Regulation of Stearoyl Coenzyme A Desaturase Expression in Human Retinal Pigment Epithelial Cells by Retinoic Acid*

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Stearoyl-CoA desaturase (SCD) is a regulatory enzyme involved in the synthesis of the monounsaturated fatty acids palmitoleate and oleate. The regulation of SCD is of physiological importance because the ratio of saturated fatty acids to unsaturated fatty acids is thought to modulate membrane fluidity. Differential display analysis of retinal pigment epithelial (ARPE-19) cells identified SCD as a gene regulated by retinoic acid. Two SCD transcripts of 3.9 and 5.2 kilobases in size were found to be expressed in these cells by Northern blot analysis. All-trans-retinoic acid (all-trans-RA) increased SCD mRNA expression in a dose- and time-dependent manner; a ~7-fold increase was observed with 1 μM all-trans-RA at 48 h. SCD mRNA expression was also increased by 9-cis-retinoic acid (9-cis-RA) as well as 4-((E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl)benzoic acid (TTNPB), a retinoic acid receptor (RAR)-specific agonist. AGN194301, a RARα-specific antagonist, suppressed the SCD expression induced by all-trans-RA, TTNPB, and 9-cis-RA. These results indicate the involvement of RARα in the induction of SCD expression by retinoic acid. However, AGN194204, an RXR (retinoid X receptor) pan agonist, also increased SCD mRNA expression. This increase was not blocked by AGN194301, suggesting that an RAR-independent mechanism may also be involved. Thus, SCD expression in retinal pigment epithelial cells is regulated by retinoic acid, and the regulation appears to be mediated through RAR and RXR.

Stearoyl-CoA desaturase (SCD, EC 1.14.99.5),1 a microsomal enzyme, catalyzes the initial desaturation of long chain saturated fatty acids into monounsaturated fatty acids. Palmitoleate and stearate are the preferred substrates for this enzyme. They are converted to palmitoleate and oleate, respectively (1,2). This oxidative reaction also requires the participation of O2, NADPH, cytochrome b5, and cytochrome b6 reductase. Two SCD genes, SCD1 and SCD2, characterized from both rat and mouse, encode functionally active proteins that share >80% sequence homology (3,4). SCD1 and SCD2 show different tissue-specific expression patterns, possibly because of marked differences in the promoter sequences of their genes (5). There are also two loci for SCD genes in the human genome, one on chromosome 10 and another on chromosome 17 (6). However, the gene on chromosome 17 appears to be a transcriptionally inactive pseudogene. The gene on chromosome 10 encodes the functionally active 359-amino acid SCD protein. This gene yields two transcripts, 5.2 and 3.9 kb in size, which differ in the length of the 3′-untranslated region.

The regulation of SCD by dietary factors, hormones, and peroxisomal proliferators has been studied in mouse and rat (7–12). The regulation of SCD is of physiological importance because changes in this enzyme activity could lead to changes in cell membrane phospholipid composition (8). Palmitoleic and oleic acids are the predominant unsaturated fatty acids present in fat depots and membrane phospholipids (9). The ratio of stearate to oleate is thought to control bilayer fluidity and thereby influence membrane function (10). Alteration in membrane fluidity leads to increased cell metabolism and differentiation (11). A high ratio of oleate to stearate is known to initiate apoptotic cell death in neonatal cardiomyocytes (12). In addition, low density lipoprotein rich in oleate showed increased resistance to oxidative damage (13). Certain fatty acid derivatives are reported to exert antiinflammatory activity by inhibiting SCD (14).

The retinal pigment epithelium (RPE) is a polarized monolayer of highly differentiated epithelial cells located between the choroid and the neural retina in the eye (15). It provides nutrients to the photoreceptor cells and carries out phagocytosis and degradation of rod outer segments tips undergoing circadian shedding. RPE cells are indispensable for the regeneration of 11-cis-retinal, the visual pigment chromophore. Retinoic acid (RA), a retinoid not involved in the visual process, is generated as a by-product in neural retina and RPE during the interaction of light with the photoreceptors (16). RA is required for many cellular functions, and its biological effects are mediated through retinoic acid receptors (RAR) and retinoid X receptors (RXR) in a ligand-dependent manner (17). In an effort to identify RA-regulated genes in human RPE cells in culture by differential display analysis, SCD was identified as one such gene. Two SCD transcripts (5.2 and 3.9 kb in size) are present in RPE cells, and both are up-regulated by RA. The regulation appears to be at the level of transcription and mediated through RAR and RXR.

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EXPERIMENTAL PROCEDURES

Materials—All-trans-retinoic acid (all-trans-RA), 9-cis-retinoic acid (9-cis-RA), stearic acid, oleic acid, and fatty acid-free bovine serum albumin were purchased from Sigma. TTNPB (4-(6′-2-(5,6,7,8-tetrahydrole-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl)benzoic acid) was from Biomol (Plymouth Meeting, PA). [1-14C]Stearic acid was purchased from Amersham Pharmacia Biotech. The retinoid receptor antagonist AGN194301 and retinoid receptor agonist AGN194204, were synthesized in the Department of Chemistry at Allergan (Irvine, CA).

Cells and Culture Conditions—Human RPE cells (ARPE-19 from ATCC) were grown in Dulbecco’s modified Eagle’s medium/nutrient mixture F-12 (Celgro, Herdon, VA) supplemented with 5% fetal bovine serum, 100 units/ml penicillin and 100 µg/ml streptomycin as described previously (18). The RPE D407 cell line (a generous gift from Dr. Richard C. Hunt, University of South Carolina Medical School, Columbia) was grown in Dulbecco’s modified Eagle’s medium supplemented with 3% fetal bovine serum, 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mM glutamine (19). Cells were seeded onto 100-mm tissue culture plates at a density of 2 × 10^5 cells/ml in complete medium and allowed to grow overnight. The culture medium was replaced next day with fresh medium containing 3% charcoal and dextran-treated fetal bovine serum (HyClone, Logan, Utah). After 24 h, when the cells were 60–70% confluent, retinoids were added to the culture medium, and the cells were allowed to grow for indicated time intervals. Retinoids were dissolved at a concentration of 10 µM in dimethyl sulfoxide before adding to the cell culture medium. The controls received the same amount of dimethyl sulfoxide. HeLa, HepG2, human gingiva fibroblast 1, human telomerase reverse transcriptase-RPE1, and monkey RPE cells were also grown in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. The cells were maintained at 37 °C in a humidified environment of 5% CO₂.

Assay of Oleic Acid Formation in ARPE-19 Cells—ARPE-19 cells were grown in 100-mm culture dishes as described before and treated with 5 µM all-trans-RA for 42 h. [1-14C]Stearic acid in fatty acid-free bovine serum albumin was added at this time, and the cells were allowed to grow for an additional 6 h. The cells were washed twice with 5 ml of ice-cold phosphate-buffered saline, pH 7.4, and then harvested in 2.0 ml of phosphate-buffered saline by the use of a rubber policeman. Cells were homogenized in a Potter-Elvehjem glass homogenizer, and lipids were extracted with chloroform/methanol (2:1) by vortexing and

Fig. 1. Identification of SCD as a differentially expressed gene in all-trans-RA-treated ARPE-19 cells. Total RNA preparations from control (lane 1) and all-trans-RA-treated (10 µM, 48 h; lanes 2 and 3, experiment performed in duplicate) ARPE-19 cells were subjected to differential display analysis. The amplification products were separated by polyacrylamide gel electrophoresis. A cDNA fragment (indicated by an arrow) representing a differentially expressed mRNA was identified as that of SCD by DNA sequencing.

Fig. 2. Expression of SCD in RPE cells and its response to all-trans-RA. SCD expression was analyzed in two human RPE cell culture systems, ARPE-19 and D407, by Northern blotting. The cDNA fragment identified as that of SCD by differential display was used as the probe. The cells were treated for 48 h with 10 µM all-trans-RA. Total RNA preparations were obtained from control (lanes 1 and 2) and treated (lanes 3 and 4) cells. Two SCD transcripts, 5.2 and 3.9 kb, were detected, and all-trans-RA treatment greatly increased SCD expression in both ARPE-19 and D407 cells. The amounts of RNA loaded are comparable as shown by the ethidium bromide staining of 28 and 18 S ribosomal RNAs.

Fig. 3. Northern blot analysis of SCD expression in human tissues and cells. Total RNA preparations from the indicated sources were analyzed by Northern blotting using 198–710-bp SCD cDNA fragments as the probe. Ethidium bromide-stained 28 and 18 S RNAs are shown to indicate that similar amounts of RNA were loaded in different lanes. Panel A, two SCD transcripts (5.2 and 3.9 kb) are expressed in all RPE cell culture systems and also in cell lines derived from other tissues. Panel B, both SCD transcripts are present in human retina and RPE. SCD expression is also detected in monkey retina, RPE, and macula (center portion of the retina). Panel C, SCD transcripts are detected in several non-ocular human tissues; the expression being highest in brain, spinal cord, and fetal liver.
centrifuged at 3,000 rpm for 15 min. The lipid phase at the bottom was collected, dried under argon, and analyzed for fatty acids using HPLC by the method of Osterroth (20). The lipids were solubilized, saponified, and derivatized (21). The resulting samples were analyzed by HPLC using a Hewlett-Packard HP1090 instrument on a 4.6 × 250-mm, 5.0-μm C8 Supelco column in line with a Packard A500 radiometric flow scintillation counter. The derivatized fatty acids were detected by absorbance at 250 nm. Individual fatty acids were identified by using labeled and unlabeled standards. Radioactive oleic acid was detected and quantified using Packard FLO-ONE software.

**RNA Extraction and Northern Blot Analysis**—Total RNA was extracted from cultured cells and tissues using RNAzol B RNA isolation kit (Tel-Test, Friendswood, TX). RNA from K-562 and Raji cells were used as positive controls for RARα and RXRα (Geneka Biotechnology, Montreal, Canada). Human multiple tissue RNA samples were purchased from CLONTECH (Palo Alto, CA).

Equal amounts of RNA samples were electrophoresed on a 1% agarose gel containing 2% formaldehyde. The RNA bands from the gel were transferred to a Nytran membrane by capillary blotting (Schleicher & Schuell, Keene, NH). Equal amounts of loading and the transfer efficiency of RNA samples were verified by ethidium bromide staining of 28S and 18S rRNAs on the gel and the membrane. The cDNA probes for SCD were generated by reverse transcription-PCR from a human brain RNA preparation using 5′-AAGTGATCCGGCAGTTCCAGGAG and 5′-GAAATGGGCTGTGACCACCCGT as sense and antisense primers, respectively. The identity of the amplification product was confirmed by sequencing, and its sequence was found to be identical to 198–710 bp of the SCD cDNA sequence (GenBank accession number AF097514). Oligonucleotide probes for RARα and RXRα were obtained from Geneka Biotechnology (Montreal, Canada). The blot was hybridized with the cDNA probe labeled with ω-32PdCTP, using QuickHyb hybridization solution (Stratagene, La Jolla, CA). Hybridization and washing were done according to the manufacturer's protocol. The blot was exposed to Kodak X-OMAT Blue autoradiography film. The relative expression of SCD was quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Normalization of the signal intensity was done using the intensity of ethidium bromide-stained 18S ribosomal RNA band. Membranes were stripped and reprobed when necessary.

**Differential Display**—Restriction fragment differential display (RFDD)–reverse transcription PCR was performed using a displayPRO-FILE kit from Display Systems Biotech (Vista, CA) (22). Briefly, 800 ng of total RNA from control and all-trans-RA-treated ARPE-19 cells was used for cDNA synthesis. The double-stranded DNA was digested with TaqI, a restriction enzyme that generates a 5′-overhanging end. The sticky ends were ligated with special adaptors, and the cDNA fragments were amplified by PCR using 32 different 32P-labeled primer sets. Denaturing polyacrylamide gels were used to separate the PCR products followed by autoradiography. The differentially expressed fragments were isolated and reamplified using the same primer combinations. Candidate cDNAs identified in the initial screen were recovered from dried gels, reamplified by PCR, and cloned into a TA-cloning vector (Invitrogen, San Diego). The cloned PCR products were analyzed by sequencing. DNA homology searches were done using the BLAST program supported by NCBI (http://www.ncbi.nlm.nih.gov/).

**Electrophoretic Mobility Shift Assay**—Nuclear extracts from control and all-trans-RA-treated ARPE-19 cells were prepared essentially as described (23). For the gel shift assay, double-stranded oligonucleotides containing RAR–RXR (DR5) binding consensus sequences or RXR–RXR (DR1) binding consensus sequences were used (Geneka Biotechnology, Montreal, Canada). The 5′-end was labeled with 3,000 Ci/mmol [32P]ATP (Amersham Pharmacia Biotech) using T4 polynucleotide kinase. 2–5 μg aliquots from control or all-trans-RA-treated nuclear extracts were preincubated in a total volume of 18 μl in the binding buffer (10 mM Hepes pH 7.4, 50 mM KCl, 1 mM dithiothreitol, 5% glycerol) containing 4 μg of poly(dI-dC) (Amersham Pharmacia Biotech). After the addition of 1 ng of 32P-labeled oligonucleotide probe, the extracts were incubated further for 20 min at room temperature. The protein-bound oligonucleotide was separated from the free oligonucleotide by electrophoresis on a 6% nondenaturing polyacrylamide gel. The gels were dried and autoradiographed at −70 °C. For the competition experiments, extracts were preincubated with a 100-fold molar excess of unlabeled oligonucleotide for 30 min before the addition of the 32P-labeled oligonucleotide probe.

### RESULTS

**Identification of SCD as a Differentially Expressed Gene in ARPE-19 Cells Treated with All-trans-RA**—In an attempt to identify genes regulated by all-trans-RA treatment in ARPE-19 cells, we performed RFDD-PCR using poly(A)+ RNA preparations from control and 10 μM all-trans-RA-treated cells. In total, we observed 13 PCR fragments that were expressed differentially between control and all-trans-RA-treated ARPE-19 cells. One of the differentially expressed mRNA products is shown in Fig. 1. This cDNA fragment was recovered from the gel and reamplified by PCR. The amplification product was cloned into the pCR II plasmid. The cloned cDNA fragment (~240 bp) was characterized by sequencing. The sequence thus obtained was compared with the nonredundant nucleotide sequence data base at the National Center for Biotechnology Information using the BLAST algorithm. The search revealed that the cDNA fragment had 100% sequence homology to 251–493 bp of human SCD (GenBank accession number AF097514).

The differential expression of SCD in ARPE-19 cells treated with all-trans-RA was verified using Northern blot analysis. The amplified cDNA fragment obtained from differential display was used as the probe. As expected for SCD, the probe hybridized to two transcripts of 3.9 and 5.2 kb (Fig. 2). Both the transcripts were increased with all-trans-RA treatment compared with the control. Identical results were obtained when the hybridization was performed with a cDNA probe designed from the 198–710 bp region of SCD cDNA or with a HindIII and Smal fragment (824 bp) obtained from a human SCD cDNA clone generated by Zhang et al. (6 and data not shown).

The expression of SCD and its response to all-trans-RA were also investigated in D407 cells, another RPE cell line (19). As shown in Fig. 2, both SCD transcripts are also present in D407 cells, and their expression is up-regulated by all-trans-RA. Thus, SCD is expressed in RPE cells, and its expression is regulated by all-trans-RA.

**SCD mRNA Expression in Cells and Tissues**—The expression of SCD was analyzed in several RPE and non-RPE cells in culture (Fig. 3A). Apart from ARPE-19 and D407, we also...
FIG. 5. Effect of all-trans-RA concentration on SCD mRNA expression. Panel A, total RNA preparations obtained from ARPE-19 cells treated for 48 h with indicated concentrations of all-trans-RA were analyzed by Northern blotting using an SCD cDNA-specific probe. Ethidium bromide-stained 28 and 18 S rRNA bands are shown to indicate that similar amounts of RNA were loaded in different lanes. Panel B, the chart shows the relative intensities of bands on the Northern blot determined using a PhosphorImager. The values were normalized using 18 S rRNA band intensity. The highest SCD expression is observed in cells treated with 1 μM all-trans-RA.

Fig. 6. Effect of all-trans-RA on oleic acid formation in ARPE-19 cells. ARPE-19 cells were treated with or without 5 μM all-trans-RA for 42 h and incubated for an additional 6 h in the presence of 50 μM [1-14C]stearic acid. Total lipids were extracted, saponified, derivatized, and analyzed for oleic acid by HPLC. Panel A, the chart shows the oleic acid formation in control and all-trans-RA (atRA)-treated cells. The dpm associated with oleic acid peak and the total dpm in the derivatized lipid fractions were determined. The oleic acid formation is shown as a percentage of total dpm. The values are the mean ± S.D., n = 3. * indicates p < 0.01 treated versus control values. Panel B, total RNA preparations from control and all-trans-RA-treated cells were analyzed by Northern blotting using an SCD cDNA-specific probe. Ethidium bromide-stained 28 and 18 S rRNA bands are shown to indicate that similar amounts of RNA were loaded in different lanes.

tested a telomerase-immortalized human RPE cell line (human telomerase reverse transcriptase-RPE1, CLONTECH) as well as monkey RPE cells in culture. SCD expression was detected in all of the RPE cell lines. SCD was also expressed in several other cell lines: COS-7 (monkey kidney fibroblast), HeLa (human adenocarcinoma), HGF-1 (human gingiva fibroblast), and HepG2 (human hepatocellular carcinoma).

SCD expression was observed in human retina and RPE (Fig. 3B). The 5.2-kb transcript was more abundant than the 3.9-kb transcript. SCD was expressed as two transcripts (5.2 and 3.9 kb) in monkey retina and RPE (Fig. 3B). The expression was also detected in the macula, the central portion of the retina. SCD mRNA expression was analyzed in various nonocular human tissues. We observed the presence of both 5.2- and 3.9-kb transcripts in most tissues tested (Fig. 3C). Adult brain, spinal cord, and fetal liver showed high level expression. The expression was moderate in liver, heart, lung, cerebellum, and fetal brain. A low level of expression was detected in kidney and trachea, whereas placenta showed no signal. The relative abundance of the two transcripts varied among the tissues. Both adult and fetal brain and cerebellum expressed more of the 5.2-kb transcript than the 3.9-kb transcript, whereas heart and trachea expressed more of the 3.9-kb transcript than the 5.2-kb transcript.

Effect of All-trans-RA on SCD mRNA Expression in ARPE-19 Cells—The effect of exogenous all-trans-RA on SCD expression in ARPE-19 cells was investigated. Total RNA preparations from cells treated with 10 μM all-trans-RA for various time intervals were analyzed by Northern blotting. The expression of both SCD transcripts increased with time until 48 h (Fig. 4). The increase in expression was about 5-fold at this time point. No noticeable change in SCD expression was observed in untreated cells used as controls for various time points (data not shown).

All-trans-RA treatment resulted in a concentration-dependent increase in the expression of both the SCD transcripts in ARPE-19 cells (Fig. 5). SCD expression increased up to a concentration of 1 μM all-trans-RA, and the increase in expression was about 7-fold at this concentration. Further increases in RA concentration were not accompanied by a corresponding increase in SCD expression, and the level of SCD expression in cells treated with 5 and 10 μM all-trans-RA was lower than that observed with 1 μM.

It was of interest to see whether an increase in SCD mRNA in all-trans-RA-treated ARPE-19 cells is associated with a corresponding increase in SCD enzyme activity. The conversion of [1-14C]stearic acid into oleic acid in intact cells was measured as an index of SCD enzyme activity. Control and all-trans-RA-treated ARPE-19 cells were incubated in the presence of [1-14C]stearic acid for 6 h, and the oleic acid formation was analyzed using HPLC. As shown in Fig. 6, the all-trans-RA treatment resulted in only a 20% increase (p < 0.01) in oleic acid formation in ARPE-19 cells. However, the treatment resulted in an ∼4-fold increase in SCD mRNA expression, as expected. Thus, it appears that the increased expression of SCD mRNA by all-trans-RA in ARPE-19 cells is associated with only a modest increase in the SCD enzyme activity.

The effect of actinomycin D, an intercalating transcriptional inhibitor, on all-trans-RA-induced SCD mRNA expression was studied (Fig. 7). ARPE-19 cells were treated with all-trans-RA in the presence of indicated concentrations of actinomycin D. The inhibition of SCD expression was concentration-dependent, and 75% inhibition was observed at a concentration of 1 ng/ml. Therefore it appears that the effect of all-trans-RA on SCD expression occurs at the transcriptional level.

Effect of 9-cis-RA on SCD mRNA Expression in ARPE-19 Cells—The effect of 9-cis-RA, an isomer of all-trans-RA, on SCD gene expression in ARPE-19 cells was studied (Fig. 8). 9-cis-RA treatments resulted in a concentration-dependent increase in SCD transcripts. The maximum increase was observed at 0.5 μM, and approximately a 3-fold increase in expression was observed at this concentration.

Regulation of SCD mRNA Expression Is Mediated through Retinoid Receptors—The role of retinoid receptors RAR and RXR in RA-induced SCD expression in ARPE-19 cells was investigated using retinoid receptor agonists and antagonists. SCD mRNA expression in ARPE-19 cells was increased by TTNPB as well as by all-trans-RA (Fig. 9). All-trans-RA is known to mediate its response through RAR (24), and TTNPB,
Presence of Retinoid Receptors in ARPE-19 Cells—The presence of retinoid receptors in ARPE-19 cells was analyzed. Total RNA preparations from these cells were analyzed by Northern blot analysis using probes specific for RARs and RXRs. Two transcripts of RARα (2.8 and 3.8 kb) were observed in the case of ARPE-19 cells (Fig. 11A). An RNA preparation from K-562 (chronic myelogenous leukemia) cells was used as the positive control. A 3.6-kb transcript for RXRα (chronic myelogenous leukemia) cells was used as the positive control. A 3.6-kb transcript for RXRα (chronic myelogenous leukemia) cells was used as the positive control.

A gel mobility shift assay was employed to characterize further the retinoid receptors in ARPE-19 cells and their response to all-trans-RA (Fig. 11C). Radiolabeled double-stranded oligonucleotides containing RAR-RXR (DR5) or RXR-RXR (DR1) binding sequences were used. The nuclear extracts prepared from ARPE-19 cells showed the ability to bind either oligonucleotide in the gel retardation assay. The specificity of the complex formation was verified by blocking the binding with a large molar excess of corresponding unlabeled oligonucleotide. Nuclear extract preparations from all-trans-RA-treated ARPE-19 cells showed increased ability to bind DR5 and DR1 sequences compared with control extracts. Thus, both RARs
and RXRs are present in ARPE-19 cells, and their expression is regulated by all-trans-RA.

**DISCUSSION**

In the present study, we report the identification of SCD as a gene regulated by RA. We employed the RFDD-PCR technique to analyze RA-regulated gene expression in ARPE-19 cells, a human RPE cell line noted for its ability to retain many structural and functional characteristics of intact RPE (29). RFDD-PCR has been reported to eliminate the reproducibility problem associated with standard differential display methods (22, 30). Northern blot analysis showed the presence of two SCD transcripts, 5.2 and 3.9 kb in size, in ARPE-19 cells, and both transcripts exhibited a time- and concentration-dependent increase in response to RA treatment.

The regulation of two SCD genes, SCD1 and SCD2, has been studied in mouse and rat, and both genes are regulated by a number of dietary factors, hormones, and peroxisomal proliferators (10, 31, 32). Unlike mouse and rat, there is only one functional SCD gene in the human (6), and the regulation of the human SCD gene has not been investigated previously. We have shown here that the SCD gene is regulated by RA in human RPE cells. The regulation of SCD expression by RA is of potential physiological importance because changes in SCD enzyme activity are reported to modulate the cell membrane phospholipid composition (8) and the ratio of saturated to monounsaturated fatty acids, which is thought to control bilayer fluidity and thereby membrane functions (10). A ~4-fold increase in SCD mRNA expression induced by all-trans-RA resulted in only a 20% increase in SCD enzyme activity. This may be because of a translational deficiency associated with certain genes in cultured RPE cells. These cells are reported to express a large amount of RPE65 mRNA with no detectable amount of RPE65 protein (33). At present, we do not know whether the regulation of SCD by RA extends to other cells and tissues as well as to other organisms, but it is interesting to note that Miller et al. (34) observed that retinol palmitate feeding increased SCD-1 expression in vitamin A-deficient mice. They have postulated that the observed effect could be mediated through RA.

Both SCD transcripts, 5.9 and 3.9 kb, were present in RPE and retina from human as well as monkey. Photoreceptor cells are known to contain high amounts of long-chain polyunsaturated fatty acids and RPE cells constantly remove large amounts of phospholipid-rich photosensitive membranes by

**FIG. 10.** Effect of RXR agonists on SCD mRNA expression. ARPE-19 cells were treated with the indicated compounds for 48 h, and the total RNA preparations were analyzed by Northern blotting using a SCD cDNA-specific probe. Ethidium bromide-stained 28 and 18 S rRNA bands are shown to indicate that similar amounts of RNA were loaded in different lanes. The relative intensities of bands on the Northern blot were determined using a PhosphorImager, and the values were normalized using the 18 S rRNA band intensity. Panel A, the cells were treated with 0.1 μM 9-cis-RA (9cRA) in the presence or absence of 1 μM RAR antagonist AGN149301. Panel B, the chart shows the relative intensities of bands on the Northern blot presented in panel A. Panel C, the cells were treated with 0.5 μM RXR-selective agonist AGN14204 in the presence or absence of 5 μM RXR antagonist AGN149301. Panel D, the chart shows the relative intensities of bands on the Northern blot presented in panel C.

**FIG. 11.** Expression of RARs in ARPE-19 cells. ARPE-19 cells were treated with 10 μM all-trans-RA for 48 h, and total RNA preparations were analyzed by Northern blotting using RARα-specific and RXRα-specific oligonucleotide probes. RNA preparations from K-562 and Raji cell lines were used as positive controls for RARα and RXRα, respectively. Ethidium bromide-stained 28 and 18 S rRNA bands are shown to indicate that similar amounts of RNA were loaded in different lanes. Panel A, Northern blot shows that RARα expression in ARPE-19 cells is induced by all-trans-RA. Lane 1, positive control; lane 2, untreated ARPE-19 cells; lane 3, all-trans-RA-treated ARPE-19 cells. Panel B, Northern blot shows that RXRα expression in ARPE-19 cells is induced by all-trans-RA. Lane 1, positive control; lane 2, untreated ARPE-19 cells; lane 3, all-trans-RA-treated ARPE-19 cells. Panel C, electrophoretic mobility gel shift assay was performed with nuclear extract prepared from control and 10 μM all-trans-RA-treated (atRA, 48 h) ARPE-19 cells. Complex formation is noticed in the RAR-RXR (DR5) heterodimer (lane 2) and RXR-RXR (DR1) homodimer (lane 10) compared with free probe (lanes 1 and 9). Nuclear extract prepared from all-trans-RA showed increased complex formation with RAR-RXR (DR5, lane 6) and RXR-RXR (DR1, lane 14). A 100-fold molar excess of the corresponding unlabeled oligonucleotide is able to inhibit the complex formation (lanes 4, 5, 7, 8, 11, 12, 15, and 16).
phagocytosis of rod outer segments undergoing circadian shedding (35). Thus, the identification of SCD in RPE cells is quite interesting because it may play an important role in the process of complex lipid metabolism and trafficking in the RPE. Moreover, because of its ability to regulate the expression of SCD, RA may also play a critical role in this process.

The regulation of gene expression by RA has been studied extensively. RA effects are mediated through the RAR and RXR (36, 37). Each of these families consists of three isoforms: α, β, and γ (38). These retinoid receptors modulate gene expression by binding to DNA sequences known as retinoid response elements, retinoid acid response elements, and retinoid X response elements, present in the regulatory region of the target genes (39). We have shown that the RA-induced SCD expression is inhibited by actinomycin D. Thus, the observed regulation of SCD appears to be at the transcriptional level.

Retinoid receptors, RARs, and RXRs form RAR-RXR heterodimers or RXR-RXR homodimers in the presence of retinoid ligands (24). These heterodimers or homodimers complexed with retinoid ligands bind to retinoid acid response elements or retinoid X response elements to regulate the transcription of target genes. Northern blot analysis showed that both RARs and RXRs are present in ARPE-19 cells, and their expression is regulated by RA. Gel shift assays using oligonucleotides containing DR5 and DR1 sequences also support the presence of RAR and RXR in RPE cells. DR5 binds selectively to RAR-RXR heterodimers, whereas DR1 preferentially binds to RXR-RXR homodimers (17). RPE cell nuclear extracts exhibited good heterodimers, whereas DR1 preferentially binds to RXR-RXR homodimers in the presence of retinoid antagonists. AGN194301 binds selectively to RAR-RXR heterodimers or RXR-RXR homodimers in the presence of retinoid antagonist AGN194301. Therefore, it is possible that RAR-RXR heterodimers are involved in the regulation of these genes.

SCD expression in ARPE-19 cells is also induced by 9-cis-RA. This retinoid ligand is able to activate target genes through an RXR homodimer or RAR-RXR heterodimer signaling pathway because of its ability to bind both RXR and RAR (17). The induction of SCD expression by 9-cis-RA is blocked completely by the RXR antagonist AGN194301. Therefore, it is possible that RAR-RXR heterodimer is involved in the 9-cis-RA-induced SCD mRNA expression.

The involvement of RAR is supported further by the observation that TTNPB increased the SCD mRNA expression. TTNPB is a synthetic, highly selective RAR agonist with no affinity for RXR (25). TTNPB induced SCD expression, and the induction was blocked completely by AGN194301, a RARα antagonist. AGN194204, an RARβ-specific agonist with no affinity toward RAR (28), also increased SCD mRNA expression; this induction was not blocked by AGN194301, a RARα-specific antagonist. Thus, a mechanism that involves RXR, but not RAR, cannot be ruled out.

In summary, we have identified SCD as a gene regulated by RA. Both the (5.2 and 3.9 kb) transcripts of SCD are expressed in human RPE cells, and their expression is greatly increased by RA. The regulation of SCD expression by RA appears to be mediated through RAR and RXR. Because SCD is a regulatory enzyme in the lipid metabolic pathway, its response to RA may play an important role in the pathophysiology of human retinal pigment epithelium. It remains to be elucidated if the regulation of SCD by RA extends to other tissues and cells.

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