). Proliferating human granulosa and theca cells were prepared and cultured as described previously by McAdams et al. (14). All cell types except human granulosa and theca cells were plated at 120,000 or 60,000 cells/well for the 6- and 12-well plates, respectively, on day 0. On day 1, cells were washed twice in DMEM alone and then transfected with LipofectAMINE (Life Technologies Inc.) plus plasmid DNA. Cells were transfected with 500 ng each of the β-galactosidase expression and reporter vectors and 500 ng of the SF-1 expression plasmid or 250 ng each of the SREBP expression plasmids. After a 2-h exposure to the DNA/LipofectAMINE complex, an equal volume of DMEM + 20% delipidated fetal calf serum or DMEM + 2× Nutridoma-SP (Boehringer Mannheim) was added to the each well and left overnight. On day 2, medium was changed to either 10% delipidated fetal calf serum or 1× Nutridoma-SP, and compounds (treatments) were added. After 24 h, cells were harvested by scraping the cells in Reporter lysis buffer (Promega) followed by a single freeze/thaw cycle. Transfection of human granulosa cells utilized the calcium phosphate coprecipitation method as described by Kirikiadou et al. (15). Human granulosa cells were transfected with 10 μg of the STAR or LDL receptor reporter constructs, 5 μg of pCH110, and 1 μg of the SREBP-1a expression plasmid. Cells were then cultured in DMEM + 10% delipidated fetal calf serum plus compounds. Cell extracts were assayed for luciferase and β-galactosidase activity. Human granulosa and theca cells (60–80% confluent) for Western and Northern analysis were cultured in DMEM minus fetal calf serum for 2 days and then treated for 24 h with oxysterols/forskolin (20 μM) before harvesting.

Enzymatic Assays—Luciferase activity was determined in a LUMAT LB 9507 luminometer (EG & G Berthold) with Promega luciferin as substrate as described previously (9). β-Galactosidase activity was determined by the standard colorimetric assay using 2-nitrophenyl-β-D-galactopyranoside as substrate. Relative luciferase activity for each well was determined by dividing luciferase relative light units by the β-galactosidase activity (A420).

Northern Blot Analysis—To determine the effect of oxysterol treatment on a SF-1-dependent gene, STAR expression was determined in cultured human granulosa and theca cells. Briefly, total RNA was isolated from cells by the method of Chomczynski and Sacchi (16). The RNA was blotted, and the blots were probed with human STAR cDNA. The membranes were later reprobed with a labeled 2885 RNA probe (700 base pairs) in order to assess the loading of lanes. Blots were visualized on a Storm PhosphorImager, and relative levels of STAR RNA and 28S RNA were determined with the ImageQuant 1.11 software (Molecular Dynamics).

Western Blot Analysis—Protein extracts from human granulosa and theca cells were generated as described previously (17). Protein concentrations of the extracts were determined by the Bio-Rad dye binding assay. Equal amounts of protein (25 μg) were loaded onto SDS-polyacrylamide gel electrophoresis gels (12.5%) for electrophoresis. After electrophoresis, gels were transferred to polyvinylidene difluoride membranes for probing with anti-STAR antibody. The rabbit polyclonal antibody was produced against recombinant human STAR protein as described previously (18). Detection of the anti-STAR antibody used the ECL kit (Amersham Pharmacia Biotech).

Metabolism of 3H-labeled 27-OHC by CV-1 Cells—Nearly confluent CV-1 cells were cultured in DMEM supplemented with 1× Nutridoma-SP. CV-1 cells in T75 flasks were incubated with unlabeled 27-OHC (10 μM), 27-[22,23-3H]OHC (2 × 106 cpm), or 27-[3H]OHC + unlabeled 27-OHC (5 μM). After 24 h of exposure to these treatments, the culture medium was collected for isolation and determination of metabolic end products. Medium harvested from cells to which only radioactive 27-OHC was added was combined with medium harvested from cells to which unlabeled 27-OHC had been added to increase the mass of metabolite, to be present and avoid analytical problems (10) occur with trace analysis. Medium from the cells cultured in the presence of both labeled and unlabeled 27-OHC and that combined as described above were reduced by evaporation and then subjected to Folch partition (chloroform/methanol 2:1); >85% of the radioactivity was recovered in the chloroform fraction. Aliquots of medium were analyzed by thin layer chromatography (Silica Gel G) in solvent of system chloroform/methanol 4:1 (v/v). Subsequently, aliquots of the media were solvolyzed for 2 h and then re-extracted with chloroform; during this procedure methyl esters were also formed. Therefore, a portion of the solvolyzed material was further treated with methanolic KOH overnight. The solvolyzed and KOH samples were then analyzed by thin-layer chromatography as described previously (19).

Detection of 7α-Hydroxylase Activity in COS-1 and CHO Cells—COS-1 and CHO cells were grown to 80–90% confluence in DMEM and a medium, respectively, supplemented with 10% fetal calf serum. The medium was then changed to DMEM plus 10% delipidated fetal calf serum containing 5 μM 27-OHC. Cells were incubated for 24 h; then, the medium was removed, and 7α-27-OHC levels were determined as described previously (20). Cellular protein concentrations were determined for normalization of the data across experiments.

RESULTS

Effect of Hydroxysterols on SF-1-dependent Promoter Activity—Fig. 1 shows the effect that several oxysterols have on SF-1-dependent STAR promoter activity in the context of CV-1 cells. 27-OHC and 25-OHC were equal in their ability to stimulate SF-1-dependent promoter activity; both oxysterols increased activity 2.3-fold over control values (p < 0.05 by analysis of variance) in a dose-dependent fashion. At 1 μM concentrations, there was no effect, whereas a 10 μM dose maximally stimulated promoter activity. Higher concentrations, 15 μM (Fig. 1) and 25 μM (data not shown), did not further stimulate STAR promoter activity. 22(R)-hydroxysterol in shown in Fig. 1 dose-dependent promoter activity, but a 15 μM dose was required to observe an effect (Fig. 1). STAR promoter activity in the transfected CV-1 cells did not respond to progesterone or cholesterol.

Because SF-1 is essential for cAMP responsiveness of the human STAR gene promoter as well as the promoters of many
of the steroid hydroxylase genes (7, 11), we examined the effect of 27-OHC on 8-bromo-cAMP-stimulated StAR promoter activity. CV-1 cells co-transfected with the SF-1 expression vector and the StAR promoter construct were treated with a maximal stimulatory dose of 27-OHC (10 μM) and increasing concentrations of 8-bromo-cAMP (Fig. 2). 27-OHC alone caused a 2.3-fold increase in StAR promoter activity. Low concentrations of 8-bromo-cAMP (0.001 to 0.1 mM) did not significantly augment StAR promoter function in the absence or presence of 27-OHC. However, 1 mM 8-bromo-cAMP alone increased StAR promoter activity 5.5-fold. The addition of 27-OHC did not further increase expression of the luciferase reporter gene, indicating that oxysterols do not have an additive or synergistic effect on cAMP-stimulated StAR promoter activity.

Effect of the Cell Host on the Ability of 27-OHC to Stimulate SF-1-dependent StAR Promoter Activity—Table I compares the SF-1-dependent StAR promoter function in different cell types. All of these cells failed to increase in response to 27-OHC. In Y-1 cells, which also expresses endogenous SF-1, 27-OHC caused a decline in SF-1-driven StAR promoter activity. In contrast to these cell types, co-transfected CV-1 cells studied concurrently responded to 27-OHC with a 2.3-fold increase in SF-1-dependent StAR promoter activity.

The fact that 27-OHC only influenced SF-1-dependent promoter activity in CV-1 cells raised several questions. Do CV-1 cells produce a unique metabolite of 27-OHC that activates SF-1? Do other cell types rapidly inactivate 27-OHC? Are there alternative mechanisms by which 27-OHC controls SF-1-dependent promoter function in CV-1 cells beyond a ligand induced activation of SF-1?

Isolation of Metabolic Products of 27-OHC Produced by CV-1 Cells—To determine whether CV-1 cells produce unique metabolites of 27-OHC, we incubated these cells with 27-[3H]OHC and characterized the metabolic products. Medium from CV-1 cells cultured in the presence of 27-[3H]OHC yielded a single radioactive band on thin-layer chromatography that remained at the origin, indicating a very polar metabolite. Cells cultured in the presence of both 27-[3H]OHC and unlabeled 27-OHC yielded the same radiolabeled polar product, but 20% of the radioactivity was recovered in a band migrating with authentic 27-OHC. Thin-layer chromatography of the solvolyzed samples yielded a radioactive band corresponding to 27-OHC and a band corresponding to the methyl ester of 3β-hydroxy-5-cholestenic acid (3β-OH-5-CA). Treatment of the sample with methanolic KOH to de-esterify the material followed by thin-layer chromatography revealed that the radioactive product migrated with 3β-OH-5-CA.

Effect of 3β-OH-5-CA and Several Other 27-OHC Metabolites on SF-1-dependent Promoter Activity—After demonstrating that 3β-OH-5-CA is the primary metabolite of 27-OHC produced by CV-1 cells, 3β-OH-5-CA and several other metabolites known to be produced from 27-OHC were tested on transfected CV-1 cells for their ability to regulate StAR promoter activity. Three concentrations (0.1, 1, and 10 μM) of 3β-OH-5-CA and the other metabolites were examined. All doses of 3β-OH-5-CA tended to reduce SF-1-dependent transactivation (Fig. 3; data for the highest dose (10 μM) are shown). 3β-OH-5-CA was also not effective in stimulating SF-1 transactivation in COS-1 cells (data not shown). 27-OHC-4-ene-3-one produced a modest increase in SF-1-dependent transactivation, whereas 27-OHC-5β-3-one and 7α,27-OHC (1, 5, 10, and 15 μM) had no effect (Fig. 3). The fact that 7α-hydroxylation of 27-OHC ablated its ability to modulate SF-1-dependent StAR promoter activity raised the possibility that other cell types rapidly inactivate 27-OHC through this pathway.

7α-Hydroxylase Activity in COS-1 and CHO Cells—COS-1 cells produced high levels of 7α,27-OHC (mean, 2514 pmol/mg of cell protein; range, 1170–4363 for five separate determinations) following incubation of cells in the presence of 5 μM 27-OHC, indicating that these cells have high endogenous 7α-hydroxylase activity. In contrast, CHO cells had no detectable 7α-hydroxylase activity. These studies failed to provide a clear
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Explanation for the ability of 27-OHC to increase SF-1-dependent StAR promoter activity in CV-1 cells but not in other cell hosts. Because oxysterols are known to be potent regulators of SREBP levels, we examined the hypothesis that SREBP inhibits StAR gene transcription and that oxysterol suppression of nuclear SREBP levels would result in increased StAR promoter activity.

Effect of SREBP-1a on StAR Promoter Activity—CV-1 cells were co-transfected with expression plasmids for the SREBP-1a, SREBP-2, or the empty pCMV-5 expression plasmid and the StAR-reporter construct. Data presented in Table II show that SREBP-1a increased (p < 0.05 by analysis of variance) StAR promoter activity > 10-fold. The ability of SREBP-1a to enhance StAR promoter activity was also observed in COS-1 cells and human granulosa cells (Table II). Remarkably, the related transcription factor, SREBP-2, was ineffective in increasing StAR promoter activity, even though SREBP-1a and SREBP-2 had equivalent stimulatory effects on the LDL receptor gene promoter construct. These findings are inconsistent with the notion that oxysterols increase StAR promoter function by suppressing nuclear SREBP.

Effects of 27-OHC on Human Granulosa and Theca Cells—Because oxysterols exert posttranscriptional actions, we examined the effects of 27-OHC on StAR protein in cells that express the endogenous StAR gene. Western analysis revealed that 27-OHC treatment of human granulosa cells increased StAR protein levels ∼5-fold over controls (Fig. 4). Stimulation of granulosa cells with forskolin increased StAR levels ∼10-fold, whereas cells treated with both forskolin and 27-OHC had the greatest StAR content. Granulosa cells treated with 3β-OH-5-CA, 27-OHC-4-ene-3-one, 27-OHC-5β-3-one, and 7α,27-OHC also exhibited similar increases in StAR protein (Fig. 4). Human theca cells responded in a manner similar to that observed for the granulosa cells (data not shown).

Northern analysis indicated that StAR mRNA levels are unaltered by oxysterol treatment, whereas forskolin caused a marked increase in StAR mRNA (Fig. 5) in both granulosa (Fig. 5A) and theca (Fig. 5B) cells. Similarly, 7α,27-OHC did not alter STAR mRNA levels in granulosa cells.

Discussion

Oxysterol Regulation of SF-1-dependent StAR Promoter Activity—The present study demonstrates that SF-1-dependent transactivation of the human StAR promoter is enhanced by certain oxysterols including 22(R)-OHC, 25-OHC, and 27-OHC in the context of CV-1 cells. Moreover, in preliminary experiments, we found that SF-1-dependent transactivation of the human P450c21, P450i7α, and the P450scx promoters was also increased 2–4-fold in the presence of 27-OHC relative to CV-1 cells treated with the ethanol vehicle. Since no stimulation was observed in CV-1 cells transfected with empty vector, reverse SF-1, or an SF-1 mutant lacking the DNA binding domain, the effect of oxysterols on StAR promoter activity was dependent on the presence of SF-1. Collectively, these findings are consistent with the recent report of Lala et al. (6) demonstrating that some oxysterols enhance SF-1-dependent activation of a construct derived from the P450c21 promoter. The lower magnitude of the oxysterol effect in our experiments (∼2-fold stimulation) compared with that described by Lala et al. (6), who reported an approximately 10-fold increase in SF-1-dependent transactivation, could be explained by their use of an “artificial” SF-1 response element (i.e. five copies of the P450c21 promoter SF-1 responsive element in series; Ref. 21). Hence, our study is the first to document that a native promoter can respond to oxysterols in the presence of SF-1 in CV-1 cells. Only certain oxysterols activated transcription, arguing for a specific interaction of these oxysterols or a metabolite...

![Graph showing relative luciferase units for different oxysterols](image)

**TABLE II**

| Cell type          | Reporter construct | Empty vector | SREBP-1a | SREBP-2 |
|--------------------|--------------------|--------------|----------|---------|
| CV-1               | pGL2-StAR         | 3015 ± 498   | 38,190 ± 7250<sup>a</sup> | 4744 ± 230 |
| COS-1              | pGL2-StAR         | 4851         | 244,964  | 11,896  |
| COS-1              | pGL2-LDLrec       | 12,153       | 225,647  | 322,560 |
| Human granulosa    | pGL2-StAR         | 1430         | 17,653   | ND      |
| Human granulosa    | pGL2-LDLrec       | 2007         | 65,744   | ND      |

<sup>a</sup> Means ± S.E. for three individual experiments with CV-1 cells are reported; results for COS-1 and human granulosa cells are representative results of a single experiment performed with triplicate cultures. Experiments with COS-1 cells and granulosa cells were carried out in duplicate and had <10% variation between experiments. ND, not determined.

<sup>b</sup> Mean is significantly different (p < 0.05) from the means for empty vector and SREBP-2.

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derived from them with factors controlling StAR promoter activity. Notably, 7α-hydroxylation of 27-OHC resulted in the loss of transcription enhancing activity. The 7α-hydroxylation of oxysterols is also associated with loss of ability to regulate sterol homeostasis and a loss of apoptosis-inducing activity (20, 22, 23).

Although our results in CV-1 cells confirm and extend those of Lala et al. (6), the oxysterol effects could not be replicated in another monkey kidney cell line (COS-1 cells), nor in two other human cell types (BeWo cells and granulosa cells), nor in Y-1 or CHO cells. Similarly, Mellon and Bair (24) recently reported that 25-OHC did not stimulate the activity of steroidogenic P450 promoters in a mouse Leydig cell line (MA-10) that expresses endogenous SF-1. The explanation for the apparently unique effects of specific oxysterols on SF-1-dependent promoter activity in CV-1 cells and the absence of such responses in other cell types was not disclosed in our studies, nor in those of Mellon and Bair (24). However, several interesting aspects of oxysterol metabolism and action emerged from our work that exclude certain explanations for the different responses in the various host cells examined.

The lack of an oxysterol response in cells other than CV-1 cells could be the result of endogenous P450c27 activity, which is known to be present in many different cells (Refs. 25 and 26 and references therein), including vascular endothelium, macrophages, hepatic cells, and ovarian cells, forming saturating amounts of oxysterol activator. In an attempt to eliminate endogenous 27-OHC as a source of ligand for SF-1 within MA-10 cells, Mellon and Bair (24) treated the cells with aminoglutethimide, an inhibitor of P450 hydroxylases. They observed no change in transactivation of a SF-1-dependent reporter following administration of 25-OHC in the presence of aminoglutethimide and used this observation to argue against a significant role for oxysterols in regulating gene expression. However, the choice of aminoglutethimide to inhibit oxysterol production may have been inappropriate, because Rennert et al. (25) previously reported that this compound, when added to isolated mitochondria from steroidogenic cells, actually increased 27-OHC synthesis by preventing formation of pregnenolone and progesterone, which are potent inhibitors of P450c27.

Thus, the experimental approach used by Mellon and Bair (24) may still leave open the possibility that endogenous oxysterols produced in certain cells maximally activate SF-1. Alternatively, 27-OHC may be converted to an inactive metabolite incapable of stimulating SF-1-dependent promoter activity in cells other than CV-1 cells. COS-1 cells converted 27-OHC to 7α,27-OHC. This finding, coupled with the observation that 7α,27-OHC had no effect on SF-1-dependent STAR promoter activity in CV-1 cells, is consistent with this hypothesis. 7α-Hydroxylation has also previously been shown to inactivate the ability of 27-OHC to regulate cholesterol synthesis (20). However, CHO cells, which lack detectable 7α-hydroxylase, did not respond to 27-OHC in terms of SF-1-dependent STAR promoter activity. Thus, rapid catabolism of 27-OHC to 7α,27-OHC does not seem to be the explanation for the insensitivity of CHO cells.

The unique response of CV-1 cells to oxysterols raised the possibility of a novel metabolic pathway leading to the formation of a SF-1 activator in these cells. CV-1 cells cultured with radiolabeled 27-OHC converted the precursor to a single product, 3β-OH-5-CA. Subsequent testing of 3β-OH-5-CA in CV-1 and COS-1 cells in the co-transfection assay indicated that 3β-OH-5-CA inhibited SF-1-dependent STAR promoter activity. The inhibitory effect of 3β-OH-5-CA does not exclude the possibility that a short-lived intermediary metabolic product derived from 27-OHC might be the activator of SF-1 in CV-1 cells. Therefore, several other metabolites of 27-OHC metabolism were also tested; 27-OHC-5β-3-one was inactive, whereas 27-OHC-4-ene-3-one enhanced SF-1-dependent transactivation when administered at concentrations equivalent to effective levels of 27-OHC. Our studies, although not establishing the identity of the SF-1 activator in CV-1 cells, do provide evidence for specificity of the process because several structurally related 27-OHC metabolites were clearly devoid of activity.

Sterol Regulatory Element Binding Protein Regulation of the STAR Promoter—Studies of other nuclear receptors indicate that true ligand(s) commonly have the ability to stimulate transactivation at submicromolar doses, often in the nanomolar range (27). The inability to observe an effect of 27-OHC or any of its derivatives on SF-1-dependent transactivation at submicromolar doses suggests that 27-OHC and/or its metabolic products might act indirectly. Oxysterols are known regulators of a large number of genes involved in maintaining cholesterol homeostasis, including 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, squalene synthase, and the LDL receptor (2, 26, 28). SREBP s are the key proteins involved in mediating cholesterol (oxysterol) dependent regulation of these genes (2). Members of a unique family of transcription factors, SREBP s, are tethered to the endoplasmic reticulum and are proteolytically cleaved when intracellular/membrane cholesterol (oxysterols) levels are reduced, releasing the transcriptionally active basic helix-loop-helix-leucine zipper tran-

![Fig. 4. Western blot analysis of STAR proteins from human granulosa cells treated with 25-OHC or 27-OHC ± forskolin (top) and 27-OHC-4-ene-3-one, 27-OHC-5β-3-one, 3β-OH-5-CA, and 7α-27-OHC ± forskolin (bottom). Human granulosa cells were cultured in medium containing 10% delipidated serum for 2 days prior to treatment with 10 µM oxysterols ± 50 µM forskolin for 24 h. The molecular mass standards are indicated at the left.](image-url)
SREBPs also regulate fatty acid metabolism by modulating the levels of key enzymes (i.e. fatty acid synthase, acetyl-CoA carboxylase, etc.) (2, 29). Our findings provide the first evidence that SREBPs could be intimately involved in the regulation of steroidogenesis through control of StAR gene expression. StAR functions in the rate-limiting step of steroidogenesis, the movement of cholesterol to the inner mitochondria membrane, where it has access to P450scc, which catalyzes the first committed step in steroidogenesis (8). Thus, StAR works at the intersection between cholesterol metabolism and steroidogenesis; its regulation by both SREBP-1a and SF-1 allows for integration of these metabolic pathways.

Our observation that the StAR gene promoter is activated by SREBP-1a and not by SREBP-2 is the first evidence for preferential activation of a promoter by a SREBP. This preferential response of the StAR promoter to SREBP-1a was observed in both COS-1 and CV-1 cells, whereas both SREBP-1a and SREBP-2 were equivalent in their ability to stimulate LDL-receptor promoter activity. Guan et al. (30) previously demonstrated that the human squalene synthase gene promoter can be differentially activated by SREBP-1a and SREBP-2, but only when various mutations were made in the sterol regulatory region of the promoter. Furthermore, a number of cell culture studies indicate that SREBP-1a and SREBP-2 undergo similar patterns of expression, and in those gene promoters studied simultaneously, SREBP-1a and SREBP-2 were equivalent in their ability to induce activation (30–33). In experiments to be described elsewhere, we have identified a nucleotide sequence in the StAR gene promoter that binds SREBP-1a in gel shift experiments and when mutated results in a reduction in SREBP-stimulated promoter activity and loss of ability to bind SREBP-1a. Thus, the StAR gene may provide a unique opportunity to delineate SREBP-1a-specific binding elements within the promoter.

The physiological significance of SREBP-1a action on StAR transcription has yet to be determined. However, it is easy to envision a role for SREBP-1a in the tropic hormone up-regulation of StAR gene expression in certain circumstances. Tropic hormones and cAMP analogs increase cholesterol utilization by steroid-producing cells, resulting in the increased synthesis of LDL receptor and HMG-CoA reductase mRNAs (34, 35), a response that is due at least in part to the action of SREBP on transcription of these genes. As granulosa cells luteinize during the periovulatory period, they dramatically increase their capacity to synthesize cholesterol de novo, take up LDL, and produce progesterone (36). This is a situation in which mRNAs for HMG-CoA reductase, LDL receptors, and StAR all rapidly increase (15, 34, 35). Coordinated expression of genes involved in cholesterol acquisition (LDL receptor and HMG-CoA reductase) and metabolism to steroid hormones (StAR) through a common transcription factor is an attractive concept.

3 L. K. Christenson, T. F. Osborne, and J. F. Strauss, unpublished observations.

FIG. 5. Regulation of StAR mRNA expression in proliferating human granulosa (A) and thecal cells (B) by 25-OHC and 27-OHC ± forskolin. Cells were cultured in medium containing 10% delipidated serum for 2 days prior to treatment with 10 μM 25-OHC or 27-OHC ± 20 μM forskolin. Total RNA (25 μg of RNA/ lane) was subjected to Northern blotting, and blots were probed with the StAR and 28S rRNA cDNAs. Histograms represent fold increases (means ± S.E. for three experiments) in StAR mRNA levels relative to cells treated under control minus forskolin conditions. Forskolin treatment increased (p < 0.05) StAR mRNA levels in both granulosa and theca cells.
**Posttranscriptional Regulation of StAR**—The present study demonstrated that oxysterols increased granulosa and theca interna cell StAR protein levels without changing StAR mRNA levels. Moreover, granulosa cells transfected with the StAR promoter construct also failed to respond to 27-OHC treatment. These observations suggest that oxysterols have posttranscriptional effects on StAR expression either by enhancing translation or by increasing protein life span. The mechanism responsible for this effect remains to be elucidated. However, it is evidently different from the transcriptional actions in CV-1 cells, which are seen with only certain oxysterols, whereas all of the oxysterols examined, including those that were not able to increase SF-1-dependent StAR promoter transactivation and that do not regulate cellular cholesterol homeostasis, increased StAR protein in granulosa cells. This raises the possibility that under certain circumstances, the rapid metabolism of oxysterols (e.g., 7α-hydroxylation of 27-OHC) to compounds that cannot suppress SREBP processing leads to increased StAR hormone-producing cells.

Specific roles for SREBP-1a and SREBP-2 in control of cholesterol homeostasis. Moreover, our finding that this response is specific to SREBP-1a will allow the dissection of specific roles for SREBP-1a and SREBP-2 in control of cholesterol, fatty acid, and now steroid metabolism. In addition to having actions at the transcriptional level, oxysterols appear to have heretofore undisclosed posttranscriptional effects on StAR expression that may promote steroidogenesis in hormone-producing cells.

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