STIMULATION OF HUMAN ENDOTHELIAL CELL PROSTACYCLIN SYNTHESIS BY SELECT LEUKOTRIENES

By LAURA G. POLOGE, EVA B. CRAMER,* NICHOLAS A. PAWLOWSKI, ELEANOR ABRAHAM, ZANVIL A. COHN, AND WILLIAM A. SCOTT‡

*From The Rockefeller University, New York 10021; the *Department of Anatomy and Cell Biology, Downstate Medical Center, Brooklyn, New York 11203; and the ‡Department of Cell Biology, The Squibb Institute for Medical Research, Princeton, New Jersey 08540*

The slow-reacting substances (SRS) consist of leukotrienes C, D, and E (LTC, -D, -E)[1] and are derived from the lipoxygenase pathway of arachidonic acid (20:4) metabolism. In examining possible mechanisms for the vasoactive effects of SRS (1–4), we found that cultures of human endothelial cells release the vasodilatory agent, prostacyclin, on exposure to 10^{-9} to 10^{-6} M LTC (5). Previous reports indicated that several structurally unrelated vasoactive substances such as histamine (6) and bradykinin (7) also stimulate prostacyclin synthesis by endothelium. Together these results suggested that the leukotrienes and other vasoactive substances may promote vasodilation through a common mechanism.

To further examine the specificity of leukotrienes in initiating 20:4 release and the synthesis of prostacyclin, we exposed human endothelial cells to the cysteine-containing leukotrienes, LTC, LTD, and LTE, and to 5(S), 12(R)-dihydroxy-eicosa-6,14 *cis*-8,10 *trans* tetraenoic acid or LTB. These studies permitted the demonstration that LTC and LTD promote the release of prostacyclin, but that LTE and LTB were inactive in stimulating endothelial cell 20:4 metabolism. The additional finding that endothelial cells metabolize LTC to LTD and LTE suggested one mechanism whereby the vasoactive effects of these substances are modulated.

Materials and Methods

Materials. Radiolabeled lipids were purchased from New England Nuclear, Boston, MA and included [5,6,8,9,11,12,14,15-^{3}H]20:4 ([^{3}H]20:4) (91.2 Ci/mmol; 1 Ci = 3.7 × 10^{10} Bq), 6-keto-[5,8,9,11,12,14,15-^{3}H(N)PGF$_{1\alpha}$ (120–180 Ci/mmol), [5,6,8,9,11-12,14,15-^{3}H(N)]thromboxane B$_2$ (100–150 Ci/mmol), [5,6,8,11,12,14,15-^{2}H(N)]-PGF$_{2\alpha}$ (150–180 Ci/mmol), and [5,6,8,11,12,14,15-^{3}H(N)]PGE$_2$ (100–200 Ci/mmol). Histamine, glutathione, γ-glutamyl transpeptidase (γ-GTPase) (bovine kidney), leucine aminopeptidase, and γ-glutamyl-*p*-nitroanilide were obtained from Sigma Chemical Co.,

[1] *Abbreviations used in this paper:* AT-125, L-(α, 5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazole-acetic acid; γ-GTPase, γ-glutamyl transpeptidase; HPLC, high performance liquid chromatography; HS, human serum; LTB, -C, -D, -E, leukotrienes B, C, D, and E; M-199, medium 199; SRS, slow-reacting substance.

St. Louis, MO. Thrombin was purchased from Ortho Diagnostic Systems, Inc., Rahway, NJ and collagenase type II from Worthington Biochemical Corp., Freehold, NJ. L-(α, 5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (AT-125) and L-γ-glutamyl-O-carboxyphenylhydrazide were gifts from Dr. Owen Griffith, Cornell Medical College.

Culture of Endothelial Cells. Endothelial cells were harvested from the vein of human umbilical cord (8) with collagenase and cultured in medium 199 (M-199) (Gibco Laboratories, Grand Island, NY) containing 20% heat-inactivated human serum (HS) as described (5). All cells examined were positive for factor VIII antigen as determined by indirect immunofluorescence (9). All experiments were performed with first passage cells grown to at least 75% confluency on 35-mm or 60-mm dishes. Endothelial cells were shown to remain viable after exposure to each of the agents used in this study by trypan blue exclusion of a representative set of cultures.

20:4 Metabolism. The phospholipids of endothelial cells in 35-mm plastic tissue culture dishes were labeled overnight with 1.0 μCi of [³H]20:4 in 1 ml of M-199 containing 20% HS. The cells were washed twice with 2 ml of M-199 (no serum), overlaid with fresh M-199, and exposed to the appropriate stimulus. Duplicate 50-μl aliquots of medium were removed and ³H content determined by liquid scintillation counting in Hydrofluor (National Diagnostics, Inc., Sommerville, NJ). Alternatively, 100 μl aliquots of medium were removed at the indicated times, spun in a microfuge to remove any floating cells, and 50 μl aliquots of the supernatant were removed for radioactivity determinations.

At the end of the experiment, the remaining medium was removed. 20:4 metabolites were extracted by the method of Unger et al. (10) as described previously (11) and separated by HPLC System I as described below. After removal of the medium, the cells were washed twice with 2 ml of phosphate-buffered saline (Ca⁺⁺ and Mg⁺⁺ free) and scraped into 1 ml of 0.05% Triton X-100. Duplicate 50-μl aliquots of the cell lysates were removed for radioactivity determinations. Protein contents of 100-μl aliquots were measured by the method of Lowry et al. (12) with bovine serum albumin as a standard.

Reverse Phase High Pressure Liquid Chromatography (HPLC). Media extracts containing 20:4 metabolites were dried to nil under a stream of nitrogen, and dissolved in 0.4 ml of the appropriate HPLC starting buffer.

HPLC System I (5) was used to separate total 20:4 oxygenated metabolites and individual leukotrienes (LTC, LTD, and LTE). HPLC System I consisted of a 4.6 mm × 25 cm column of 5-μm Ultrasphere ODS (Altex Instruments, Wolburn, MA) eluted at 1 ml/min with (*a*) 60 ml of methanol/water/acetic acid (65:34.9:0.1, vol/vol/vol) adjusted to pH 5.4 with ammonium hydroxide, followed by (*b*) 40 ml of methanol/water/acetic acid (75:25:0.01, vol/vol/vol), and then (*c*) 40 ml of methanol/acetic acid (100:0.01, vol/vol). Column effluents were monitored for absorption at 280 nm with a Kratos Spectroflow 773 Monitor (Kratos, Inc., Schoeffel Instrument Division, Westwood, NJ). LTC, LTD, and LTE were quantitated by their absorption at 280 nm, assuming an extinction coefficient of 40,000 M⁻¹ · cm⁻¹ (13). Where appropriate, the radiolabel contents of column fractions were determined. The elution times of LTC, LTD, and LTE were 19–21, 43–45, and 49–51 min, respectively. For identification of individual cyclooxygenase products, the contents of fractions 4–16 from System I were pooled, taken to dryness, and redissolved in the starting buffer for System II.

HPLC System II consisted of a 3.6 mm × 30 cm Waters fatty acid analysis column (Waters Associates, Inc. Milford, MA) eluted at 2 ml/min with 50 ml of water/acetonitrile/benzene/acetic acid (76.7:23.0:0.2:0.1, vol/vol/vol/vol), followed by 20 ml of methanol/acetic acid (100:0.01, vol/vol) (5). Fractions of 2 ml were collected and the radiolabel content of each was determined as described above. The elution times of 20:4 oxygenated metabolites on HPLC Systems I and II were determined using commercial radiolabeled standards (cyclooxygenase products) or radiolabeled standards prepared in this laboratory (lipoxygenase products).

Preparation of Leukotrienes. LTC was extracted from culture medium of mouse peritoneal macrophages prelabeled with [³H]20:4 stimulated with the maximal phagocytic

load of unopsonized zymosan (11) (ICN K & K Laboratories Inc., Plainview, NY) or 10 μg/ml calcium ionophore A23187 (Calbiochem-Behring Corp., La Jolla, CA). [³H]LTC was extracted from medium of mouse peritoneal macrophages that were prelabeled with [³H]20:4 for 16 h and stimulated as described above. LTD and [³H]LTD were prepared by treatment of LTC and [³H]LTC with γ-GTPase (14) and the reaction was stopped by extraction at 4°C.

LTE was prepared from LTD by a modification of the procedure of Sok et al. (14). Briefly, γ-GTPase-treated LTC was extracted at 4°C, dried under nitrogen, and redissolved in 1 ml of 0.05 M Tris, pH 8.5, containing 10 mM MgCl₂. The leukotrienes were treated with 0.8–1.0 U of leucine aminopeptidase for 30 min at room temperature. The reaction was stopped by extraction at 4°C. The leukotrienes were purified by HPLC System I.

LTC Metabolism. Endothelial cell cultures were washed twice with 2 ml of M-199 without serum, overlaid with 1 ml of fresh, serum-free M-199 or RPMI 1640 medium (Gibco Laboratories), and incubated at 37°C in the indicated concentrations of [³H]LTC. Controls consisted of [³H]LTC incubated in M-199 or RPMI 1640 medium in the absence of cells. No conversions of LTC to LTD or LTE were observed in the controls. At the specified times, media were removed from the endothelial cell cultures and controls, extracted for 20:4 metabolites, and the leukotrienes separated by HPLC System I. The percentage of each leukotriene was determined from the ³H content of the appropriate fractions.

Assay of γ-GTPase. γ-GTPase activity of endothelial cells was measured by a modification of the method of Szasz (15). Briefly, endothelial cell lysates were prepared by scraping cells from 60-mm culture dishes into 100 μl of Triton X-100 (0.5% wt/vol). Control samples contained 100 μl of Triton X-100 only. Reactions were initiated by the addition of 0.9 ml of 0.5 M Tris buffer, pH 8.5, containing 25 mM glycylglycine and 2.5 mM of L-γ-glutamyl-*p*-nitroanilide. After vortexing, the mixture was incubated at 37°C. The reactions were stopped on ice and centrifuged to remove cellular debris. Formation of the cleavage product, *p*-nitroaniline, was quantitated spectrophotometrically at 405 nm, assuming an extinction coefficient of 9,900 M⁻¹ · cm⁻¹ (15). Activity is expressed as μmoles of *p*-nitroaniline released per minute.

In some experiments, the reaction mixture was added directly to endothelial cell cultures in 60-mm dishes. The Tris buffer was replaced by RPMI 1640 medium (RPMI Select-Amide Kit; Gibco Laboratories), pH 7.1, less glutathione, vitamins, and pH indicator. The reaction volume was generally 4 ml. At the indicated times, 1-ml aliquots were removed to determine the quantity of *p*-nitroaniline (above). Controls consisted of reaction mixtures incubated in dishes without cells.

Results

20:4 Release. As noted previously, 10^{-3} to 10^{-6} M LTC promoted the release of 20:4 from endogenous phospholipid stores in endothelial cell cultures (5). Fig. 1 shows the time course of ³H release by endothelial cell cultures prelabeled with [³H]20:4 and exposed to various concentrations of LTD. Release occurred at 10^{-8} M LTD and reached maximal levels of 3% of the cell-associated radiolabel at $1-4 \times 10^{-7}$ LTD. Similar to the endothelial cell response to an LTC stimulus (5), the amount of radiolabel released into the culture medium increased during the first 15–30 min of exposure to LTD and leveled off thereafter.

Fig. 2 compares the release of radiolabel by endothelial cell cultures exposed to 10^{-7} M LTC, LTD, or LTE. This concentration of LTC (5), as well as LTD, elicited maximal release of radiolabel. Because the amount of release promoted by individual leukotrienes varied, their potency was directly compared within
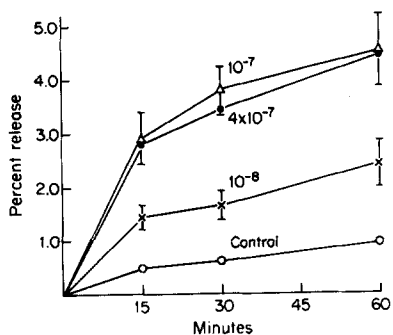
FIGURE 1. Time course of radiolabel release by endothelial cells as a function of LTD concentration. Cultures were labeled overnight with [^{3}H]20:4 in M-199 and 20% HS. After washing with M-199, the cultures were overlaid with M-199 ± the indicated concentration of LTD. Release is expressed as the percent of total cell radiolabel recovered in the culture medium. At each time point, values are corrected for all counting aliquots removed at previous time points. Control cultures were incubated in M-199 in the absence of any stimulus. Values are mean ± range ($n = 2$).
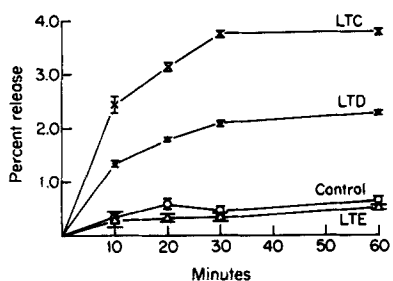

FIGURE 2. Time course of radiolabel release by endothelial cells in response to 10^{-7} M leukotrienes or no stimulus (control). The experimental conditions were similar to those described in Fig. 1. Data are expressed as the mean ± range of percent release ($n = 2$).

the same experiments. The percent of radiolabel released in response to the LTC challenge was consistently higher than in response to LTD. In this experiment, 3.8% of the total cell-associated radiolabel was recovered in the medium of LTC-treated cultures compared with 2.5% for LTD-treated cultures. In addition, LTC promoted a higher level of release than did LTD at all concentrations tested (1 × 10^{-3} to 4 × 10^{-7} M) (data not shown). In a separate experiment, the 11-trans isomer of LTC at 10^{-7} M caused 3.12 ± 0.26% release of radiolabel compared with 3.52 ± 0.48% with the equivalent concentration of LTC.

In contrast, no release of radiolabel above control levels was observed when endothelial cell cultures were exposed to LTE in concentrations of up to 10^{-6} M (Fig. 3). LTB at 10^{-6} M was also ineffective in stimulating 20:4 release by endothelial cells (Fig. 3), and this concentration of LTB failed to block 20:4 release by cultures subsequently challenged with 10^{-7} M LTC.

If endothelial cells were challenged with a maximal LTC stimulus (10^{-7} M) for 30 min ($n = 5$), the response of these cells to a second such challenge of LTC was reduced >90% compared with the level of radiolabel released by cells not
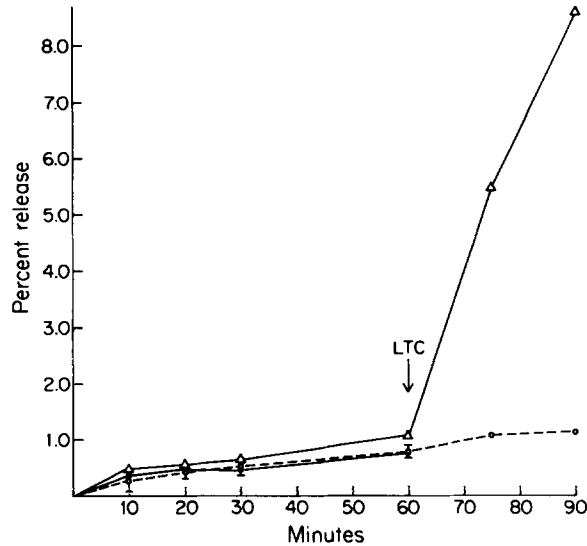
FIGURE 3. Time course of radiolabel release by endothelial cells in response to 1×10^{-6} M ($\triangle$) LTB (0–60 min), ($\bullet$) LTE, or ($\bigcirc$) no stimulus. The experimental conditions are those described in Fig. 1. At 60 min, 1×10^{-7} M LTC (arrow) was added to the culture incubated in LTB.
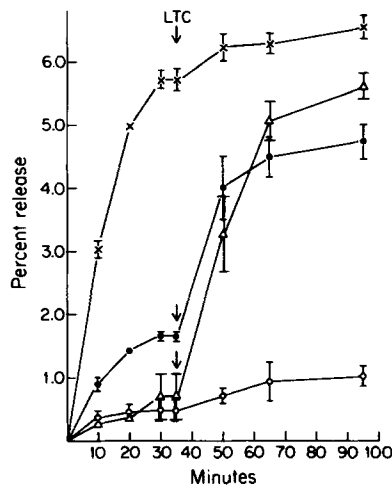

FIGURE 4. Time course of radiolabel release by endothelial cells in response to sequential exposures to LTC. Data are expressed as the mean ± range (n = 2). At time 0, cultures were challenged with 1×10^{-7} M LTC ($\times$), 1×10^{-8} M LTC ($\bullet$), 1×10^{-9} M LTC ($\triangle$), or no stimulus ($\bigcirc$). At 35 min, the cultures indicated by the arrows were given a second challenge of 1×10^{-7} M LTC.

previously exposed to LTC (Fig. 4). However, endothelial cells retained the capacity to respond to multiple challenges of SRS by the release of endogenous 20:4, provided that the level of the stimulus was submaximal (Fig. 4). After a 30 min exposure to 10^{-8} M LTC in serum-free medium, additional release of radiolabel was evident when cultures were challenged with a second dose of 10^{-7}

M LTC. In preliminary experiments, additional release of radiolabel was noted when cells exposed to 10^{-7} M LTC were challenged with 1 U/ml of thrombin.

20:4 Metabolites. Prostacyclin was the major 20:4 metabolite synthesized by endothelial cells exposed to either LTC or LTD. Fig. 5 shows HPLC separations of the radiolabeled products released into the culture medium. The prominent peak eluting in fractions 4–16 from HPLC System I consisted primarily of cyclooxygenase products. The other major peak in fractions 114–124 contained unreacted 20:4. Separation of individual cyclooxygenase products by HPLC System II indicated that the predominant peak of radiolabel had elution characteristics of 6-keto $PGF_{1\alpha}$, the breakdown product of prostacyclin.

Leukotriene Metabolism. The fate of [^{3}H]LTC added to culture medium was assessed by HPLC after a 1 h exposure to endothelial cells. No uptake of LTC by these cultures was evident as determined by the absence of cell-associated radiolabel at the end of the experiment.

The elution profile of the radiolabel extracted from culture medium is shown in Fig. 6 along with the profile of radiolabel obtained after a 1 h incubation of [^{3}H]LTC in medium without cells. >97% of the radiolabel extracted from control medium (no cells) eluted in a single peak as LTC. Two additional peaks of radiolabel with elution times corresponding to LTD (44 min) and LTE (49 min) were evident after exposure of LTD to cells. LTC, LTC, and LTE accounted for 57, 33, and 10% of the total radiolabel recovered, indicating that 43% of the LTC was metabolized during the 1 h exposure to endothelial cells.

Fig. 7 shows the time course of LTC metabolism by endothelial cells. Cultures
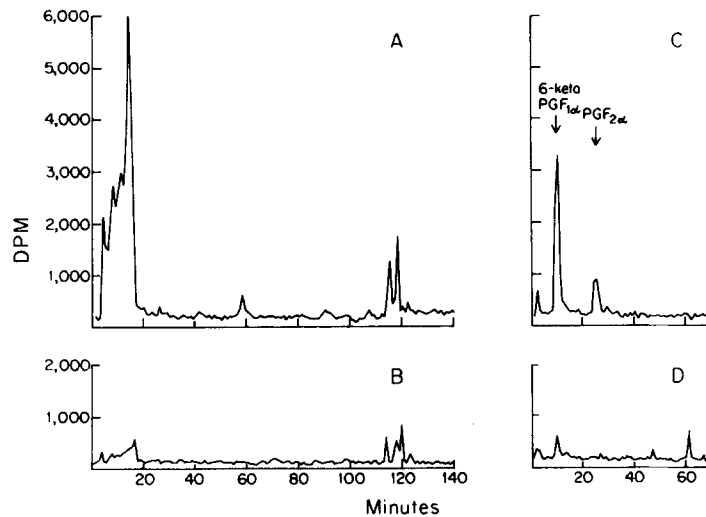

FIGURE 5. HPLC elution profiles of the [^{3}H]20:4 metabolites released by endothelial cells in response to a 60 min exposure to 1×10^{-7} M LTD or in M-199 (no stimulus). Total 20:4 metabolites released by cells exposed to (A) LTD and (B) no stimulus were extracted from culture media and separated on HPLC System I. Cyclooxygenase products eluted in 4–16 min, lipoxygenase products in 17–110 min, and free 20:4 in 110–140 min. Cyclooxygenase products released by (C) cells exposed to LTD and (D) by control cells were pooled separately and rechromatographed using HPLC System II. Elution times of radiolabeled standards were: 6-keto $PGF_{1\alpha}$, 10 min; and PGE, 24–25 min.
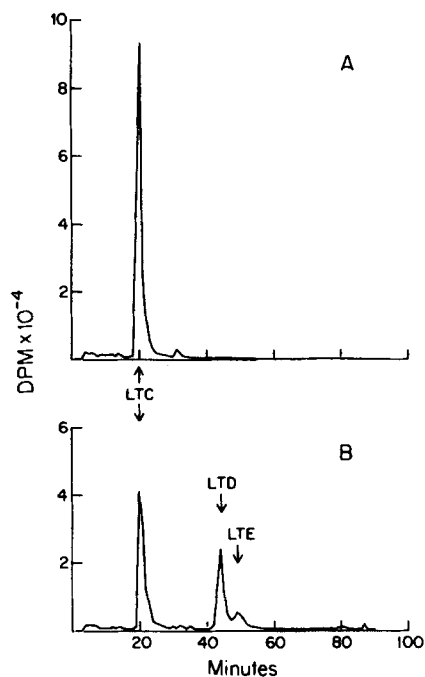
FIGURE 6. Metabolism of LTC by endothelial cells. HPLC System I profiles showing the fate of [³H]LTC incubated for 1 h. (A) M-199 (no cells) and (B) M-199 plus cells. Radiolabeled LTC, LTD, and LTE standards eluted at 20, 44, and 49, respectively.
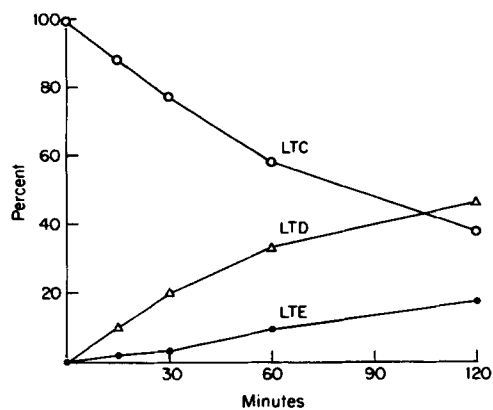

FIGURE 7. Time course of LTC metabolism by endothelial cells. Cultures were incubated for the indicated times with 1×10^{-7} M LTC. The culture media were extracted and the leukotrienes separated by HPLC System I. The level of each leukotriene was determined from the radiolabel content of appropriate peaks in the HPLC elution profiles.

containing an average of 133 μg of cell protein were incubated with 10^{-7} M LTC for the indicated times. The rate of LTC disappearance was approximately linear for the first 30 min, and slowed thereafter. Concomitant increases in the levels of LTD and LTE were evident. By 120 min, 63% of the LTC was metabolized in this experiment.

γ-GTPase. γ-GTPase catalyzes the conversion of LTC to LTD (16). Levels of γ-GTPase were measured using the chromogenic substrate L-γ-glutamyl-*p*-nitroanilide (15) and Triton X-100–solubilized cell extracts. At pH 8.5, the pH optimum of the enzyme (17), the level of γ-GTPase activity was $6.5 \pm 0.4 \times 10^{-7}$ U/mg cell protein ($n = 2$).

Using the same substrate we were also able to detect γ-GTPase activity in intact cells maintained in medium (RPMI) at pH 7.1, but only if the transpeptidase acceptor glycylglycine was present. In three experiments, the activity was $4.8 \pm 3.5 \times 10^{-8}$ U/mg cell protein. Endothelial cells hydrolyze LTC under these same conditions in the absence of an acceptor. Although the activity is 14-fold less in whole cells than in Triton X-100 extracts, it is nevertheless sufficient to account for the rate of LTC metabolism as calculated from the data in Fig. 7.

We questioned whether inhibitors of γ-GTPase also block LTC metabolism by intact endothelial cells. Glutathione inhibited (>80%) the conversion of LTC (10^{-7} M) to LTD and LTE but only when present in vast molar excess (10 mM) to the LTC substrate. Preincubation of cells with 1 mM AT-125, an irreversible γ-GTPase inhibitor (18), for 1 h achieved the same level of inhibition of LTC metabolism as for hydrolysis of γ-glutamyl-*p*-nitroanilide. However, no significant inhibition of LTC breakdown was noted with the specific and reversible γ-GTPase inhibitor L-γ-glutamyl-*O*-carboxyphenylhydrazide (19), although this compound (1 mM) blocked (>93%) the hydrolysis of the colorimetric substrate by intact cells.

Histamine. It was of interest to directly compare prostacyclin synthesis by endothelial cells stimulated with LTC and a structurally unrelated vasoactive agent. For this purpose we chose histamine, which has been reported (20) to stimulate the release of prostacyclin by endothelium. At 10^{-7} M, a concentration at which both LTC and LTD induced maximal release of 20:4 from endothelial cells, histamine had no effect. Release of radiolabel from endothelial cells above basal levels was evident at 10^{-6} M histamine; however, maximal levels of release required 5×10^{-6} M histamine. HPLC profiles of the 20:4 metabolites released by histamine-challenged cells were similar to those shown in Fig. 5. Consistent with the kinetics of prostacyclin synthesis reported by others (20), the histamine-mediated 20:4 release plateaued in 10 min and was more rapid than the 15–30 min release promoted by LTC. In this regard, the histamine-mediated response more closely paralleled that elicited by thrombin (5) than the leukotrienes.

Discussion

LTC, 11-trans LTC, and LTD, but not LTE and LTB, promote the release of radiolabel by cultured human endothelial cells prelabeled with [³H]20:4. The radiolabel recovered in the culture medium was primarily 6-keto $PGF_{1\alpha}$, the breakdown product of prostacyclin. Direct comparisons of the leukotrienes indicated that LTC and 11-trans LTC, on a molar basis, were more effective in eliciting prostacyclin synthesis than LTD. LTE and LTB within the same concentration range (10^{-9} to 10^{-6} M) as LTC and LTD failed to elicit prostacyclin synthesis above basal levels. The rank order of the leukotrienes in eliciting

prostacyclin release by cultured human endothelium is: LTC $\approx$ 11-trans LTC > LTD >>> LTE = LTB.

The relative effectiveness of the leukotrienes in promoting prostacyclin synthesis indicated that specific structural features of the leukotriene molecule are essential for stimulation of prostacyclin synthesis by cultured endothelium. Removal of the glutamyl residue from LTC caused ~50% loss in activity, and removal of the glycyl moiety (conversion of LTD to LTE) rendered the molecule inactive. The necessity of the peptide backbone was further indicated by the fact that LTB was also inactive as a stimulus for prostacyclin synthesis by endothelial cells. However, a change in the fatty acid moiety of LTC to the 11-trans isomer had little effect on leukotriene-stimulated prostacyclin synthesis. It remains to be determined whether receptors for LTC and or LTD exist on the surface of endothelial cells. However, the evident requirement for structural features of the peptide backbone and the low concentrations of these lipid mediators required for initiation of prostacyclin formation suggest this possibility.

The potency of leukotrienes in causing prostacyclin synthesis was compared with that of histamine, an important vasoactive agent. It is well established that histamine promotes prostacyclin synthesis by cultured endothelial cells, and it is likely that this effect is mediated via an H1 receptor (20). As with LTC and LTD, histamine-induced 20:4 release is dose dependent; however, higher concentrations (1–5 μM) are required to promote levels of prostacyclin synthesis comparable to those elicited by 0.1 μM LTC. The kinetics of histamine-mediated prostacyclin synthesis are more rapid than in leukotriene-stimulated cultures, with a time course of <10 min.

Endothelial cell cultures metabolized LTC to LTD and LTE at concentrations (10^{-7} M) that promote prostacyclin synthesis. Thus, these cells have the capacity to inactivate the vasoactive leukotrienes. This may be one mechanism for controlling the level of prostacyclin formation and may explain the transient effects of these lipid mediators on the vasculature. However, the rate of LTC metabolism by these cells is slow compared with the rate of prostacyclin synthesis. It is likely that other mechanisms are also involved in either inhibiting prostacyclin synthesis or the binding of leukotrienes to endothelium.

Anderson et al. (16) have shown that purified γ-GTPase catalyzes the conversion of LTC into LTD. Recently, this enzyme was localized by cytochemical techniques to endothelial cell plasma membranes in rat hyperplastic liver (21). Levels of γ-GTPase activity in intact endothelial cells and Triton X-100 extracts of endothelial cells were sufficient to account for LTC metabolism.

LTC metabolism in intact endothelial cells was blocked by AT-125, an irreversible γ-GTPase inhibitor (19), and also by glutathione when in considerable molar excess to the substrate. Hammarstrom (22) previously reported that glutathione inhibits the formation of LTD from LTC by partially purified γ-GTPase from swine kidney. However, in intact endothelial cells, no inhibition was seen with the specific inhibitor of γ-GTPase, GCPH (18), at a 400-fold molar excess over the LTC concentration. The explanation for this discrepancy remains to be determined.

It is interesting that all three cysteinyl-containing leukotrienes induce plasma

transudation from microvasculature (3, 23), but only LTC and LTD trigger prostacyclin formation by endothelial cells. Prostacyclin induces dilation in postcapillary venules, but is not known to lead to plasma transudation. Together these observations suggest that vascular dilation and permeability may not be causally related. A second factor or mechanism may control vessel permeability and the quantity of plasma exudate (24).

Summary

Cultured endothelial cells from human umbilical cord labeled with [^{3}H]20:4 release radiolabel when exposed to leukotrienes C or D (LTC or LTD). The major radiolabeled 20:4 metabolite recovered in the culture medium was prostacyclin. Both leukotrienes produced a dose-dependent synthesis of prostacyclin, with a maximal response at 10^{-7} M leukotriene. LTC promoted a twofold greater response than did LTD at all concentrations tested (10^{-9} to 10^{-7} M). In contrast, no release of radiolabel above basal levels was evident with a challenge of LTE or LTB at the same concentrations.

Endothelial cells metabolize ~40–50% of exogenously supplied LTC to LTD and LTE in 60 min. Levels of α-glutamyltranspeptidase (γ-GTPase), the ectoenzyme reported to convert LTC to LTD, were detected in intact endothelial cells with the chromogenic substrate L-γ-glutamyl-p-nitroanilide at levels sufficient to account for the observed rate of LTC metabolism. High concentrations of the γ-GTPase inhibitors, glutathione and AT-125, blocked the metabolism of LTC by endothelium. These results suggest that degradation of leukotrienes by endothelium may be one mechanism for inactivation of these lipid mediators.

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