Regulation of human apolipoprotein A-I expression in Caco-2 and HepG2 cells by all-*trans* and 9-*cis* retinoic acids

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Abstract Retinoids are reported to stimulate apolipoprotein (apo) A-I gene promoter activity (Rottman et al. 1991. *Mol. Cell. Biol.* **11:** 3814–3820) and apoA-I protein secretion by monkey hepatocytes (Kaptein et al. 1993. *Arterioscler. Thromb.* **13:** 1505–1514). In this study we have assessed the effects of retinoids on parameters of apoA-I biosynthesis in human cell lines. Caco-2 and HepG2 cells (human intestinal and hepatoma cell lines, respectively, both known to express and secrete apoA-I) were stably transfected with a reporter gene construct containing 1.3 kb of the 5-' flanking region of the human apoA-I gene linked to the firefly luciferase coding region. These cells were incubated for 48 h with 10 μM all-*trans* retinoic acid (RA) or 9-*cis* RA. The cells were then assayed for luciferase activity, for apoA-I mRNA level, and for secretion of apoA-I protein in the medium. Secretion of apoB was monitored as well. In Caco-2 cells, all-*trans* and 9-*cis* RA increased luciferase activity, mRNA content, and protein secretion by 40% to 80% above control. Strikingly, in HepG2 cells all-*trans* and 9-*cis* RA caused a more marked stimulation of luciferase activity (by 100–150%) but a weaker increase of mRNA content and protein secretion (by 25–30%). In contrast, apoB secretion was inhibited by the two retinoids in Caco-2 cells and not changed in HepG2 cells. We conclude *i)* that retinoids can activate apoA-I gene transcription and protein secretion in these human cell lines, and *ii)* that in HepG2 cells (in contrast to Caco-2 cells) apoA-I promoter activation by these retinoids does not correlate with their final effect on apoA-I protein production.—**Giller, T., U. Hennes, and H. J. Kempen.** Regulation of human apolipoprotein A-I expression in Caco-2 and HepG2 cells by all-*trans* and 9-*cis* retinoic acids. *J. Lipid Res.* 1995. **36:** 1021–1028.

Supplementary key words retinoids • apoA-I gene promoter activity • apoA-I mRNA • luciferase • nuclear run-on assay

The plasma levels of HDL-cholesterol and apoA-I, its major protein constituent, are inversely correlated to the incidence or severity of coronary heart disease (1–3). In adult organisms, apoA-I is synthesized and secreted by liver and small intestine. Transgenic mice overexpressing apoA-I have higher plasma levels of HDL and apoA-I, and are protected against the atherogenic effect of a high-cholesterol diet (4), suggesting that up-regulation of apoA-I synthesis may afford a new approach for the prevention or therapy of atherosclerotic diseases.

Within the promoter region of the apoA-I gene, different regions have been identified as essential for transcriptional activity (5–8). An enhancer required to express the gene in liver cells is located in this apoA-I promoter between positions −220 and −110 with respect to the transcriptional start site (9). However, for efficient expression of the human apoA-I gene in intestinal epithelial cells in transgenic mice, an element within the promoter region of the adjacent apoC-III gene (located 3' of the apoA-I gene) was found to be needed as well (10). Within the hepatic enhancer, three regions were specified that are simultaneously required for promoter activation. Among these, the best characterized is site A (−214 to −190 bp), a DNA sequence of 24 bp containing the motif TGAACCctTGACCC. This element was characterized as a retinoic acid receptor responsive element (RARE) (11), which opened up the possibility that plasma levels of HDL and apoA-I may be controlled by a retinoid.

Biological effects of retinoids and expression of RA-responsive genes are mediated by different receptors, namely RARα, RARβ, RARγ, as well as by RXRα, RXRβ and RXRγ, in homodimeric or heterodimeric constellations (12). In transient transfection experiments, the RARE in the apoA-I gene promoter was described to be activated more strongly by RXRα than by RARα or RARβ (11). In transactivation assays, RARs can be activated by all-*trans* RA and 9-*cis* RA whereas RXRs are potentially activated by 9-*cis* RA (13–16) and not (15) or with much less potency (16) by all-*trans* RA. The RARE present in the apoA-I promoter can bind both RAR-RXR heterodimers and RXR-RXR homodimers (17), and in transfected cells this element responds to RXR-selective

Abbreviations: HDL, high density lipoprotein; apo, apolipoprotein; APL, apoA-I promoter/luciferase reporter plasmid; CAT, chloramphenicol acetyl transferase; DMSO, dimethyl sulfoxide; FCS, fetal calf serum; PCR, polymerase chain reaction; RA, retinoic acid; RARE, retinoic acid responsive element.

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retinoids when RX receptors are cotransfected (18). Effects of RAR-selective retinoid agonists so far have not been described.

Recently, direct evidence for stimulation of apoA-I production by retinoids has been reported. In cultured cynomolgus monkey hepatocytes, Kaptein, De Wit, and Princen (19) found apoA-I secretion to be stimulated 2- to 3-fold by all-*trans* RA, 9-*cis* RA, and various synthetic retinoids, paralleled by a similar increase in mRNA content and in the rate of apoA-I gene transcription. Second, Katocs, Largis, and Karathanasis (20) reported that plasma levels of apoA-I and HDL-cholesterol were increased in rabbits after oral administration of all-*trans* RA.

It was the aim of the present study to determine whether and to what extent retinoids can stimulate apoA-I promoter activity, mRNA content, and protein secretion by human cells. We first assessed whether retinoids could increase expression of luciferase from a construct containing the coding region of luciferase under the control of the apoA-I promoter. (This construct was stably transfected in Caco-2 and HepG2 cells in order to get a screening tool for rapid identification of retinoids and other compounds able to activate the apoA-I gene promoter.) Luciferase activity was increased to a moderate extent by all-*trans* and 9-*cis* RA in both transfected cell lines, stimulation being stronger in HepG2 cells. The level of apoA-I mRNA and secretion of apoA-I were stimulated to a similar degree as the luciferase activity in Caco-2 cells but to a lesser degree in HepG2 cells. In contrast, secretion of apoB was suppressed (Caco-2 cells) or not affected (HepG2 cells).

MATERIALS AND METHODS

Materials

All-*trans* RA was from Sigma and 9-*cis* RA was a gift from Dr. M. Klaus, Hoffmann-La Roche. HepG2 cells and Caco-2 cells were obtained from ATCC, Rockville, MD). Suppliers of other reagents are given in the Methods sections below.

DNA constructs, plasmid isolation, and sequencing

A 1.3-kb apoA-I promoter fragment was amplified with the PCR technique using human genomic placental DNA (Oncogene Science Inc.) as template and the synthetic oligonucleotides 5'-aagcttgtgctgcaaacaccaacgg-3' and 5'-agatctgaagggccgtgggggacc-3' as primers. For subcloning the primers were designed with an upstream HindIII and a downstream BglII restriction site. After 20 cycles the DNA fragment was separated on a low-melt agarose gel, purified, and cut with HindIII and BglII. To construct the reporter plasmid APL (apoA-I-promoter-luciferase cDNA) this fragment was inserted into the vector pBluc2 cut with the same restriction enzymes. The plasmid pBluc2, created by replacing the CAT coding sequence from pBLCAT (21) by the coding sequence of firefly luciferase as reporter gene, was kindly provided by Dr. W. Hunziker, F. Hoffmann-La Roche, Basel. Four arbitrarily chosen APL clones were sequenced using the UBS sequenase DNA sequencing kit (United States Biochemical Corporation, Cleveland, OH). The apoA-I exon 3 DNA fragment used as probe for the northern blot analysis was isolated by the PCR method from human genomic DNA purified from Caco-2 cells, using the oligonucleotides 5'-ctgggacagcgtgacctc-3' and 5'-tctgagcaccgggaaggg-3' as primers. The DNA fragment was ligated into the SmaI site of the vector pSPT19 to create the plasmid papoex3. The plasmid pHcGAP carrying the human glyceraldehyde-3-phosphate dehydrogenase cDNA (GAPDH) was purchased from the American Type Culture Collection (ATCC number 57090). A 780 bp PstI/XbaI fragment isolated from low-melting agarose was used for labeling.

Cell culture and isolation of stably transfected Caco-2 and HepG2 cells

Caco-2 and HepG2 cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS), 100 units/ml penicillin, and 100 μg/ml streptomycin, in an atmosphere of 5% CO_2 in air. Cells were split 24 h prior to transfection. Caco-2 cells grown in petri dishes (9 cm) were transfected with 8 μg reporter plasmid APL and 0.5 μg selection plasmid pSV2neo providing geneticin (G-418) resistance to cells, using Transfectam (Sepracore, France) as described earlier (22). After 1 h the cells were incubated again in full growth medium, which was then replaced every 48 h by growth medium containing 800 μg/ml G-418 sulfate (Gibco BRL). Colonies of surviving cells were picked after 3 weeks and split at low density to run another round of clonal isolation. Three stably transfected cell lines called Caco-2/7B5, 7B6, and 7B7 were established. The line 7B6 was chosen for these experiments and was propagated over 1 year without losing luciferase activity.

HepG2 cells were transfected with 6 μg APL and 0.5 μg pSV2neo per 9-cm dish, by the $CaPO_4$ method, using the ProFection Kit of Promega. Selection was made with 600 μg/ml G-418 for 4 weeks using medium that was conditioned for 24 h by HepG2 cells. Three stably transfected clones referred to as 10D1, 10D2, and 11A1 were established, of which 10D2 was chosen for further in-depth experiments.

Substance application

Retinoids were tested on confluent Caco-2/7B6 cells precultured for 12 days or HepG2/10D2 cells grown for 5 days, in six-well plates (Costar, precoated with gelatine for HepG2 cells). After 24 h preincubation in DMEM without phenol red and containing 10% FCS hormone

depleted by charcoal treatment (medium A), the retinoids were diluted into medium A from 10 mM stock solutions in DMSO. From a six-well plate three wells were used as controls (DMSO) and three for one of the retinoids tested.

Assay systems: luciferase, nuclear run-on, northern blot analysis, ELISA

To determine luciferase activity, cells were washed once in PBS and lysed in 100 mM potassium phosphate buffer (pH 7.8) supplemented with 0.1 mM DTT, by three cycles of freezing and thawing. Lysates were centrifuged for 10 min at 12,000 rpm to precipitate cell debris and 15 μl of the cleared supernatant was analyzed in a Luminoscan apparatus (Labsystems) by injecting 100 μl of luciferase assay buffer (Promega) and measuring light production for 10 sec. None of the compounds tested directly interfered with the luciferase reaction. Protein concentration of the lysates was measured using the BCA protein assay reagent (Pierce) and the specific luciferase activity was calculated. Nuclear run-on assays were performed essentially as described by Kaptein et al. (19).

Total cellular RNA was isolated from cells by the method of Chomczynski and Sacchi (23), by adding 0.5 ml of the guanidinium thiocyanate lysis buffer directly to one 3-cm culture dish. Six μg of total cellular RNA was separated on a 1.5% agarose gel containing 2.2 M formaldehyde and electrotransferred to Hybond N nitrocellulose membranes (Amersham). After 5 min UV fixation, the membranes were prehybridized at 42°C for 1 h in a buffer containing 50% deionized formamide, 5× SSC, 50 μg/ml sheared herring sperm DNA, 0.1% SDS, 1 mg/ml Ficoll 400, and 1 mg/ml polyvinylpyrolidone. Probes were created by labeling DNA fragments of the apoA-I and GAPDH genes (obtained as described above) with [α^{32}P]dCTP (Amersham), using a random priming labeling kit (Prime-It, Stratagene). The probes were boiled for 5 min and added to prehybridization buffer that was supplemented with 1/4 volume of 50% dextransulfate. The filters were hybridized at 42°C for 48 h, and finally washed with a buffer containing 0.1 × SSC, 0.1% SDS at 60°C for 90 min. Signal quantification was done using a Phosphorimager (Molecular Dynamics).

Concentrations of human apoA-I and human apoB in conditioned cell culture media were determined by enzyme-linked immunosorbent assays (ELISA), based on the solid phase sandwich principle and performed in a single-step procedure. Appropriately diluted media samples, in parallel with plasma standards and controls, were added to microtiter plates precoated with either sheep anti-human apoA-I or sheep anti-human apoB IgG (The Binding Site, Birmingham UK). Incubation overnight was carried out together with the same soluble horseradish peroxidase-conjugated immunoglobulin. After washing, the enzymatic reaction was carried out using tetramethylbenzidine-H_2O_2 as substrate mixture. The absorbance at 405 nm was measured photometrically within 15 min against standards with known concentrations of pure human apoA-I or human apoB run in parallel with the media samples (Roche Diagnostics, Kaiseraugst, Switzerland). The linear portion of the standard curve was used for linear interpolation of the unknown values. The assay methods had an intra-assay coefficient of variation of 5% for apoA-I and of 7% for apoB. Specificity of the antibodies was verified by immunoprecipitation experiments using conditioned medium of HepG2 cells incubated with [^{35}S]methionine. The immune-complexes were collected using protein A, and electrophoresed by SDS-PAGE. Radiolabeled bands in the gel, detected by the Phosphorimager, were only at 28 kD (apoA-I) with the anti-apoA-I IgG, and only at 250 (unspecific) and 500 kD (apoB) with the anti-apoB IgG.

Calculation and statistical evaluation

As mentioned above, on each of the six-well plates there were triplicate incubations for control (DMSO) and for a substance. The means of these triplicates were considered as a single experimental value, and the mean for the retinoid tested on a plate was expressed as percentage of the mean of the controls on the same plate. For each of the retinoids at least six of such percentage values were obtained in separate experiments on different days. To judge whether a retinoid had any effect, it was tested when the mean of these percentage values was different from 100 (Student's *t*-test for paired observations). To judge whether effects were different between the two retinoids tested, an unpaired *t*-test of these percentage values was performed. We used the program Statview 4.0 (Abacus Concepts, Berkeley, CA) on a Macintosh IIcx computer.

RESULTS

Isolation of the human apoA-I promoter fragment and characterization of stably transfected cell lines

A 1.3 kb DNA fragment (sequence deposited in EMBL database, see ref. 24) containing specific promoter motifs, the transcriptional start site, and the first intron of the apoA-I gene was amplified by PCR technology from human genomic DNA. Sequencing of four independent clones obtained revealed that three were identical and the fourth had a one base pair exchange as compared to the first three. All four differed in seven positions from the sequence published by Higuchi et al. (25). The changes are not located in the promoter elements identified thus far including the RARE (11) but their influence on transcriptional activity was not analyzed.

We established three stably transfected lines of both Caco-2 and HepG2 cells that all expressed luciferase activity. We confirmed by Southern blotting that a copy of

the reporter cassette from the plasmid APL was integrated into the genome, without affecting the integrity of the endogenous apoA-I gene (data not shown).

Effect of all-*trans* RA on apoA-I promoter-driven luciferase activity in stably transfected cell lines

In preliminary experiments we tested the ability of all-*trans* retinoic acid and of other retinoids to stimulate luciferase expression with all stably transfected cell lines of Caco-2 or HepG2 origin. Although there were some differences in the absolute levels of luciferase activity, the relative increases upon treatment with retinoids was similar among the three cell lines of either origin (data not shown). We selected the lines Caco-2/7B6 and HepG2/10D2 for the experiments described in this paper.

Next, the time- and concentration-dependency of luciferase activation by all-*trans* RA in the Caco-2/7B6 cells were assessed. For that purpose cells were seeded in 96-well plates and grown for 8 days until confluence. As shown in **Fig. 1A,** stimulation of luciferase activity started to become visible after 24 h incubation in the presence of 10 μM all-*trans* RA, and then increased with time in a linear way without reaching a plateau even after an incubation period of 96 h. Similar results were obtained with HepG2/10D2 cells (not shown). As shown in Fig. 1B, the stimulation of luciferase expression in Caco-2/7B6 cells by all-*trans* RA was only observed with concentrations of 1 μM or higher.

Effect of all-*trans* RA and 9-*cis* RA on parameters of apoA-I expression and on apoB secretion in Caco-2/7B6 cells or HepG2/10D2 cells

The time course of apoA-I and apoB secretion by the two cell lines during a 48-h incubation period is shown in **Fig. 2.** ApoA-I was secreted by both Caco-2/7B6 and HepG2/10D2 cells at a nearly steady rate during this period. The rate of apoB secretion by the Caco-2/7B6 cells was slow during the first 6 h but then increased and was constant for the remaining period; in contrast, apoB secretion by HepG2 cells was stable during the first 30 h but then rapidly decreased to zero. As shown in the figure, HepG2 cells secrete about 2-fold more apoA-I and 3-fold more apoB than Caco-2/7B6 cells at comparable total cell protein per well in the first 24-h period.

After having found that all-*trans* RA is an activator of the apoA-I promoter in these cell lines, we checked whether this translated into an increase of the apoA-I mRNA content and secretion of apoA-I protein by these cells. We compared the effects of all-*trans* RA with those of 9-*cis* RA, the retinoic acid isomer with high activating potency on RXR-type receptors (13–16). Secretion of apoB was also measured in order to assess the specificity of the RA effect. These studies were done in six-well plates to obtain sufficient material for RNA determination.

As shown in **Fig. 3,** all-*trans* RA and 9-*cis* RA at 10 μM had similar stimulatory effects on luciferase activity,

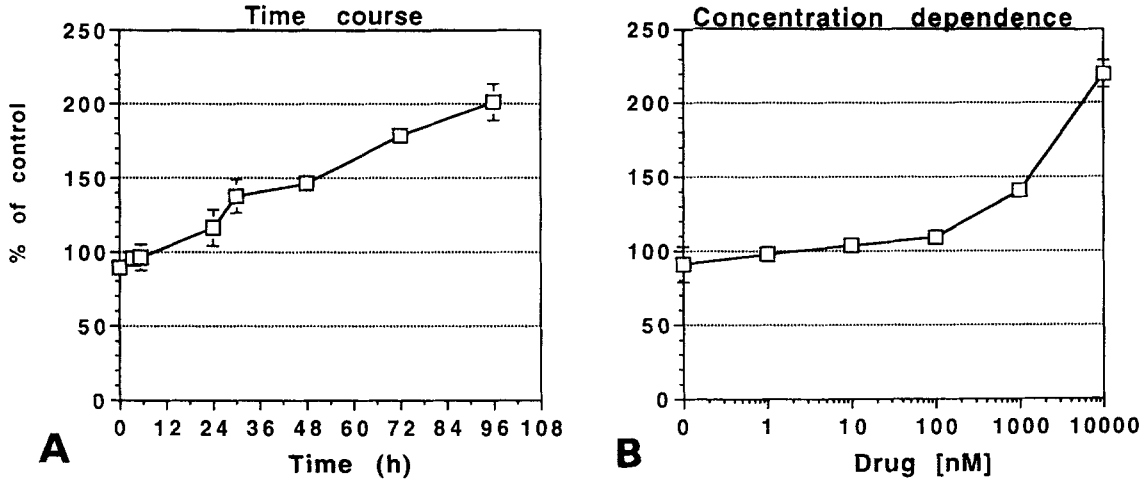

Fig. 1. Effect of all-*trans* RA on luciferase activity in transfected Caco-2 cells. A: Caco-2/7B6 cells which were stably transfected with the apoA-I-luciferase reporter construct were grown to confluence for 8 days. After 24 h adaptation to the incubation media, the cells were incubated for various periods with 10 μM all-*trans* RA or with 0.1% DMSO as control. The stimulation of luciferase activity by RA (percent of DMSO control) as function of incubation time is shown. Mean and standard error of triplicates were determined as described in materials and methods. B: Caco-2/7B6 cells grown as described above where incubated for 72 h with increasing concentrations of all-*trans* RA. The specific luciferase activity is displayed as percent of control. The data points represent means and standard errors for five separate incubations.

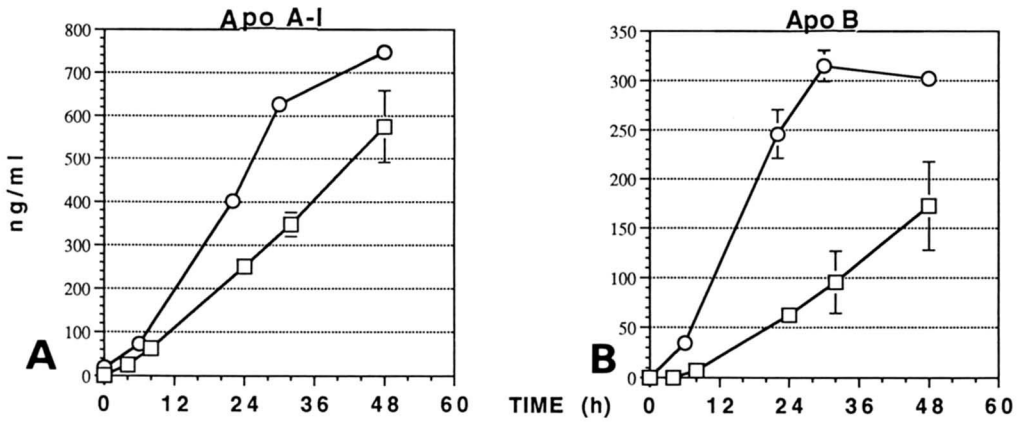

Fig. 2. Time course of apoA-I and apoB secretion by Caco-2 and HepG2 cells. The time course of apolipoprotein A-I and B secretion by the stably transfected Caco-2 and HepG2 cells was investigated in 6-well plates. Caco-2/7B6 cells were precultured for 12 days, HepG2/10D2 cells for 7 days. Then the cells were washed in PBS and cultured for 48 h in 6 ml of fresh DMEM. At the time points indicated in the figure, 200 μl of Caco-2 (open squares) or HepG2 (open circles) cell supernatant was removed, stored at −20°C, and analyzed for apoA-I and apoB. The values given in the figure are means of three wells ± standard error. At the end of the incubations the wells contained 1.09 mg (Caco-2/7B6 cells) and 1.05 mg (HepG2 cells) of cell protein.

apoA-I mRNA, and apoA-I protein secretion in Caco-2/7B6 cells. Both compounds caused increases of about 40%, 80%, and 60% above control of these parameters, respectively, after 48 h incubation. In contrast, secretion of apoB was inhibited by 50% by both retinoic acids (Fig. 3).

In HepG2/10D2 cells, the two natural retinoic acids were considerably more active in stimulating luciferase activity than in Caco-2/7B6 cells: 100% and 150% above control with all-*trans* RA or 9-*cis* RA, respectively (**Fig. 4**). However, the two retinoic acids increased apoA-I mRNA content and apoA-I protein secretion by only about 25–30% (all significantly different from control at

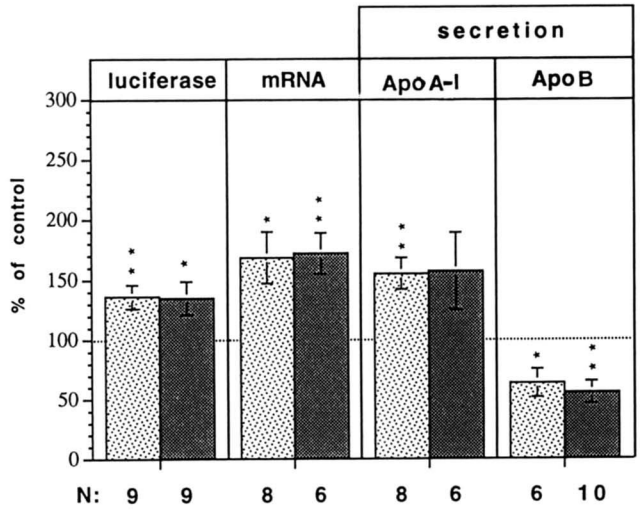

Fig. 3. Effect of retinoids on apoA-I promoter activity, apoA-I mRNA content, and apoA-I and apoB secretion in Caco-2/7B6 cells. Caco-2/7B6 cells grown for 8 days in 6-well dishes were incubated 48 h with 10 μM all-*trans* RA (dotted bars) or 9-*cis* RA (cross-hatched bars), in 2 ml medium per well. Luciferase activity, apoA-I mRNA levels, and secreted apoA-I and apoB protein were measured as described in Materials and Methods, and the average value for the wells with compounds expressed as percent of the average of the control wells. The bars represent means and standard errors of these "percent-of-control" values, with the number of separate experiments given below the bars. Asterisks indicate probability of mean being not different from 100%; *, $P < 0.05$; **, $P < 0.01$.

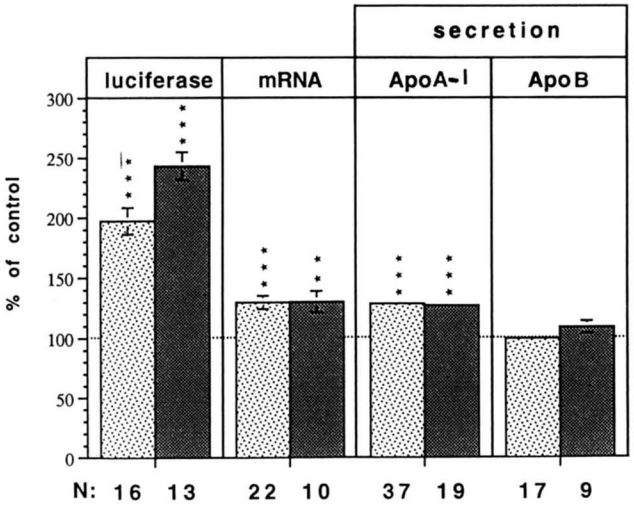

Fig. 4. Effect of retinoids on apoA-I promoter activity, apoA-I mRNA content, and apoA-I and apoB secretion in HepG2 cells. HepG2 cells grown 5 days in 6-well dishes were incubated 48 h with 10 μM all-*trans* RA (dotted bars) or 9-*cis* RA (cross-hatched bars), as described in the legend of Fig. 3. The bars represent means and standard errors of the "percent-of-control" values (calculated as described in Fig. 2), with the number of separate experiments given below the bars. Asterisks indicate probability of mean being not different from 100%; **, $P < 0.01$; ***, $P < 0.0001$.

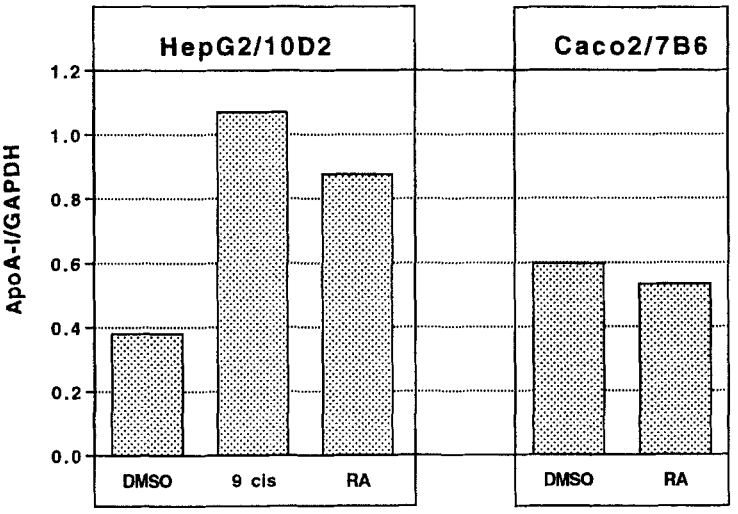

Fig. 5. Effect of retinoids on rates of nuclear apoA-I gene transcription (run-on assays) in HepG2/10D2 and Caco-2/7B6 cells. Cells were grown in 75-cm^2 flasks for 5 days and then incubated for 48 h with 10 μM retinoids or DMSO as indicated. The nuclei were collected as reported elsewhere (18). The in vitro incorporation of [^{32}P]UTP into apoA-I RNA relative to the GAPDH signal is shown in the figure. The signal intensity was quantified using a Phosphor Imager.

$P < 0.01$). ApoB secretion by these cells was not affected by the retinoic acids.

We observed whether the parameters for apoA-I biosynthesis (luciferase expression, apoA-I mRNA, and apoA-I protein) were correlated, using the data of those experiments in which the effects of these retinoids on at least two of these parameters were measured. The only significant correlation found was that between luciferase and apoA-I mRNA in Caco-2 cells ($r = 0.65$, $n = 16$, $P = 0.005$).

Nuclear run-on experiments

Finally, because the response of luciferase in HepG2/10D2 cells was so divergent from the response of apoA-I mRNA or apoA-I protein, we checked the effects of the two retinoic acids on the rate of apoA-I gene transcription in these cells measured by nuclear run-on assays. As shown in **Fig. 5,** the retinoic acids stimulated apoA-I gene transcription by HepG2/10D2 cells by about 150% and 90% above control after the cells were incubated for 48 h with 9-*cis* RA or all-*trans* RA, respectively. In contrast, after incubation of Caco-2 cells with all-*trans* RA the transcription rate of the apoA-I gene was not altered (Fig. 5).

DISCUSSION

Changes in luciferase activity as reporter for apoA-I promoter activation

The data presented show for the first time that all-*trans* and 9-*cis* RA are able to stimulate apoA-I promoter activity in human cells, as assessed by luciferase activity, and for HepG2/10D2 cells by nuclear run-on assays. As the stimulation obtained in the luciferase assays (Fig. 4) is comparable in size with those seen in the run-on experiments (Fig. 5), the luciferase reporter construct seems to be a reasonable indicator of the rate of apoA-I gene transcription in the HepG2/10D2 cells.

Significant stimulation of luciferase activity by all-*trans* RA was only observed when >100 nM concentrations were applied to the cells (Fig. 1B), which contrasts with the EC50 of about 100 nM for all-*trans* RA in other transactivation assays (16). The long incubation time needed to see stimulation of luciferase activity (Fig. 1A) suggests that further processes, e.g., induction of other genes, are required in order to develop this stimulation.

In transient transfection experiments using CV-1 cells and the apoA-I RARE linked via the thymidine kinase-promoter to chloramphenicol acetyl transferase (CAT) as the reporter gene, Rottman et al. (11) found RXRα to be a 6-fold stronger stimulator of the apoA-I RARE than RARα or RARβ in the presence of all-*trans* RA. On the other hand, in similarly designed experiments, Lehmann et al. (18) found activation of this RARE by RARα (+ all-*trans* RA or 9-*cis* RA) to be about equal with that by RXRα (+ 9-*cis* RA). Our experiments presented here, using a 1.3 kb long 5' flanking region of the apoA-I gene transfected into cells competent to express and secrete apoA-I, indicate that the two natural ligands activate apoA-I gene expression equally well, which is in agreement with the findings of Lehmann et al. (18).

The promoter/reporter cassette that we used contains the known regulatory elements required to express the apoA-I gene in liver. However, it does not contain the regulatory sequences 3' to the apoA-I gene, described to be important for the efficient expression of human apoA-I gene in intestinal epithelial cells of transgenic mice (10). Nevertheless, we obtained RA-inducible luciferase expression in our Caco-2 transfectants. The discrepancy may be related to the incorporation into the human genome and expression in human cells with an environment of homologous nuclear factors in our study.

Presently we cannot explain why the stimulatory effects of the retinoids on luciferase activity were noticeably greater in HepG2/10D2 cells than in Caco-2/7B6 cells (compare Figs. 3 and 4). As we added the retinoids during a rather long (48 h) incubation time, luciferase activity may also be determined by factors involved in its intracellular turnover. Luciferase protein has been described to be targeted towards peroxisomes (26), which may decrease its turn-over. If the amount of peroxisomes would be higher in HepG2 cells than in Caco-2 cells, the higher stimulation of luciferase in HepG2 cells may be the result of a higher degree of peroxisomal sequestration as compared to Caco-2 cells.

Regulation of apoA-I mRNA content and apoA-I and apoB secretion

Our findings qualitatively confirm a very recent paper by Berthou et al. (27) showing that both all-*trans* and 9-*cis* RA are able to increase the apoA-I mRNA content and apoA-I protein secretion by HepG2 cells, and extend this to Caco-2 cells. The stimulation seems to be specific for apoA-I because it was not observed for apoA-II (27) or for apoB (present study). In our experiments the stimulatory effects on apoA-I mRNA and secretion are weaker than those reported by Berthou et al. (27), perhaps because they performed incubations with retinoids in a serum-free rather than in serum-stripped medium.

We also have confirmed the finding of Wagner et al. (28) that retinoids decrease apoB secretion from Caco-2 cells by about 50%. The fact that they used only 1 μM all-*trans* RA may explain the lack of effect on apoA-I secretion in their experiments.

In Caco-2 cells, the increase of apoA-I mRNA content was highly correlated with the stimulation of luciferase activity, which suggested that in these cells the retinoids regulate this mRNA by an increased rate of apoA-I gene transcription. The finding that this rate was not increased in the nuclear run-on experiment (Fig. 5) suggests that the retinoid effect on the transcription rate is transient and has waned after 48 h incubation.

We found no significant correlation between apoA-I mRNA level and apoA-I protein secretion for either cell type, supporting earlier contentions that apoA-I protein secretion is not only determined by the amount of apoA-I mRNA available but that additional regulation occurs at translational or post-translational sites (29, 30).

Our data differ from those of Kaptein et al. (19) who found 2.5-fold increases of apoA-I secretion by 10 μM all-*trans* RA, 9-*cis* RA, and various synthetic retinoids using cultured cynomolgus monkey hepatocytes. In our human cell lines, 10 μM all-*trans* RA stimulated apoA-I secretion by only 50% (Caco-2 cells) and 30% (HepG2 cells). The reason for these differences between human and monkey hepatocytes is currently unknown. In any case, the low stimulation found by us with these compounds in human cells suggests that retinoic acids are not very promising to increase plasma HDL levels. This might already have been surmised from the absence of an effect on the plasma apoA-I level in patients treated with etretinate or isotrenoin (31, 32). More potent compounds may become available in the future.

The similarity in effect of both retinoic acids suggests that they act predominantly through the RAR-type receptors. Nevertheless, it will be interesting to see whether RXR-specific retinoids also have some potential to activate apoA-I biosynthesis. Studies to that effect are underway.

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