Sterol 27-hydroxylase: expression in human arterial endothelium

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Abstract Human endothelium obtained from both the aorta and the pulmonary artery has been evaluated for the presence of the messenger RNA coding for the expression of sterol 27-hydroxylase. Unique oligomers were designed to detect the mRNA by reverse transcription followed by the polymerase chain reaction. The amplified product was sequenced and was found to be identical to the published sequence for nucleotides 491 to 802 of the human sterol 27-hydroxylase cDNA. Northern blot analysis confirmed the presence of 27-hydroxylase mRNA in pulmonary artery and aortic endothelium. As part of these studies, enzymatic activity was assayed in cultured arterial endothelium using cholesterol as a substrate and isotope ratio gas–liquid chromatography–mass spectrometry to identify the metabolites, 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid, in the medium. Localization of sterol 27-hydroxylase to vascular endothelium indicates intracellular production of the biologically active metabolite 27-hydroxycholesterol.—**Reiss, A. B., K. O. Martin, D. E. Rojer, S. Iyer, E. A. Grossi, A. C. Galloway, and N. B. Javitt,** Sterol 27-hydroxylase: expression in human arterial endothelium. *J. Lipid Res.* 1997. **38:** 1254–1260.

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Sterol 27-hydroxylase is a polyfunctional mitochondrial cytochrome P-450 (1) that is found in the liver and other tissues. In the liver, it initiates the side chain oxidation of C_{27} sterol intermediates in bile acid synthesis derived from the initial 7α-hydroxylation of cholesterol (2).

The cloning of sterol 27-hydroxylase led to characterization of its pattern of expression with the surprising finding that the enzyme is widely distributed in tissues (3). This wide tissue distribution implies that its role in sterol metabolism extends beyond the liver. We previously reported in this journal that bovine aortic endothelial cells synthesize 27-hydroxycholesterol (4). We now report the detection of the mRNA for sterol 27-hydroxylase in human aortic and pulmonary artery endothelium through RT-PCR using a unique oligonucleotide pair. Detection of 27-hydroxylase mRNA and catalytic activity in human arterial endothelium denotes a novel activity in this cell type which may represent a regulatory step in vascular lipid accumulation and atherogenesis.

METHODS

Cell culture

Growth conditions: all cells were grown at 37°C in a 5% CO_2 atmosphere. Growth medium for endothelial cells (ECs) was Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY) that also included 10% fetal bovine serum (FBS), 100 μg/ml penicillin, 100 units/ml streptomycin, 2 mmol/L L-glutamine, 100 μg/ml heparin, and 25 μg/ml endothelial cell growth supplement (Collaborative Biomedical Products, Bedford, MA). The harvesting medium for ECs was the same as the growth medium, except that it contained 20% FBS and 50 μg/ml endothelial cell growth supplement. Human aortic endothelial cells (HAECs) were obtained from Clonetics (San Diego, CA) at passage 3.

Human pulmonary artery endothelial cells (HPAECs) were obtained either from Clonetics or from pulmonary arteries of cadaveric organ donors for whom no tissue-compatible recipient was available. These specimens were a gift from Dr. Edith Robbins. The HPAECs were harvested using sterile technique as fol-

Abbreviations: EC, endothelial cell; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; HAEC, human aortic endothelial cell; HBSS, Hank's balanced salt solution; HPAEC, human pulmonary artery endothelial cell; CHO, Chinese hamster ovary cell.

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lows (5). The pulmonary artery was separated from the lung by dissection and was immediately immersed in ice-cold Hank's balanced salt solution (HBSS) for transport to the tissue culture lab, where it was transferred to a petri dish filled with prewarmed HBSS. It was then opened lengthwise with a scalpel and incubated at 37°C for 10 min in a 0.06% solution of Type I collagenase (Sigma Chemical Co., St. Louis, MO) in HBSS/glucose (0.5 mg/ml). After removal of the artery from the collagenase solution, the HPAECs were collected by repeatedly scraping the artery's intimal surface with a sterile cotton swab and stirring the swab into a tube containing harvesting medium for ECs. The cells were pelleted by centrifugation, resuspended in 3 ml of harvesting medium, plated onto a 60-mm dish, and incubated until confluent. They were then split 1:2, incubated in growth medium for ECs, and passaged until sufficient cells were obtained for analysis. Cells were used at passage 3 or 4. Their identity as vascular endothelial cells was confirmed using Sigma test kits for Factor VIII (present only in vascular endothelium) and α-actin (present only in smooth muscle and not in ECs).

Confluent monolayers of HPAECs and HAECs were rinsed twice with HBSS; then 3 ml of DMEM containing 10% delipidated FBS and either 20 μmol/L cholesterol dissolved in 2-hydroxypropyl-β-cyclodextrin (6) or an equivalent amount of vehicle alone were added to each 60-mm dish. Media and cells were harvested at 72 h. The medium collected from each dish was analyzed for cholesterol metabolites of the sterol 27-hydroxylase. The cells were subjected to mRNA extraction followed by RT-PCR, or to total RNA extraction followed by Northern blotting using the full-length human 27-hydroxylase cDNA as a probe. The protein concentration of cell extracts was measured using the BCA Protein Assay Reagent (Pierce, Rockford, IL).

Sterol 27-hydroxylase expression

The presence of sterol 27-hydroxylase mRNA in cultured HPAECs and HAECs was shown by reverse transcription of mRNA from these sources followed by PCR (7, 8). The design of the protocol takes advantage of the high degree of sequence homology between human and rabbit sterol 27-hydroxylase cDNAs (1). This homology permits a single set of primers to amplify a segment of identical length and nearly identical sequence in both species. A known amount of rabbit sterol 27-hydroxylase mRNA that had been transcribed in vitro was amplified, as were the human 27-hydroxylase mRNAs derived from the specimens of interest. The exogenously synthesized rabbit mRNA served as a standard. The primers used for amplification span a 311 base-pair sequence encompassing nucleotides 491–802 of both the rabbit and human sterol 27-hydroxylase cDNAs. A search of the GenBank (9) confirmed that the primers were homologous only with the sterol 27-hydroxylase sequence. To ensure that only RNA-derived products are of the appropriate size, the amplification product crosses the junction of the third and fourth exons of the genomic sequence (10). The product obtained by direct PCR of genomic DNA is approximately 0.1 kb larger than the RT-PCR product.

Construction of the rabbit 27-hydroxylase mRNA standard

The template for in vitro transcription is a recombinant plasmid called pGEM26-OH that consists of the pGEM4 vector (Promega, Madison, WI) harboring a BAM HI fragment corresponding to nucleotides 7 through 1766 of the rabbit 27-hydroxylase cDNA in the SP6 orientation (gift from Dr. D. Russell) (3). This plasmid was linearized by digestion with the restriction enzyme Hind III and then transcribed in vitro using the Riboprobe II Core System (Promega, Madison, WI). Concentration of the mRNA was determined to be 140 ng/μl by ultraviolet absorption spectrophotometry.

Oligonucleotide synthesis

Oligonucleotide primers were synthesized by the New York University General Clinical Research Center using an Eppendorf D300 DNA synthesizer (Eppendorf, Madison, WI).

The composition of the 20-mer oligonucleotide sequences is as follows (A = adenine, G = guanine, T = thymine, C = cytosine):

Upstream primer:
5′ TGCGCCAGGCTCTGAACCAG 3′

Downstream primer:
5′ TCCACTTGGGGAGGAAGGTG 3′

Isolation of sample RNA and DNA

mRNA from HPAECs, HAECs, Chinese hamster ovary (CHO) cells, and HepG2 cells was prepared using the Micro-FastTrack mRNA Isolation Kit Version 2.0 (Invitrogen, San Diego, CA). Total RNA was isolated using the RNeasy Total RNA Kit (QIAGEN Inc., Chatsworth, CA) and genomic DNA was prepared using the Easy-DNA Genomic DNA Isolation Kit (Invitrogen, San Diego, CA).

Reverse transcription

All reagents were supplied by Perkin Elmer Cetus (Norwalk, CT) and all reverse transcription and PCR reactions took place in a DNA Thermal Cycler 480 (Perkin Elmer Cetus, Norwalk, CT).

For each assay 0.5 μg of mRNA was reverse transcribed using 50 U of Moloney Murine Leukemia Virus

reverse transcriptase in the presence of 20 U of RNase inhibitor. The reaction mixture contained 5 mM $MgCl_2$, PCR buffer, and 1 mM each of dATP, dGTP, dCTP, and dTTP, total volume 20 μl. The reaction was primed with the specific 27-hydroxylase downstream primer. Reaction conditions were 42°C for 25 min, followed by 99°C for 5 min to inactivate the reverse transcriptase, and finally cooling to 5°C for 5 min.

Polymerase chain reaction

Amplification of the specific segment of the reverse-transcribed cDNA required an adjustment of buffer conditions and the addition of AmpliTaq polymerase (2.5 U/reaction) and the sterol 27-hydroxylase upstream primer. The dNTPs and downstream primer remained from the previous reverse transcription reaction. The final PCR amplification occurred in a volume of 100 μl, a final $MgCl_2$ concentration of 2.0 mM, and a final upstream primer concentration of 1.5 ng/μl (150 ng/tube). The tubes were then heated to 94°C for 2 min to achieve a "hot start" for the PCR by melting the Ampliwax PCR Gem 100 that had been added to separate the AmpliTaq polymerase from other components of the mixture. A "hot start" was especially desirable because of the relatively high guanine and cytosine content of the primers. Amplification proceeded for 35 cycles, each consisting of 1 min at 94°C for denaturation, 1 min at 62°C for annealing, and 1 min at 72°C for extension. Cycling was followed by a final 7-min soaking at 62°C.

Separation quantification and sequencing of PCR products

PCR products were loaded directly onto a 1% agarose gel and electrophoresed at 5 V/cm for 1.5–2 h. DNA was visualized and photographed under ultraviolet light (320 nm) after ethidium bromide staining. The 310 base pair amplification product derived from HAECs was gel purified and both strands were sequenced using the DyeDeoxy Terminator Cycle Sequencing Kit (Perkin-Elmer/Applied Biosystems Division, Foster City, CA).

PCR of genomic DNA

PCR amplification of the targeted segment of the 27-hydroxylase gene was carried out using 20 ng of genomic DNA isolated from each of the following cell types: HAECs, HPAECs, CHO, and HepG2. The reactions took place in a final volume of 100 μl, in a $MgCl_2$ concentration of 2.5 mM with 200 μM dNTPs, PCR buffer, and a concentration for both upstream and downstream primer of 1.5 ng/μl. Five units/100 μl of AmpliTaq DNA polymerase was added over a layer of AmpliWax according to manufacturers directions in order to achieve a "hot start" for the PCR. PCR reaction conditions were identical to those described above for the PCR portion of the RT-PCR.

Northern blot analysis

Fifteen μg of total cellular RNA from each sample of HAECs and HPAECs was analyzed for the presence of the 27-hydroxylase message by Northern blot hybridization after electrophoresis through a 1% formaldehyde gel. The RNA was transferred from the gel to a nylon filter by turboblotting and the RNA was UV-linked. The hybridization probe consisted of the full-length 1.8 kb human 27-hydroxylase cDNA inserted in the pCMV expression vector (1). Approximately 30 ng of this probe was random primed with ^{32}P dCTP to a specific activity of 2.2×10^9 dpm per μg. After a 2-h prehybridization at 42°C in hybridization buffer (50% formamide, 6 × SSPE, 0.5% SDS, 5 × Denhardt's solution and 0.3 mg/ml of sheared herring testes denatured DNA), the filter was probed with the radiolabeled denatured plasmid in 10 ml of hybridization buffer at 1×10^7 dpm per ml for 18 h at 42°C. The filter was then washed three times (25 min per wash) in 1 × SSC with 0.1% SDS at 50°C. Finally, the filter was exposed for 7 days using Kodak XOMAT AR film.

Sterol 27-hydroxylase activity

Media collected at the completion of each study were analyzed for 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid content using methods previously described in detail (11). In brief, deuterated internal standards of 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid (500 ng) were added to 1-ml aliquots of medium, which were saponified and then brought to pH 1 before extraction of the steroids into ethyl acetate. The dried extract was applied to a silica gel G plate. After development (chloroform–acetone 97:3, v/v), which removes cholesterol and permits elution of each compound of interest using authentic standards to identify the appropriate zones, the eluates were taken to dryness and methylated with diazomethane and/or acetylated with pyridine/acetic anhydride. Isotope ratio mass spectrometry was done using a Hewlett-Packard GLC-MS (Model #5890-5970) and a fused silica capillary column (CP-sil 19 CB, 0.25 mm i.d., 25 m length; Chrompack, Raritan, NJ). For 27-hydroxycholesterol diacetate the ratio of the areas m/z 426 (protium) to m/z 430 (deuterated) was used to calculate the mass, and for the C_{27} acid the respective pair was m/z 412/m/z 418.

RESULTS

Figure 1 shows the presence of a band on a 1% agarose gel after RT-PCR of mRNA isolated from HPAECs

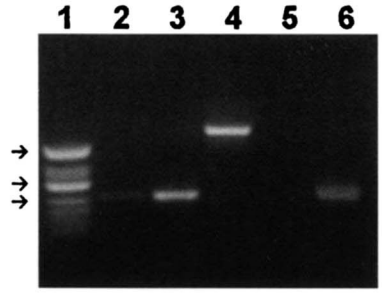

Fig. 1. Agarose gel electrophoresis of the product after RT-PCR with sterol 27-hydroxylase-specific primers of mRNA from HepG2 cells (lane 3), CHO cells (lane 5), and HPAECs (lane 6). Lane 2 is a positive control consisting of the product of RT-PCR with sterol 27-hydroxylase-specific primers of 0.02 ng of rabbit sterol 27-hydroxylase mRNA in vitro transcribed from a recombinant plasmid harboring the sterol 27-hydroxylase cDNA. Lane 4 contains the product after RT-PCR with β-actin-specific primers of mRNA from CHO cells. Molecular weight standards in lane 1 are indicated by arrows from top to bottom: 1300, 600, and 270 base pairs.

(lane 6) using sterol 27-hydroxylase-specific primers that were constructed to amplify a 311 base-pair fragment spanning nucleotides 491–802. The band is identical in size to that obtained with mRNA derived from HepG2 cells (lane 3) but is not found after RT-PCR using mRNA derived from CHO cells (lane 5). Using oligonucleotide primers specific for β-actin sequence, a band was found in CHO cells (lane 4).

Figure 2 illustrates the presence of the sterol 27-hydroxylase-specific band after RT-PCR of mRNA derived from HAECs, HPAECs, and HepG2 cells along with their corresponding β-actin controls.

The 310 base-pair amplification product derived from HAECs was gel-purified and both strands were sequenced and found to be 100% homologous to the published sequence (**Fig. 3**).

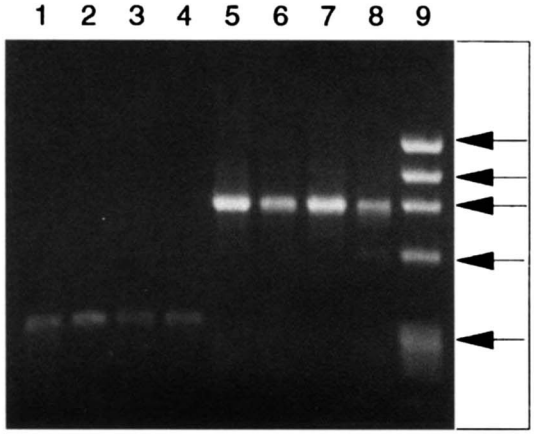

Fig. 2. Amplification product after RT-PCR with sterol 27-hydroxylase primers of mRNA from HPAECs (lane 1), two different samples of HAECs (lanes 2 and 3), and HepG2 cells (lane 4). Lanes 5 through 8 contain the product after RT-PCR with β-actin-specific primers of mRNA corresponding to lanes 1 through 4. Molecular weight standards in lane 9 are indicated by arrows from top to bottom: 1353, 1078, 872, 603, and 270 base pairs.

Figure 4 illustrates that the product of RT-PCR of genomic DNA with the 27-hydroxylase primers is clearly larger than the product obtained by RT-PCR using a mRNA template. The 0.1 kb difference in the size of the amplified fragment is exactly as predicted based on the characterization of the genomic DNA by Leitersdorf et al. (10).

Figure 5 is a Northern blot analysis of total RNA isolated from HAECs and HPAECs after a 24-h incubation in medium containing either delipidated serum and 20 μM cholesterol or delipidated serum and vehicle (2-hydroxypropyl-β-cyclodextrin) alone. The probe used in these studies was a ^{32}P-radiolabeled double-stranded plasmid consisting of a pCMV vector harboring the full-length human 27-hydroxylase coding sequence. A single 2.0 kb species of sterol 27-hydroxylase mRNA is detected, just as it was by Cali et al. (12) in their Northern analysis of COS cells transfected with the 27-hydroxylase cDNA. The relative amounts of 27-hydroxylase mRNA detected in both the HAECs and HPAECs are approximately equal independent of whether cholesterol was added to the media. Thus, the higher conversion of cholesterol to 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid in the presence of cholesterol is likely substrate-driven and not a result of transcriptional upregulation.

Table 1 indicates that sterol 27-hydroxylase metabolites of cholesterol were found in the medium after a 72-h incubation in the presence of either HAECs or HPAECs. The amounts of both 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid were greater when cholesterol was added to the medium.

DISCUSSION

Initial interest in a sterol 27-hydroxylase related to the synthesis of bile acids from cholesterol in the liver. The finding of a mitochondrial 27-hydroxylase with broad substrate specificity for all the 7α-hydroxylated C_{27} steroid intermediates that are generated in the liver provided the basis for the currently held concept of hepatic bile acid synthesis (13). However, it was known that cholesterol is also a substrate for this enzyme but, because of its very low turnover rate compared with the 7α-hydroxylated steroid intermediates, it was not considered of physiologic importance in the metabolic pathway for bile acid synthesis in the liver (2).

The demonstration by Northern blot analysis that the 27-hydroxylase enzyme is present in organs not known to produce 7α-hydroxylated C_{27} sterol intermediates (3) justifies a renewal of interest in the cholesterol-metabo-

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A TGCGCCAGGCTCTGAACCAGCGGTTGCTGAAGCCAGCGGAAGCAGCGCTC 540
B tgcgccaggctctgaaccagcggttgctgaagccagcggaagcagcgctc

A TATACGGATGCTTTCAATGAGGTGATTGATGACTTTATGACTCGACTGGA 590
B tatacggatgctttcaatgaggtgattgatgactttatgactcgactgga

A CCAGCTGCGGGCAGAGAGTGCTTCGGGGAACCAGGTGTCGGACATGGCTC 640
B ccagctgcgggcagagagtgcttcggggaaccaggtgtcggacatggctc

A AACTCTTCTACTACTTTGCCTTGGAAGCTATTTGCTACATCCTGTTCGAG 690
B aactcttctactactttgccttggaagctatttgctacatcctgttcgag

A AAACGCATTGGCTGCCTGCAGCGATCCATCCCCGAGGACACCGTGACCTT 740
B aaacgcattggctgcctgcagcgatccatccccgaggacaccgtgacctt

A CGTCAGATCCATCGGGTTAATGTTCCAGAACTCACTCTATGCCACCTTCC 790
B cgtcagatccatcgggttaatgttccagaactcactctatgccaccttcc

A TCCCCAAGTGGA 802
B tccccaagtgga
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Fig. 3. Comparison of the nucleotide sequence of the 310 base-pair fragment generated by RT-PCR of mRNA derived from HAECs with 27-hydroxylase-specific primers (panel A) to the published sequence for nucleotides 491 to 802 of the human sterol 27-hydroxylase cDNA (panel B). The 5′ underlined sequence marks the location of the sense primer while the 3′ underlined sequence marks the location of the antisense primer. Portion of sequence in panel A confirmed by both upstream and downstream primers is in **boldfaced** letters while portion of 5′ and 3′ sequence derived from only the downstream or upstream primer, respectively, is in regular typeface. Nucleotides are numbered on the right.

lizing capacity of sterol 27-hydroxylase. Our finding that 27-hydroxycholesterol accumulates in the medium of bovine aortic cells in culture (4), particularly when cholesterol is added to the medium, indicated the existence of enzyme activity as this sterol, in contrast to 25-hydroxycholesterol, is not generated by autooxidation (14). Other than our studies, the only published data on 27-hydroxylase activity in specific cell types demonstrated an activity in human alveolar macrophages of approximately 15–20 μg 27-hydroxycholesterol/10^6 cells per 48 h (15). This represents a 50-fold greater activity than that found in either arterial endothelium or HepG2 cells. The reason for this high capacity of alveolar macrophages to convert cholesterol to 27-hydroxycholesterol is not known.

Our current findings using RT-PCR to detect 27-hydroxylase mRNA establish that the enzyme initially detected in the liver is also present in vascular endothelium. The finding of the metabolites 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid further establishes that the mRNA is being transcribed

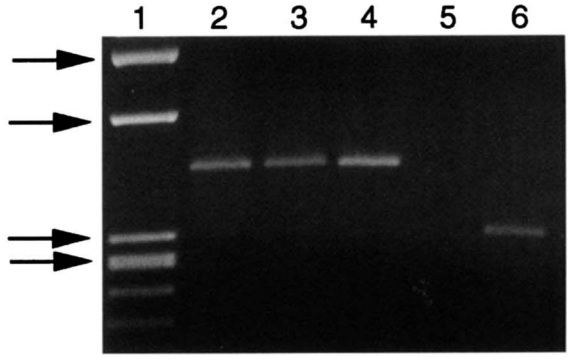

Fig. 4. PCR amplification using 27-hydroxylase primers of genomic DNA derived from HAECs (lane 2), HPAECs (lane 3), HepG2 cells (lane 4), and CHO cells (lane 5). For size comparison, the 310 base-pair RT-PCR product with 27-hydroxylase-specific primers of pure rabbit sterol 27-hydroxylase mRNA is shown (lane 6). Molecular weight standards (lane 1) are indicated by arrows from top to bottom: 872, 603, 310, and 270 base pairs.

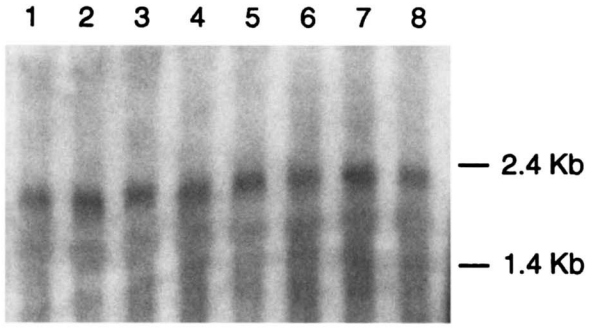

Fig. 5. Northern blot hybridization analysis of HAEC and HPAEC total RNA. Fifteen μg of total RNA was isolated from four individual samples of HAECs (lanes 1–4) and four individual samples of HPAECs (lanes 5–8). Lanes 1 and 2 show total RNA from HAECs after a 24-h incubation in media containing cholesterol to a concentration of 20 μM. Lanes 3 and 4 show total RNA from HAECs after a 24-h incubation in media without cholesterol-containing vehicle (2-hydroxypropyl-β-cyclodextrin) alone. Lanes 5 and 6 show total RNA from HPAECs after a 24-h incubation in media containing cholesterol to a concentration of 20 μM. Lanes 7 and 8 show total RNA from HAECs after a 24-h incubation in media without cholesterol-containing vehicle (2-hydroxypropyl-β-cyclodextrin) alone. The total RNA samples were electrophoresed, blotted, and probed with a pCMV recombinant plasmid harboring the full-length human 27-hydroxylase cDNA, as described in Materials and Methods. The numbers on the right side indicate the positions of RNA size markers.

to produce the enzyme that utilizes the cholesterol that was added to the medium.

It is beyond the scope of these studies to define further the source of cholesterol for the enzyme in the vascular endothelium. We have utilized hydroxypropyl-β-cyclodextrin as a vehicle for adding cholesterol to the medium (6). It is known to enhance apparent enzyme activity when cholesterol is the substrate (11). In considering the possible physiologic implications, it would be of interest to know whether apoproteins play a specific role in delivering cholesterol to the enzyme site. As part of these considerations it is also necessary to consider the role of various cholesterol carrier proteins that may occur intracellularly (16). In particular, the newly identified steroidogenic acute regulatory (StAR) protein, specific for cholesterol as a ligand and essential for normal adrenal hormone synthesis (17), has also been found to enhance 27-hydroxycholesterol synthesis in a doubly transfected cell model (18). Thus, to define fully the physiologic role of the enzyme in vascular endothelium, further information is needed.

Although a role for the sterol 27-hydroxylase in atherogenesis has not been established experimentally, we hypothesize that 27-hydroxylase activity in arterial endothelium provides a local mechanism of defense against cholesterol accumulation within the arterial wall by serving as a pathway for elimination of intracellular cholesterol by conversion to more polar metabolites. These metabolites are then transported to the liver for further metabolism to bile acids. The importance of local 27-hydroxylase activity has only recently been recognized due to the discovery of 27-hydroxylase expression in extrahepatic tissues including macrophages (15) and vascular endothelial cells (4). An example of the importance of local effects of 27-hydroxylase activity is in the study of the neurologic manifestations of the disease cerebrotendinous xanthomatosis (CTX) (12) which results from the lack of a functional 27-hydroxylase enzyme. Manifestations of this autosomal recessive genetically determined deficiency (19,20) range from accelerated atherosclerosis to progressive neurologic impairment. Initially, when it was thought that the 27-hydroxylase was expressed exclusively in the liver, the clinical manifestations of CTX were attributed solely to the high rates of cholestanol and cholesterol synthesis in the liver followed by the transport and deposition of these sterols in other tissues. Administration of chenodeoxycholic acid to CTX patients reduces the high rates of hepatic cholestanol and cholesterol synthesis and reduces xanthoma formation (21, 22). However, this form of therapy has been less useful for the management of the neurologic symptoms (23, 24). It is important to our understanding of the pathogenesis of CTX to distinguish the biologic effects of lack of local 27-hydroxycholesterol synthesis from the high rates of cholesterol production in the liver. The specific probes that we have designed and validated provide a tool for evaluating the

TABLE 1. Cholesterol metabolites of the sterol 27-hydroxylase

Cell Type	Culture Medium	27-Hydroxycholesterol		3β-Hydroxy-5-Cholestenoic Acid	
HAEC	Delipidated FCS	301.5 ± 41.5	(2)	6.7	(1)
HAEC	+ cholesterol[a]	1672.0 ± 156.1	(6)	46.8 ± 3.7	(3)
HPAEC	Delipidated FCS	233.1 ± 45.1	(3)	5.7 ± 1.5	(3)
HPAEC	+ cholesterol[a]	932.8 ± 37.2	(9)	43.0 ± 9.6	(9)
HepG2	+ cholesterol[a]	1622.1 ± 130.1	(6)	471.1 ± 56.4	(6)
CHO	+ cholesterol[a]	not detected	(3)	2.2 ± 0.71	(3)

Medium was collected after a 72-h incubation and analyzed for cholesterol metabolites by isotope ratio mass spectrometry using deuterated internal standards. Values given as mean ± standard error of the mean. Number of studies in parentheses.

[a]Twenty nmol/ml of medium.

expression of the enzyme in different tissues and therefore the possibility of distinguishing between the metabolic role of sterol 27-hydroxylase in the liver and in other tissues that express the enzyme.

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