Stimulation of Vascular Smooth Muscle Cell Prostacyclin and Prostaglandin E₂ Synthesis by Plasma High and Low Density Lipoproteins

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SUMMARY. We studied the effects of plasma high density and low density lipoproteins upon the synthesis of prostacyclin and prostaglandin E₂ by vascular smooth muscle cells. Prostaglandin synthesis was measured in 24-hour cultures by radioimmunoassay of the stable metabolites of prostacyclin, 6-keto-prostaglandin F₁α, and of prostaglandin E₂. High density lipoproteins induced dose-dependent increases in the release of 6-keto-prostaglandin F₁α, and of prostaglandin E₂ from smooth muscle cells to values 14- and 50-fold above control. Incubations with low density lipoproteins at comparable cholesterol concentrations also induced dose-dependent release of 6-keto-prostaglandin F₁α, and prostaglandin E₂, but to a lesser extent. Rat high density lipoprotein, which contained 2.5 times more cholesteryl arachidonate than human high density lipoproteins, stimulated 6-keto-prostaglandin F₁α, and prostaglandin E₂ release 2- to 3-fold more than human high density lipoproteins, whereas the delipidated apoproteins of high density lipoproteins had no significant effect on prostaglandin synthesis. Recombinant high density lipoproteins containing cholesteryl-[l-¹⁴C]arachidonate stimulated release of [¹⁴C]-6-keto-prostaglandin F₁α, and [¹⁴C]-prostaglandin E₂ by smooth muscle cells. The ionophore, A 23187, released labeled 6-keto-prostaglandin F₁α, and prostaglandin E₂ from cells preincubated with recombinant high density lipoprotein containing cholesteryl-[l-¹⁴C]arachidonate. Unlabeled high density lipoproteins, in contrast, did not cause release of radioactive prostaglandins from cells preincubated with [¹⁴C]-arachidonate. Phospholipase activators were synergistic (bradykinin) or additive (angiotensin II) with high density lipoprotein in stimulation of prostaglandin synthesis. The data indicate that both high and low density lipoproteins stimulate the synthesis of prostacyclin and prostaglandin E₂ by vascular smooth muscle cells. The results suggest that the lipoproteins provide arachidonate to a phospholipase-sensitive pool accessible to cyclooxygenase. (Circ Res 54: 554-565, 1984)
aggregation can initiate the formation of experimental atherosclerotic lesions, prostaglandin formation is initially reduced (Eldor et al., 1981). However, medial smooth muscle cells migrate through the internal elastic membrane and proliferate to form a neointima; these cells are capable of synthesizing PGI₂ and are in direct contact with plasma HDL and LDL (Eldor et al., 1981). The effects of plasma lipoproteins upon prostaglandin synthesis by vascular smooth muscle cells are unknown. The present study was designed to investigate the influence of plasma lipoproteins, particularly HDL, upon the synthesis of PGI₂ and PGE₂ by vascular smooth muscle cells grown in tissue culture.

Methods

Reagents

Plasma lipoproteins were obtained by preparative ultracentrifugation of human and rat plasma in a Beckman 40.3 rotor at densities between 1.019 and 1.063 g/ml (LDL), 1.125 and 1.21 g/ml (human HDL₃), and 1.075 and 1.21 g/ml (rat HDL). Lipoproteins were centrifuged once at the upper density limit, and were assayed for protein by the method of Lowry et al. (1951), and for cholesterol by the method of Zlatkis and Zak (1969). Before incubation with cells, the lipoproteins were dialyzed once against 200 volumes of 0.9% saline overnight, and once against 100 volumes of M-199 tissue culture media (Grand Island Biological Company [Gibco]) containing penicillin (100 U/ml), streptomycin (100 µg/ml), and 25 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES, Sigma Chemical Company), pH 7.4, at 4°C. Apo-HDL was prepared by delipidation of native HDL in 20 volumes of ethanol:diethyl ether (3:2), followed by washing in diethyl ether at -15°C. Lipoprotein-deficient serum was prepared as previously described (Havel et al., 1955).

All reagents were prepared in M-199 tissue culture media containing 25 mM HEPES (pH 7.4) containing 1% penicillin-streptomycin, 0.06 M N-2-hydroxyethylpiperazine- N-2-ethanesulfonic acid (HEPES, Sigma Chemical Company), pH 7.4, at 4°C. Apo-HDL was prepared by delipidation of native HDL in 20 volumes of ethanol:diethyl ether (3:2), followed by washing in diethyl ether at -15°C. Lipoprotein-deficient serum was prepared as previously described (Havel et al., 1955).

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Verification of Cells

Cells were determined to be vascular smooth muscle by (1) their hill-and-valley configuration at confluence (Ross, 1971); (2) positive fluorescence staining for smooth muscle actin and myosin (Groschel-Stewart et al., 1975; Chamley et al., 1977), and (3) the presence of thick and thin filaments, and dense bodies, as demonstrated by electron microscopy (Gimbrone and Cotran, 1975).

For phase contrast microscopy, cell monolayers on coverslips were prefixed in calcium-flushed glutaraldehyde for 15 seconds, then exposed to 0.25% NP-40 (Shell Oil Co.) in phosphate-buffered saline (PBS), pH 7.0, for 1–5 minutes, rinsed in PBS, and the fixation continued for 10 minutes more in 2% glutaraldehyde in 0.06 M cacodylate buffer, pH 6.8, containing 170 mM NaCl, 3 mM KCl, 1 mM CaCl₂, 0.5 mM MgCl₂, and 0.5% tannic acid. After fixation, washed coverslips were postfixed in osmium tetroxide, stained for 60 minutes in 1% aqueous uranyl acetate, mounted in buffer glycero, and observed with a Leitz Ortholux phase contrast microscope.

For immunocytology, cells were fixed in 2.5% purified formalin in phosphate-buffered saline, made permeable with acetone:water (1:1) and then acetone at -20°C before staining. Sites of F-actin were identified with rhodamine-phalloidin (Molecular Probes). Myosin was visualized by indirect immunofluorescence, using as primary reagents rabbit antibody to human platelet myosin (Godman et al., 1980). For electron microscopy, cells growing in plastic dishes were fixed in phosphate-buffered 2.5% glutaraldehyde (pH 7.2) containing 1% sucrose for 20 minutes, postfixed in 2% osmium tetroxide, stained in 0.25% aqueous uranyl acetate overnight, dehydrated in ethanol, and embedded in Epon 812. After overnight incubation at 55°C, the
partially polymerized casts containing the monolayer was removed from the dish and polymerization was continued for 48 hours at 60°C. Selected areas of the cast were cut out, glued to Epon blocks, and sectioned on an LKB Ultramicrotome II. Sections were stained with uranyl acetate and lead acetate and viewed in a Phillips 200 electron microscope.

Incubations

For each experiment, cells were thawed rapidly, and plated into 24-well cluster plates in M-199 (containing 10% fetal bovine serum, 1% penicillin-streptomycin, 25 mM HEPES, pH 7.4), and incubated at 37°C. The media were changed 24 hours after plating, and every 3 days thereafter. Cell viability, as determined using the trypan blue exclusion technique 24 hours after plating, was 88% with a plating efficiency of 40%. Before study, subconfluent cells (5.2 x 10^4 cells/well) were washed twice with 1.0 ml of serum-free M-199. Control cultures (in triplicate wells) received 1.0 ml of M-199 (which did not contain 10% fetal bovine serum), and experimental cultures (in triplicate wells) received 1.0 ml of M-199 containing the test agent. After 24 hours of incubation at 37°C, media were removed and assayed for 6-keto-PGF_1α, the stable hydrolysis product of prostacyclin, and PGE_2 by radioimmunoassay (RIA). Results of these experiments were analyzed by analysis of variance after log transformation of the data. Data are reported as mean ± se. Cell number was determined by counting the cells directly, using a calibrated ocular within the phase-contrast microscope (Olympus).

Radioimmunoassay

The RIA for 6-keto-PGF_1α was performed essentially as previously described (Fleisher et al., 1982a), with the modification that M-199 was used for the preparation of the standard curves in place of Dulbecco's modified Eagle's medium (DME).

PGE_2 was measured by RIA as previously described (Oliver et al., 1980). The PGE_2 antibody (Sigma) achieved approximately 40% binding of [3H]PGE_2 in the absence of unlabeled PGE_2. Cross-reactivity with other prostanoids was as follows: PGE_3, 3.2%; PGA_2, 0.2%; 13,14-dihydro-PGE_2, 0.15%; 13,14-dihydro-15-keto-PGE_2, 0.11%; and less than 0.1% for 6-keto-PGF_1α, PGA_2, 6-keto-PGF_2α, PGB_1, and PGB_2. All samples were assayed in duplicate without extraction. Each assay tube received 100 μl of standard [2.0–200 pg of authentic PGE_2 (Upjohn Co.)] in M-199, or unknown, 100 μl of 0.1 M phosphate-buffered saline (pH 7.4) containing 0.1% gelatin (PBS-saline) with [3H]PGE_2 [5,6,8,11,12,14,15-3H(N)-PGE_2, 8000 cpm (New England Nuclear)] and 100 μl of antiserum prepared in PBS-G. Samples were incubated for 2 hours at 4°C. Antibody-bound PGE_2 was separated from free ligand by addition of 1.0 ml of ice-cold PBS-G containing 0.025% dextran T-70 (Pharmacia Fine Chemicals) and 0.25% activated charcoal (Norit “A,” Amend Drug and Chemical Co.). After 15 minutes, samples were centrifuged at 5000 rpm (4810 g) for 10 minutes at 4°C. The supernatant was decanted into polypropylene scintillation vials (Kimble); after addition of 15 ml of Hydrofluor (Nuclear Diagnostics) radioactivity was counted in a Tri-Carb model 3330 liquid scintillation spectrometer.

Radioisotope and Recombinant HDL Experiments

Smooth muscle cells were prelabeled with [1,14C]arachidonate (50–60 μCi/mmole) (Amersham/Searle) by meth-
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Figure 2 illustrates the dose-response relationship of HDL and LDL on prostaglandin release by vascular smooth muscle cells. Control cells (incubated in media alone) released 0.24 ± 0.07 ng 6-keto-PGF$_{1alpha}$/ml, and 0.16 ± 0.03 ng PGE$_2$/ml (Fig. 2B), into the media at the end of the 24-hour incubation period. HDL$_3$ induced a dose-dependent stimulation of 6-keto-PGF$_{1alpha}$ and PGE$_2$ release by the smooth muscle cells (Fig. 2, A and B). At highest doses, incubation with HDL$_3$ (0.375 mg cholesterol/ml),
induced a 14-fold increase of 6-keto-PGF\(_{1\alpha}\) production to 3.48 ± 0.06 ng/ml (P < 0.01). PGE\(_2\) synthesis was increased by this concentration of HDL\(_3\) to values 50 times control (8.07 ± 0.75 ng/ml, P < 0.001). LDL, at equivalent cholesterol concentrations, also produced dose-dependent stimulation of 6-keto-PGF\(_{1\alpha}\) and PGE\(_2\) (Fig 2, A and B). However, the stimulatory effect of LDL on prostaglandin release was considerably less than that of HDL\(_3\). The concentrations of 6-keto-PGF\(_{1\alpha}\), and PGE\(_2\) found in incubations containing LDL were approximately 4-10 times those of the control cultures.

Figure 3 summarizes three experiments depicting the time-course of lipoprotein-induced prostacyclin release by the smooth muscle cells. In the control cultures, the concentration of 6-keto-PGF\(_{1\alpha}\) in the media (0.25 ± 0.03 ng/ml) did not increase significantly over the 24-hour time period. In contrast, both HDL\(_3\) (0.15 mg cholesterol/ml) and LDL (0.15 mg cholesterol/ml) produced a slowly progressive accumulation of 6-keto-PGF\(_{1\alpha}\) in the media. HDL\(_3\) produced a 10-fold, and LDL a 4-fold, increase in 6-keto-PGF\(_{1\alpha}\) concentration in the media at 24 hours.

The relationship between cell density and lipoprotein-induced 6-keto-PGF\(_{1\alpha}\) release is depicted in Figure 4. Cells were plated at variable density and exposed to media alone (control) or media containing HDL\(_3\) (0.15 mg cholesterol/ml), LDL (0.15 mg cholesterol/ml), or sodium arachidonate (10 \(\mu\)M). The stimulation of 6-keto-PGF\(_{1\alpha}\) per 10\(^4\) cells, induced by HDL\(_3\), LDL, and arachidonate was greater in cells at lower densities than in cells at higher densities. As the cells approached confluence (10\(^5\)/well), the synthesis of prostacyclin induced by all three agents was significantly less than at lower cell densities.

Gospodarowicz et al. and other investigators have reported that plasma factors, including the lipoproteins, may influence the growth characteristics and morphology of vascular smooth muscle cells grown in tissue culture (Gospodarowicz et al., 1981; Tauber et al., 1980, 1981a). Figure 5 shows phase-contrast photomicrographs illustrating the effects of serum-free M-199, and serum-free M-199 containing HDL\(_3\), LDL, or lipoprotein-deficient serum (LPDS), on the morphology of the cultured vascular smooth muscle cells over a 24-hour incubation period. In serum-free M-199, the cells lost their spindly shape and appeared rounded, but were not detached from the plating surface (panel A). When HDL\(_3\) (0.15 mg cholesterol/ml) was present in the media, the normal morphology of the cells was preserved (panel B). In contrast, when LDL (0.15 mg cholesterol/ml) was
FIGURE 4. Effect of cell density on lipoprotein and arachidonate-induced 6-keto-PGF₁α release. Cells were plated at variable densities in M-199 containing 10% fetal bovine serum, and incubated for 5 days at 37°C. Subsequent cell densities ranged from 3.1 x 10⁴ to 1.1 x 10⁵ cells/well. Cells were washed twice in serum-free M-199 and exposed to media alone (open circles), or media containing HDL (0.15 mg cholesterol/ml, closed squares), LDL (0.15 mg cholesterol/ml, closed triangles), or sodium arachidonate (10 μM, open triangles) for 24 hours at 37°C. Data are expressed as ng 6-keto-PGF₁α/10⁶ cells/24 hours.

FIGURE 5. Phase-contrast photomicrographs illustrating the effects of HDL, LDL, and lipoprotein-deficient serum (LPDS) on smooth muscle cell morphology. Cells were grown in M-199 containing 10% fetal bovine serum for 5 days. The medium was removed, and the cells were washed twice in serum-free media. Cultures were then exposed for 24 hours to M-199 alone (panel A), or media containing HDL₁ (0.15 mg cholesterol/ml, panel B), LDL (0.15 mg cholesterol/ml, panel C), or LPDS (2.5 mg protein/ml) (170X, bar μm).

present in the media, the cells appeared vacuolated, irregular, and swollen (panel C). LPDS ameliorated the adverse effects of media containing LDL on cell morphology (panel D). HDL₁ had similar effects as LPDS (not shown). However, despite these changes in morphology, neither basal nor lipoprotein-induced PGI₂ and PGE₂ release was altered by the presence of LPDS (Fig. 6). When the cells were incubated in 5% platelet-poor lipoprotein-deficient serum (2.5 mg protein/ml), they did not secrete significantly more 6-keto-PGF₁α into the media than in the basal state (control = 0.18 ± 0.07 vs. LPDS = 0.37 ± 0.05 ng/ml (n = 2)).

To determine whether lysosomal degradation is necessary for lipoprotein-induced prostaglandin synthesis, cells were incubated with HDL₁ (0.15 mg cholesterol/ml) and LDL (0.15 mg cholesterol/ml) during exposure to chloroquine (45 μM), an inhibitor of lysosomal enzyme activity (Goldstein et al., 1975). Chloroquine did not cause a significant alteration in either basal or lipoprotein-induced 6-keto-PGF₁α release (Fig. 7). In further experiments, LDL coincubated with dextran sulfate stimulated 6-keto-PGF₁α release to the same degree as native LDL (2.59 vs. 2.21 ng/ml, respectively).

Table 1 summarizes experiments in which the effects of two cyclooxygenase inhibitors on HDL₁-induced 6-keto-PGF₁α release were evaluated. Cultures were pretreated with aspirin (20-100 μg/ml), and meclofenamate (5.0, 10.0, and 20.0 μg/ml) for 30 minutes before exposure to media alone (control) or media containing HDL₁ (0.15 mg cholesterol/ml). Aspirin and meclofenamate inhibited the basal and HDL₁-induced release of 6-keto-PGF₁α by vascular smooth muscle cells.

The effects of agents which stimulate phospholi-
pases upon prostaglandin synthesis by the vascular smooth muscle cells were evaluated. Cells were exposed to bradykinin, (1.0 µg/ml), and angiotensin II (5.0 µg/ml), in the presence and absence of HDL3, (0.15 mg cholesterol/ml) and to the calcium ionophore, A-23187 (10 µM) and sodium arachidonate (10 µM) (Table 2). In the basal state, the cells released similar amounts of PGI2 and PGE2. All agents stimulated PGI2 and PGE2 release significantly. Bradykinin, angiotensin II, and sodium arachidonate induced an approximately equal stimulation of PGI2 and PGE2 while A-23187 stimulated PGI2 release more than PGE2. In contrast, HDL3 stimulated PGE2 synthesis more than that of PGI2. In coincubations with HDL3, the effects of HDL3 and of angiotensin II on prostaglandin synthesis were additive, whereas the effects of bradykinin and HDL3 were synergistic.

In order to determine whether esterified arachidonic acid in HDL3 might be involved in the stimulation of prostaglandin synthesis, we examined the effects of rat HDL, which has a greater content of arachidonate in its cholesteryl esters than human HDL3. GLC analysis of fatty acid methyl esters revealed that rat HDL contained 38% of its cholesteryl esters as arachidonate, compared to 12% for human HDL3. The arachidonate content of phospholipids was similar in the rat and human particles, 10%. Smooth muscle cells were exposed to human and rat HDL and their respective apoproteins at a constant protein concentration (0.4 mg protein/ml—equivalent to 0.15 mg cholesterol/ml in native HDL3). Control cultures received media alone. At comparable protein concentrations, rat HDL induced significantly greater synthesis of PGI2 and PGE2 (approximately 2-fold) (P < 0.05) than did human HDL3, whereas their respective apoproteins did not stimulate significantly the synthesis of either prostaglandin (Fig. 8).

To investigate more directly whether HDL lipids might provide arachidonate to smooth muscle cells as substrate for prostaglandin synthesis, recombinant human HDL containing cholesteryl [1,14C]arachidonate was prepared and incubated with the smooth muscle cells for 24 hours. At 24 hours, the media was removed, acidified, extracted and analyzed by thin layer chromatography. Figure 9A shows the densitometric analysis of an autoradiogram of the TLC plate. Radioactivity was incorporated by the cells into the 6-keto-PGF1α and PGE2 peaks. The cells then were washed and incubated

![Figure 6. Effect of LPDS on basal, HDL3, and LDL-induced 6-keto-PGF1α release. Supernatants from the cells described in Figure 5 were assayed for 6-keto-PGF1α as described in Methods.](image)

![Figure 7. Effect of chloroquine on HDL3 and LDL-induced 6-keto-PGF1α release from smooth muscle cells. Cells were grown as described in Figure 2, and were then washed twice in serum-free M-199. Cells were exposed to media alone, or media containing either HDL3 (0.15 mg cholesterol/ml), or LDL (0.15 mg cholesterol/ml) in the absence (open bars) and presence (striped bars) of chloroquine (45 µM) for 24 hours at 37°C. Cells exposed to chloroquine were treated 30 minutes prior to and during incubations with the lipoproteins (n = 3, bars indicate mean ± SE).](image)

![Table 1. Effect of Aspirin and Meclofenamate on HDL3-Induced 6-Keto-PGF1α Release](table)
for 30 minutes in medium alone (control) or medium containing A-23187. Figure 9B shows that, after incubation with the ionophore, significant amounts of label appeared in the 6-keto-PGF<sub>1α</sub> and PGE<sub>2</sub> bands. Cells incubated with media alone did not elaborate prostaglandins containing label. Analysis of the cell lipids in these experiments indicated that 78% of the <sup>14</sup>C was present in cell phospholipids; only 3% was present in cellular cholesteryl esters. These data suggest that HDL cholesteryl arachidonate undergoes hydrolysis upon interaction with smooth muscle cells and provides cell phospholipids with arachidonate which may be available for prostaglandin synthesis.

Experiments were also performed to investigate whether HDL mobilized cellular arachidonate stores for prostaglandin synthesis. Smooth muscle cells were preincubated with [1-<sup>14</sup>C]arachidonate for 18 hours, then washed and incubated with unlabeled HDL for 24 hours or with A23187 for 15 minutes; the media were extracted and analyzed by TLC. Table 3 indicates that there was no measurable incorporation of radioactivity into 6-keto-PGF<sub>1α</sub> or PGE<sub>2</sub> after incubation with HDL, in contrast to the significant incorporation of label into prostaglandins after exposure to A23187.

**Discussion**

These results indicate that plasma high and low density lipoproteins stimulate the release of 6-keto-PGF<sub>1α</sub> and of PGE<sub>2</sub> from vascular smooth muscle cells grown in tissue culture. Since 6-keto-PGF<sub>1α</sub> is the major stable product of prostacyclin (Johnson et al., 1976) and since prostaglandins are not stored in cells (Piper and Vane, 1971), the data indicate that HDL₃ and LDL stimulate the synthesis of PGI₂ and PGE₂ by the cultured vascular smooth muscle cells. Incubation of the cells with HDL₃ for 24 hours produced a significant dose-dependent accumulation of 6-keto-PGF<sub>1α</sub> and PGE₂ in the culture media. Although the release of 6-keto-PGF<sub>1α</sub> by untreated cells was larger than the release of PGE₂, the release of PGE₂ from the cells induced by HDL₃ was proportionately larger than that of 6-keto-PGF<sub>1α</sub>. LDL also induced dose-dependent increases in the release of 6-keto-PGF<sub>1α</sub> and of PGE₂ by the vascular smooth muscle cells into the culture medium; the degrees of stimulation of 6-keto-PGF<sub>1α</sub> release and PGE₂ release induced by LDL were equivalent. However, the magnitude of the vascular smooth muscle prostaglandin synthesis induced by LDL was significantly less than that produced by HDL₃ at the same cholesterol concentrations. The in vitro stimulation of prostaglandin synthesis produced by both lipoproteins in these experiments was observed over a range of concentrations of HDL₃ and LDL that was similar to those present in normal serum and lymph.

In our previous study, HDL₃ produced a marked stimulation of prostaglandin synthesis by vascular endothelial cells grown in tissue culture (Fleisher et al., 1982a). The magnitude of 6-keto-PGF<sub>1α</sub> release induced by HDL₃ was greater in endothelial cells
FIGURE 9. Panel A: production of [1-14C]prostaglandins by rabbit aortic smooth muscle cells exposed to recombinant HDL-containing cholesteryl-[1-14C]arachidonate. Confluent smooth muscle cells were incubated with recombinant HDL-containing cholesteryl-[1-14C]arachidonate for 24 hours at 37°C. The media were removed, acidified, extracted, and analyzed by TLC. An autoradiogram was prepared and analyzed by densitometry. Panel B: A-23187-induced [1-14C]prostaglandin release by cells preincubated in recombinant HDL-containing cholesteryl-[1-14C]arachidonate. Cells as described in panel A were washed and exposed to media alone or media-containing 10 μM A-23187 (solid line) for 30 minutes at 37°C. Media were analyzed by TLC as described above.

than in smooth muscle cells; this observation is consistent with the previously reported data of Moncada, who reported that there was a gradient of 6-keto-PGF1α synthesis from the intima toward the media and adventitia of intact blood vessels (Moncada et al., 1977). In contrast to the modest stimulation of 6-keto-PGF1α and PGE2 release by smooth muscle cells induced by LDL in the present study, LDL at equivalent cholesterol concentrations (0.015–0.30 mg/ml) produced no significant stimulation of 6-keto-PGF1α release by endothelial cells cultured under similar conditions (Flesher et al., 1982b). In subsequent studies, however, higher doses of LDL (>0.3 mg cholesterol/ml) have been found to stimulate PGI2 synthesis by endothelial cells. However, even at similar cholesteryl arachidonate content, HDL stimulated prostaglandin synthesis to a greater extent than LDL (Pomerantz et al., unpublished observations). These observations indicate that this is a relative but not absolute specificity of the effect of HDL on prostaglandin synthesis.

Inhibition of HDL3-induced PGI2 synthesis by cyclooxygenase inhibitors implies an effect of lipoproteins prior to cyclooxygenase, the enzyme responsible for the conversion of arachidonate to the endoperoxide precursors of PGI2 and PGE2. Our data differ from those showing divergent effects of HDL and LDL on PGI2 synthesis by aortic microsomes, where effects of the two lipoproteins on prostacyclin synthase were suggested (Beitz and Forster, 1980). The slow progressive time course of lipoprotein induced prostaglandin synthesis differs markedly from the rapid effects of the phospholipase activators, angiotensin II, bradykinin and A23187 (Whorton et al., 1982; Hong and Deykin, 1982), and the rapid stimulation of prostaglandin production produced by arachidonate coupled to human serum albumin, suggesting a different more complex mechanism of action.

Results from several of the present experiments suggest that HDL stimulates prostaglandin synthesis, at least in part by providing the cells with arachidonate. The observations that the delipidated apoproteins of human and rat HDL did not significantly stimulate the production of 6-keto-PGF1α and PGE2 by the smooth muscle cells—whereas the native HDLs from both species had significant stimulatory effects—suggest that the lipid portion of the HDL particle is important in the stimulation of prostaglandin synthesis. When incubated at equivalent protein concentration, rat HDL induced significantly greater release of 6-keto-PGF1α and PGE2 than hu-
man HDL$_3$ (Fig. 8). Rat HDL contains a much higher proportion of arachidonate esterified in its cholesteryl esters than human HDL (38 vs. 12%), whereas the phospholipid arachidonate content of rat and human HDL are similar (~10%); this suggests that the marked effect of rat HDL may be due to its higher content of cholesteryl arachidonate.

Direct evidence for incorporation of arachidonate from HDL as substrate for smooth muscle cell prostaglandin synthesis was obtained in the experiments showing the incorporation of label from cholesteryl [1-14C]arachidonate from HDL recombinants into 6-keto-PGF$_{1\alpha}$ and PGE$_2$ (Fig. 9A). The experiments with cells preincubated with the labeled recombinant that were exposed to A23187 (Fig. 9B) indicate that [1-14C]arachidonate was transferred from the lipoprotein into cellular phospholipid pools that were available for prostaglandin synthesis via the action of phospholipases. The studies with cells prelabeled with [1-14C]arachidonate indicate that native HDL does not significantly liberate arachidonate from endogenous cellular stores for prostaglandin synthesis. Additional evidence that lipoprotein stimulates prostaglandin synthesis by providing arachidonate has been obtained in experiments with endothelial cells: (1) when HDL$_3$ recombinants containing cholesteryl [3H]arachidonate were incubated with cells, the label was incorporated into newly synthesized 6-keto-PGF$_{1\alpha}$, and (2) the degree of HDL-induced 6-keto-PGF$_{1\alpha}$ release was directly proportional to the cholesteryl arachidonate content of the HDL particle (Fleisher et al., 1982b).

Data from two other experiments are also consistent with the hypothesis that lipoproteins may provide arachidonate to the smooth muscle cells for prostaglandin synthesis. First, the differential response of prostaglandin release induced by the lipoproteins (PGE$_2$ > 6-keto-PGF$_{1\alpha}$) differed from that induced by the three activators of phospholipases (PGE$_2$ ≤ 6-keto-PGF$_{1\alpha}$, Table 2), suggesting that HDL does not act in a similar fashion. Second, phospholipase activators were either synergistic (bradykinin) or additive (angiotensin II) with HDL in stimulating prostaglandin release (Table 2). These results can be explained by incorporation of lipoprotein arachidonate into a pool of cellular lipid which is susceptible to the action of the phospholipases and accessible to cyclooxygenase.

Although the potential mode of entry of lipoprotein lipid containing arachidonate into the cells remains to be explored, several observations of the present study indicate that it is not via receptor-mediated endocytosis involving the LDL receptor. First, HDL$_3$ induced significant stimulation of PGI$_2$ and PGE$_2$ synthesis by the smooth muscle cells, despite the fact that HDL$_3$ does not contain the apoproteins recognized by the LDL receptor, i.e., apoB and apoE (Innerarity et al., 1978). Second, chloroquine, an agent which inhibits the activity of lysosomal enzymes such as acid cholesteryl hydro-
ease, whereas the concentration of plasma HDL correlates negatively with risk of coronary artery disease (Miller and Miller, 1975). The mechanism of apparent protective effect of HDL is unknown, but could conceivably relate to a reduction in thrombotic complications of atherosclerotic disease (Heiss et al., 1980) or to effects of HDL on mobilization of cholesterol from peripheral tissue (Carew et al., 1976). In this regard, Stein et al. (1975, 1980) have shown that HDL can mobilize cholesterol esters from lipid-laden smooth muscle cells and macrophages (Stein et al., 1975, 1976, 1980), and Hajjar et al. showed that PGl₂, 6-keto-PGE₁, and 6-keto-PGF₁α activated acid-cholesteryl hydrolase and implicated PGI₂ in the process of cholesterol removal from smooth muscle cells.

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