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AGONIST SPECIFIC DESENSITIZATION OF LEUKOTRIENE C_4-STIMULATED PGI_2 BIOSYNTHESIS IN HUMAN ENDOTHELIAL CELLS

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SUMMARY: Leukotriene C_4 (LTC_4) and, to a lesser extent, leukotriene D_4 (LTD_4) concentration dependently stimulate prostacyclin (PGI_2) biosynthesis in cultured human umbilical vein endothelial cells. PGI_2 biosynthesis was quantitated by radioimmunoassay and its structure confirmed by gas chromatography/mass spectrometry. Preincubation of endothelial cells with LTC_4 resulted in desensitization to subsequent LTC_4 stimulation. However, PGI_2 biosynthesis in response to thrombin, PGH_2 and arachidonic acid was not inhibited by preincubation with LTC_4. The C-6-sulfidopeptide leukotriene receptor level antagonist FPL-55712 attenuates LTC_4, but not thrombin-stimulated PGI_2 biosynthesis. These data suggest that human umbilical vein endothelial cells have a C-6-sulfidopeptide leukotriene receptor, and that stimulation of this receptor results in PGI_2 biosynthesis.

The synthesis of prostaglandin I_2 (PGI_2) by cultured human endothelial cells was first observed by Weksler et al. (1,2). Later, Baenziger et al. (3) observed that histamine stimulates PGI_2 biosynthesis, and Marcus et al. (4) showed that exogenously added prostaglandin H_2 could serve as a precursor for PGI_2 biosynthesis.

Another family of vasoactive lipids that are derived from arachidonic acid are the leukotrienes. Leukotriene B_4 is a potent stimulator of human neutrophil chemotaxis, aggregation, and degranulation, but has little intrinsic cardiovascular activity (5-9). The C-6-sulfidopeptide leukotrienes (LTC_4, LTD_4) are vasoactive and contract a variety of smooth muscles (10-14). Both LTC_4 and LTD_4 induce a transient contraction of arterioles, and exudation of fluid from post capilliary venules (6). LTD_4 also causes a decrease in coronary blood flow in anesthetized sheep and dogs (14,15). Since cyclo-oxygenase inhibitors have been reported to attenuate some of the cardiovascular activities of the sulfido-

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peptide leukotrienes (16), we examined the influence of LTC_4 and LTD_4 on PGI_2 biosynthesis in human umbilical vein endothelial cells.

MATERIALS AND METHODS

Leukotrienes C_4, D_4, and arachidonic acid, were obtained from The Upjohn Company, Kalamazoo, MI. PGH_2 was biosynthesized according to Gorman et al. (17). Thrombin (200 U/ml) and histamine were purchased from The Sigma Chemical Company, St. Louis, MO. Indomethacin was obtained from Merck and Co., Rahway, NJ. FPL55712 was obtained from Fisons Co., Leicestershire, England. ^{3}H-6-keto-$PGF_{1\alpha}$ (120.0 Ci/mmol) was purchased from New England Nuclear, Boston, MA. 6-Keto-$PGF_{1\alpha}$ antibody was purchased from Collaborative Research, Waltham, MA.

Endothelial cells were derived from human umbilical cord veins as described by Jaffe et al. (18). Gas chromatography/mass spectrometry of 6-keto-$PGF_{1\alpha}$ was done as previously described (20).

PGI_2 biosynthesis was studied by measuring 6-keto $PGF_{1\alpha}$ by radioimmunoassay (19). Data are represented as the mean $\pm$ SEM of triplicate determinations. Cells pretreated with 10 μM indomethacin were incubated for 10-20 minutes before the addition of agonist.

RESULTS AND DISCUSSION

Incubation of human umbilical vein endothelial cells with LTC_4 and, to a lesser extent, LTD_4 results in a concentration dependent stimulation of PGI_2 biosynthesis (Figure 1). Maximal stimulation by LTC_4 is observed between 145 and 480 nM, LTD_4 approaches the maxima at 2 μM (Figure 1).

Since our measurement of PGI_2 biosynthesis was done using a radioimmunoassay for 6-keto-$PGF_{1\alpha}$, we wanted to be sure that we were actually studying PGI_2 biosynthesis. We therefore incubated endothelial cells with 480 nM LTC_4 for 30 min, extracted the lipid products from the incubation medium, and performed gas chromatography-mass spectrometry on the extract. The principal component of the extract after base treatment and derivatization displayed prominent ions at m/z 115, m/z 378, m/z 418, m/z 508, and the molecular ion at 629. The spectrum corresponds exactly with the spectrum of the authentic 6-keto-$PGF_{1\alpha}$ standard, and proves without equivocation that prostacyclin is produced in response to LTC_4. Unstimulated cultures did not produce enough 6-keto-$PGF_{1\alpha}$ to detect by GC/MS techniques.

An analysis of the time course of LTC_4-stimulated PGI_2 synthesis shows that significant levels of PGI_2 are produced during the first 5 minutes of incubation, and that essentially maximal synthesis is achieved within 15 minutes

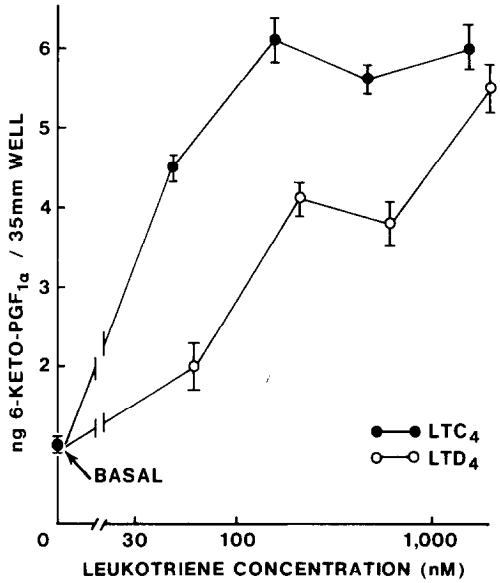

Figure 1: Concentration Dependent Stimulation of Prostaglandin I_2 Biosynthesis by Leukotriene C_4 and D_4.

Human endothelial cells (0.67 x 10^6 cells/well), were washed and incubated for 30 min at 37 °C with either LTC_4 or LTD_4 at the indicated concentrations. 6-keto-$PGF_{1\alpha}$ was measured in the incubation medium by radioimmunoassay. The basal level of 6-keto-$PGF_{1\alpha}$ did not significantly increase during the 30 min incubation period.

(Figure 2). Thrombin-stimulated PGI_2 synthesis is maximal within 10 minutes, and is much more pronounced than the stimulation observed with LTC_4 (Figure 3). It should be noted that incubation of the endothelial cells with 10 μM indomethacin (a potent prostaglandin cyclooxygenase inhibitor) completely inhibits both LTC_4 and thrombin-stimulated PGI_2 synthesis (Figure 2).

If the LTC_4-stimulation of PGI_2 biosynthesis is a receptor mediated activity, it should display agonist-specific desensitization. To test this, we incubated endothelial cells for 20 min at 37 °C with 16 μM LTC_4, washed the cells 3 times, and restimulated the cells with from 48 to 480 nM LTC_4 (Figure 3). This response was then compared to naive cells which were not preincubated with LTC_4, but were washed in the same manner. The naive cells exhibited a marked stimulation of PGI_2 biosynthesis in response to LTC_4, but the cells that were preincubated with LTC_4 did not respond to the second addition of 48 to 480 nM LTC_4 (Figure 3). The desensitization is agonist specific, because

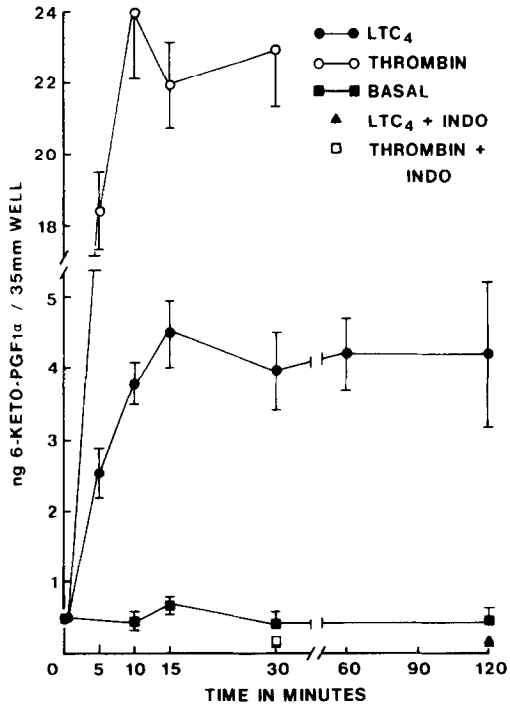

Figure 2: Time Course of LTC_4 and Thrombin-Stimulated PGI_2 Biosynthesis.

Human endothelial cells (0.91 x 10^6 cells/well) were exposed to 0.48 μM LTC_4, and 6-keto-$PGF_{1\alpha}$ measured at 5, 10, 15, 30, 60 and 120 min post addition. Some cells were stimulated with 2.0 Units/ml thrombin and 6-keto-$PGF_{1\alpha}$ measured at 5, 10, 15, and 30 min post addition.

preincubation of cells with 16 μM LTC_4 did not alter subsequent stimulation by 2 U/ml thrombin, 3 μM arachidonate, or 1.4 μM PGH_2 (Figure 4). These data show that both the prostaglandin cyclooxygenase and the prostacyclin synthase are still active, even though the system is desensitized to LTC_4.

To investigate further the specificity of the LTC_4 response we used the purported leukotriene receptor level antagonist FPL 55712. FPL 55712 from 5.6 μM to 56 μM did antagonize LTC_4-stimulated PGI_2 biosynthesis in endothelial cells (Figure 4). However, only about 50% of the response could be blocked by the highest concentration of FPL 55712. Although FPL 55712 did not completely inhibit the response, it did display some specificity for the LTC_4 response, as thrombin-stimulated PGI_2 biosynthesis was only marginally inhibited by FPL 55712 (Figure 4).

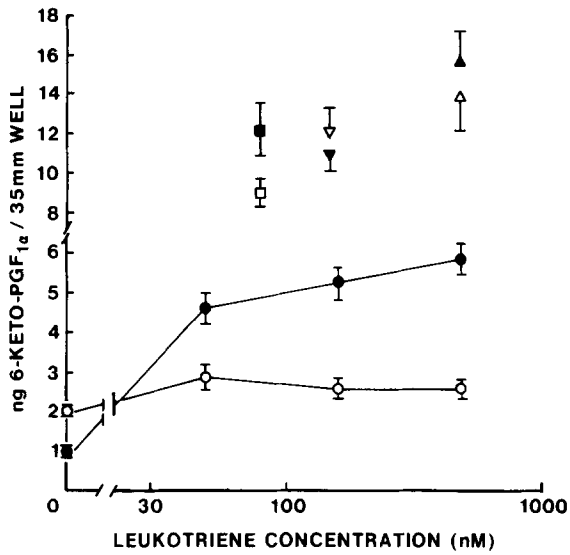

Figure 3: Desensitization of LTC_4-Stimulated PGI_2 Biosynthesis in Endothelial Cells.

Human endothelial cells (0.62 x 10^6 cells/well) were preincubated for 20 min at 37°C with 16 μM LTC_4, washed 3 times with fresh buffer, and exposed to from 48 to 480 nM LTC_4 for 30 min at 37°C (○—○). The 6-Keto-$PGF_{1\alpha}$ levels produced by these cells was then compared to the synthesis of 6-keto-$PGF_{1\alpha}$ in response to LTC_4 in naive cells that had not been preincubated with LTC_4 (●—●). Another group of cells were preincubated with 16 μM LTC_4, washed and challenged with 2 Units/ml thrombin (■), 3 μM arachidonate (▲), or 1.4 μM PGH_2 (▼), and compared to the respective naive cells represented by the corresponding open symbols (□, △, ▽).

DISCUSSION

LTC_4 and, to a lesser extent, LTD_4 stimulate PGI_2 biosynthesis in cultured human umbilical vein endothelial cells. Essentially maximum LTC_4 stimulation is achieved between 145 and 480 nM, but 2 μM LTD_4 is required for maximal stimulation.

In a quantitative sense, 2 Units/ml of thrombin (a maximally stimulating concentration) releases 3 to 5 times more PGI_2 than a maximally stimulating level of LTC_4.

The time course of LTC_4-stimulated PGI_2 biosynthesis is slightly slower than thrombin-stimulated activity. Maximal PGI_2 synthesis is achieved with thrombin in 10 minutes, while LTC_4 requires approximately 15 minutes.

The stimulation of PGI_2 biosynthesis by LTC_4 is a specific response to the leukotriene. Cells preincubated with LTC_4 display agonist specific

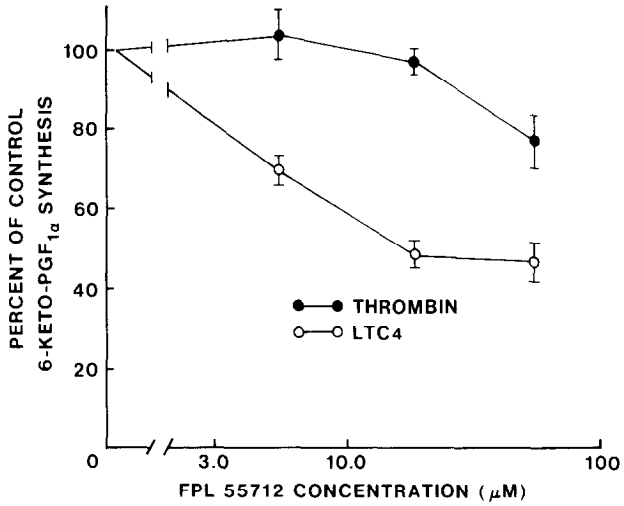

Figure 4. Influence of FPL-55712 on Thrombin and LTC_4-Stimulated PGI_2 Biosynthesis.

Human endothelial cells (0.68 x 10^6 cells/ml) were preincubated for 10 minutes at 37 °C with from 5.6 to 56 μM FPL-55712. The cells were then challenged with either 2 Units/ml thrombin or 48 nM LTC_4, and 6-keto-$PGF_{1\alpha}$ levels measured. Data are presented as a percent of the control (no FPL-55712) LTC_4 or thrombin-stimulated response.

desensitization to subsequent LTC_4 challenge. Thrombin-stimulated PGI_2 biosynthesis is not altered by preincubation with LTC_4, and although the data is not shown, the same is true for histamine as well. Desensitization of endothelial cells to LTC_4-stimulation does not alter the ability of endothelial cells to synthesize PGI_2 in response to exogenous arachidonate or PGH_2. These data show that the enzymes required for PGI_2 synthesis are still functional, but the cells cannot respond to LTC_4. These findings suggest that the loss in LTC_4 sensitivity is at the level of the LTC_4 receptor, or in the transduction of the LTC_4 signal. It is unlikely that a loss in phospholipase activity accounts for desensitization, since the cells still respond to thrombin and histamine.

The physiological relevance of PGI_2 synthesis in response to LTC_4 is not known. Several other vasoactive molecules such as histamine, bradykinin, and angiotensin II also stimulate endothelial cell PGI_2 biosynthesis (3, 21, 22). However, since some of the agents are vasoconstrictors, while others are vasodilators, no clear relationship between vascular tone and the synthesis of PGI_2 exists.

It is known that PGI_2 exacerbates plasma exudation in response to zymosan or bradykinin (23), yet PGI_2 itself is essentially inactive. Since PGI_2 has been reported to inhibit leukotriene biosynthesis in human neutrophils by stimulating cyclic AMP accumulation (24), it is possible that the synthesis of PGI_2 retards further LTC_4 synthesis by granulocytes or mast cells at the site of inflammation. While this manuscript was in preparation, Cramer et al. (25) reported that LTC_4 stimulates PGI_2 biosynthesis in human umbilical vein endothelial cells. However, no attempt to quantitate the 6-keto-$PGF_{1\alpha}$ was made, and no GC/MS analysis was done. In addition, our observation that LTC_4 induces agonist specific desensitization in neutrophils is novel, and suggests that human endothelial cells have a C-6-sulfidopeptide leukotriene receptor.

Regardless of the exact physiological significance of LTC_4-stimulated PGI_2 biosynthesis, both LTC_4 and PGI_2 are potent vasoactive molecules which are involved in acute inflammatory reactions. The finding in this report suggests that the synthesis of the two molecules are directly linked to one another.

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