Identification and Characterization of Leukotriene C₄ Receptors in Isolated Rat Renal Glomeruli

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SUMMARY. The immediate reduction of renal blood flow and glomerular filtration rate in response to intravenous infusion of leukotriene C₄ in the rat prompted an analysis of isolated rat renal glomeruli for the presence of specific receptors for leukotriene C₄. Specific binding of [³H]leukotriene C₄ to glomeruli increased in a time-dependent manner, reached equilibrium after 60 minutes of incubation at 4°C, and was 80% reversible upon addition of excess unlabeled leukotriene C₄ at equilibrium. Specific binding of [³H]leukotriene C₄ to glomeruli increased in a dose-dependent manner, approaching saturation at concentrations of 40–60 nM. Inhibition of binding of [³H]leukotriene C₄ with increasing concentrations of unlabeled leukotriene C₄ was dose dependent. The equilibrium dissociation constant for [³H]leukotriene C₄ binding to glomeruli, calculated from saturation and competitive binding-inhibition studies, was 25 ± 7 nM and 35 ± 16 nM (mean ± SEM), respectively, and glomerular leukotriene C₄ receptor density was 8.5 ± 1.5 and 9.0 ± 3.0 pmol/mg protein, respectively. The other natural vasoactive sulfidopeptide leukotrienes, leukotriene D₄ and leukotriene E₄, the chemotactic agent, leukotriene B₄, and the sulfidopeptide leukotriene antagonist, FPL 55712, competed for the receptor at concentrations 2–3 orders of magnitude higher than the homoligand, leukotriene C₄. The binding and specificity characteristics of the glomerular leukotriene C₄ receptor are similar to those previously reported for the D₁ receptor, a nonspecific smooth muscle cell line derived from hamster vas deferens, for guinea pig ileum smooth muscle, and for a subcellular fraction of rat lung homogenate, and represent the first characterization of such a receptor in a vascular tissue.

Further characteristics of the glomerular leukotriene C₄ receptor are similar to those previously reported for the D₁ receptor, a nonspecific smooth muscle cell line derived from hamster vas deferens, for guinea pig ileum smooth muscle, and for a subcellular fraction of rat lung homogenate, and represent the first characterization of such a receptor in a vascular tissue. (Circ Res 56: 324–330, 1985)
Methods

Leukotrienes Κ₄, Α₄, Ε₄, and Β₄ were prepared synthet-ically (Corey et al., 1980a, 1980b) and were stored under argon at −80°C in 0.05 M phosphate buffer, pH 6.8, containing 20% ethanol. [3H-Κ₄]LTC₄ (35.7-40 Ci/mmol) was supplied by New England Nuclear, and FPL55712 by Fisons. High performance liquid chromatography (HPLC) grade methanol (Fisher Scientific Co.), L-serine, N-2-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid (HEPES), reduced glutathione, sodium tetraborate, arachidonic acid (Sigma Chemical Corp.), and prostaglandin E₂ (Seragen) were purchased from the manufacturers.

Isolation of Glomeruli

Male Sprague-Dawley rats, 250-300 g (Gofmoor Farms), were decapitated and exsanguinated, and the kidneys were removed and placed in ice-cold phosphate-buffered saline (PBS), pH 7.4; all subsequent steps were carried out at 4°C. Glomeruli were isolated by the following method modified from that of Misra (1972). The kidneys were bisected longitudinally and the medulla, papilla, capsule, and any visible connective tissue were removed by macroscopic dissection. The remaining cortical rims were then minced with a razor blade and pressed through a stainless steel sieve of 90-μm pore size. The resulting suspension was sequentially poured through a stainless steel sieve of 300-μm and a 40-μm sieve and the fraction retained on the 90-μm sieve was used for experiments. The suspension was then rinsed with PBS three times and resuspended in 20 mM HEPES buffer, pH 7.4, containing 125 mM NaCl and 5 mM CaCl₂. Sedimentation and resuspension were repeated twice. Two 50-μl portions of the final suspension were examined microscopically for each preparation. Glomeruli were then rinsed from the surface of the sieve into a 50-ml centrifuge tube, sedimented at 100 g for 2 minutes, and resuspended in 20 mM HEPES buffer, pH 7.4, containing 125 mM NaCl and 5 mM CaCl₂. Sedimentation and resuspension were repeated twice. Two 50-μl portions of the final suspension were examined microscopically for each preparation. Glomeruli were then obtained as described above.

Radioligand Binding

Because glomeruli were found to adhere to polyethylene tubes under the assay conditions to be described, binding experiments were carried out in borosilicate glass tubes in which retention of glomerular protein was less than 5%. Unless otherwise stated, between 500 and 1000 glomeruli were incubated in duplicate or triplicate with constant concentrations of [3H]LTC₄ in 0.5 ml of assay buffer consisting of 20 mM HEPES, pH 7.4, 125 mM NaCl, 5 mM CaCl₂, and 1 mM serine-borate complex at 4°C for 60 minutes on a rotary shaking platform. Serine-borate complex was included to prevent LTC₄ metabolism by peptidase cleavage (Tate and Meister, 1978; Orning and Hammarström, 1980). Bound radioactivity was separated from free by suction-filtration through prewetted glass-fiber filters (Boehringer-Mannheim) on a single Hoeffer filtration manifold, followed by four successive washings with 2.5-ml portions of ice-cold assay buffer: the filtration step and all of the washes were complete within 10 seconds. Timed experiments (n = 2), each done in triplicate, at 10-second intervals, demonstrated that total and nonspecific, and thus also specific [3H]LTC₄ binding, did not change during 40 seconds of continuous washing with ice-cold assay buffer. Filters were placed into scintillation vials with 1 ml of ethanol:water, 4:1 (vol/vol) at room temperature for 12-16 hours. Five milliliters of Aquasol (NEN) were added and, after an additional 2-4 hours of incubation at room temperature, bound radioactivity was determined in a Tracor scintillation count with 50% counting efficiency for tritium. Total radioactivity per assay tube was determined in buffer solution replicates without glomeruli; two 100-μl portions of the mixture were added directly to scintillation vials containing 1 ml of 80% ethanol, and were processed and counted in parallel with the filters. Specific binding was defined as bound radioactivity which was inhibited by 3 μM unlabeled LTC₄. Nonspecific binding was found to be 2-3% of the total radioactivity added, and ranged from 10-20% of the total bound radioactivity in competition experiments; in saturation studies, nonspecific binding reached 30-50% at the highest [3H]LTC₄ concentrations used.

The time course of specific [3H]LTC₄ binding to glomeruli was assessed in six separate experiments for concentrations of [3H]LTC₄ of 0.3, 0.6, 1.0, 3.3, 6.7, and 8.5 nM. Glomeruli were incubated with these concentrations of [3H]LTC₄ with and without 3 μM unlabeled LTC₄ for periods from 1-120 minutes at 4°C. In four experiments, we assessed reversibility of the binding at equilibrium by adding 3 μM unlabeled LTC₄ after 60 minutes of incubation with [3H]LTC₄ and determining remaining bound radioactivity as a function of time.

Specific [3H]LTC₄ binding as a function of increasing glomerular concentration was assessed at fixed concentrations of [3H]LTC₄. In four separate experiments in which the [3H]LTC₄ concentration was 1.9, 2.5, 5.0, and 7.7 nM, the glomerular concentration was varied in each from 200-2000/ml. The influence of Ca²⁺ on [3H]LTC₄ binding to glomeruli was examined at concentrations of 1, 2, 5, and 10 mM, added as its chloride salt by comparison to specific binding in the absence of added divalent cations. The heat sensitivity of the glomerular LTC₄-binding site was determined by boiling glomeruli for 15 minutes before assessing specific and nonspecific [3H]LTC₄ binding.

To determine the saturation, affinity, and density of LTC₄-binding sites, 250-300 glomeruli were incubated with increasing concentrations of [3H]LTC₄ (0.5-60 nM) in an incubation volume of 250 μl for 60 minutes at 4°C. Total and nonspecific binding were determined for each concentration of [3H]LTC₄. Data were analyzed using the LIGAND program (Munson and Rodbard, 1980).

We carried out competitive binding-inhibition studies by incubating a fixed number of glomeruli with [3H]LTC₄ in a volume of 0.5 ml in the presence of increasing concentrations of unlabeled LTC₄ (0.1-3000 nM). Data for competition by unlabeled LTC₄ were analyzed with the LIGAND program to determine the number of distinct sites, binding affinity, and receptor density. Competition
of LTD₄ (0.01–100 μM), LTE₄ (0.01–100 μM), LTB₄ (0.01–100 μM), FPL 55712 (0.1–100 μM), reduced glutathione (0.001–10 mM), prostaglandin E₂ (0.001–10 mM), or arachidonic acid (0.001–10 mM) for [³²P]LTC₄ binding was also determined.

Bioconversion and/or oxidative inactivation of [³²P]LTC₄ was examined by incubating [³²P]LTC₄ in the absence and presence of glomeruli for 90 minutes at 4°C. Glomeruli were separated by centrifugation in a Beckman microfuge at 8,000 g for 30 seconds at 4°C, and the supernatant extract was removed. The glomeruli were resuspended and incubated for 30 minutes at 4°C with buffer containing 3 μM unlabeled LTC₄ in order to displace bound [³²P]LTC₄; the glomeruli were then sedimented with the microfuge, and the eluate supernatant was removed. The residual tissue-associated radioactivity was determined as usual and compared with replicates in which specific binding of [³²P]LTC₄ to glomeruli was determined in the routine fashion. Solutions of [³²P]LTC₄ incubated without glomeruli, the glomerular [³²P]LTC₄ supernatant, and the glomerular eluate each were adjusted to the solvent for reverse-phase-HPLC, namely, methanol:assay buffer (65:34.9:0.1, vol/vol/vol, pH 5.6). One-milliliter fractions were collected individually with 200–500 ng of unlabeled LTC₄ standard onto an Altex C₄ reverse-phase HPLC column (Rainin) and eluted isocratically at a flow rate of 1 ml/min with methanol:water:acetic acid (65:34.9:0.1, vol/vol/vol, pH 5.6). One-milliliter fractions were collected in scintillation vials, mixed with 5 ml aquasol, and assessed for radioactivity. The retention times of synthetic LTC₄, and of LTD₄, which were chromatographed separately, were determined by on-line monitoring of the UV absorbance at 280 nm.

Protein was determined by the method of Lowry et al. (1951) after the glomeruli were washed three times by centrifugation in PBS to remove HEPES buffer and were digested in 1 N NaOH at 60°C for 30 minutes.

Data are presented as means ± SEM with n = 3 being the minimum number of separate experiments for any experimental condition; data for triplicate determinations within a single experiment are presented as means ± 5.

Results

Time-course and Reversibility of [³²P]LTC₄ Binding at 4°C

Specific binding of [³²P]LTC₄ at all concentrations (0.3–8.5 nM) tested, increased in a time-dependent manner at 4°C, reached binding equilibrium by 60 minutes of incubation, and remained stable for up to 120 minutes. Nonspecific binding reached its maximum at 5 minutes of incubation and remained stable thereafter. In four time-course experiments, an excess 3 μM unlabeled LTC₄ was added at binding equilibrium, resulting in rapid reversal of specific [³²P]LTC₄ binding which was 86 ± 4% (mean ± SEM) complete 60 minutes later. A representative time-course of [³²P]LTC₄ binding and reversal by displacement is shown in Figure 1.

Effect of Ca²⁺ Concentration, Renal Perfusion, Heat Treatment, and Varying Glomerular Concentration on Specific [³²P]LTC₄ Binding

Specific [³²P]LTC₄ binding relative to no added calcium was 94 ± 4% at a calcium ion concentration of 1 mM, and at 2, 5, and 10 mM, 103 ± 7, 105 ± 4, and 105 ± 3% (mean ± SEM, n = 4), respectively. As these differences were not significant, further experiments were arbitrarily carried out with 5 mM Ca²⁺ in the buffer.

Specific [³²P]LTC₄ binding to glomeruli from saline-perfused kidneys was identical at 105 ± 7% (mean ± SEM, n = 3) to that obtained with glomeruli isolated from nonperfused kidneys, which was expressed as 100%. Hence, there was specific [³²P]LTC₄ binding to glomerular tissue, and not to retained blood products.

Specific [³²P]LTC₄ binding to glomeruli which were boiled for 15 minutes was reduced to 5.1 ± 2.4% (n = 3) of that of control glomeruli, demonstrating that the specific LTC₄-binding site in glomeruli is heat sensitive.

Specific [³²P]LTC₄ binding to glomeruli increased linearly when the glomerular concentration was varied from 200–2000/ml. Regression analysis of this relationship for four separate experiments yielded a correlation coefficient of 0.988 ± 0.004 (mean ± SEM). An experiment that is representative of this linear relationship is shown in Figure 2.
Binding of \(^{3}H\)LTCA to Rat Ileum Smooth Muscle

With a radioligand concentration of 2.22 nM, rat ileal longitudinal smooth muscle strips bound 37 ± 4 fmol \(^{3}H\)LTCA/mg protein in the absence of unlabeled LTCA, and 79 ± 11 fmol/mg protein in the presence of 0.3 \(\mu\)M unlabeled LTCA. In glomeruli from the same rats, which were processed concurrently, total \(^{3}H\)LTCA binding was 568 ± 69 fmol/mg protein in the absence of unlabeled LTCA, and 252 ± 23 fmol/mg in the presence of 0.3 \(\mu\)M unlabeled LTCA. Thus, specific binding of radiolabeled LTCA was not observed in rat ileal smooth muscle, a tissue known not to contract in response to LTCA (Goldenberg and Subers, 1982).

Equilibrium Binding Parameters Derived from \(^{3}H\)LTCA Binding as a Function of Increasing Concentrations of Radioligand

Specific \(^{3}H\)LTCA binding to isolated glomeruli with increasing concentrations of \(^{3}H\)LTCA ranging from 0.5–60 nM reached a plateau between 40 and 60 nM in a representative experiment (Fig. 3), demonstrating saturability of the glomerular LTCA-binding site. Scatchard transformation of these data (Fig. 3) resulted in a linear plot, indicating the detection of one population of binding sites. Equilibrium-binding parameters derived by Scatchard analysis were: an equilibrium dissociation constant (Kd) of 23.5 nM and a receptor density (Ro) of 8.4 pmol/mg glomerular protein. Saturation-binding data from six separate experiments were consistently found to best fit a one-site binding curve when analyzed by the LIGAND program. The binding parameters derived by computer analysis were Kd = 25 ± 7 nM (mean ± SEM) and glomerular LTCA receptor density = 7.8 ± 2.0 ± 10^8 sites/gglomerulus or 8.0 ± 1.5 pmol/mg protein.

Competitive Binding Inhibition Studies

Incubation of glomeruli with 0.5–1 nM \(^{3}H\)LTCA in the presence of increasing concentrations of unlabeled LTCA (0.1–3000 nM) resulted in dose-dependent displacement of \(^{3}H\)LTCA from its binding site (n = 5), as illustrated in Figure 4A. Because increasing the concentration of unlabeled LTCA above 3 \(\mu\)M did not result in further inhibition of \(^{3}H\)LTCA binding, \(^{3}H\)LTCA binding in the presence of 3 \(\mu\)M unlabeled LTCA was taken as nonspecific binding. Competitive binding-inhibition data from five separate experiments depicted in Figure 4A were best resolved by a one-site binding curve. Binding parameters for the five experiments were
Radioligand-binding techniques were used to identify and characterize specific binding sites for LTC₄ in isolated rat renal glomeruli. Association of [³H]LTC₄ at 4°C with the glomerular binding site was time-dependent and reached equilibrium at 60 minutes of incubation for concentrations of [³H]LTC₄ ranging from 0.3 nM to 8.5 nM, and was stable for at least 60 minutes thereafter (Fig. 1). Specific [³H]LTC₄ binding to isolated renal glomeruli was rapidly reversible when excess unlabeled LTC₄ was added at equilibrium, showing that the association of [³H]LTC₄ with glomeruli is not merely due to incorporation of this polar lipid into the phospholipid bilayer of plasma membranes. Because equilibrium conditions were verified for even the lowest concentrations of radioligand used in subsequent saturation and competitive binding studies, calculation of equilibrium-binding parameters was feasible with the LIGAND program (Munson and Rodbard, 1980).

Specific [³H]LTC₄ binding was to glomerular tissue and not to retained blood elements, since specific [³H]LTC₄ binding was identical in glomeruli isolated from saline-perfused and nonperfused kidneys within the same animal. Specific [³H]LTC₄ binding was nearly undetectable in glomeruli that had been boiled for 15 minutes before the binding studies, indicating that the binding site is heat sensitive. Specific [³H]LTC₄ binding to isolated glomeruli increased linearly with increasing glomerular concentration (Fig. 2), and was saturable with increasing [³H]LTC₄ concentrations (Fig. 3), fulfilling two of the criteria that are usually required for a binding site to be considered as a receptor.

Because of the previous observation that guinea pig ileal segments (which are routinely used in the bioassay of slow-reacting substance of anaphylaxis) express specific LTC₄ receptors (Krilis et al., 1984), rat intestinal tissue, which does not respond to LTC₄ (Goldenberg and Subers, 1982), was used for comparison, with negative results.

With the LIGAND program, saturation data were consistently (n = 6) found to best fit a one-site binding model, indicating one population of LTC₄ receptors with a single affinity in isolated glomeruli. The linear Scatchard plot shown for one such experiment with increasing concentrations of [³H]LTC₄ standard when subjected to reverse-phase HPLC with a recovery of 73 ± 5% (mean ± SEM, n = 3) of the applied counts. [³H]LTC₄ incubated in the presence of glomeruli and [³H]LTC₄ previously bound to glomeruli and then dissociated by excess unlabeled LTC₄ each eluted at the retention time of authentic LTC₄ with less than 2% of the radioactivity appearing at the retention times of LTD₄ and LTE₄ standards (Fig. 5). Overall recovery of [³H]LTC₄ in the supernatant and eluate of glomeruli was 74 ± 15% (mean ± SEM, n = 6).

**Discussion**

[LTC₄ Degradation during and after Receptor Binding](#)

[³H]LTC₄ incubated in assay buffer alone at 4°C for 90 minutes co-eluted with the synthetic LTC₄ standards when subjected to reverse-phase HPLC with a recovery of 73 ± 5% (mean ± SEM, n = 3) of the applied counts. [³H]LTC₄ incubated in the presence of glomeruli and [³H]LTC₄ previously bound to glomeruli and then dissociated by excess unlabeled LTC₄ each eluted at the retention time of authentic LTC₄ with less than 2% of the radioactivity appearing at the retention times of LTD₄ and LTE₄ standards (Fig. 5). Overall recovery of [³H]LTC₄ in the supernatant and eluate of glomeruli was 74 ± 15% (mean ± SEM, n = 6).

**FIGURE 5.** RP-HPLC profile of [³H]LTC₄ after 90 minutes of incubation of 11 nM LTC₄ in assay buffer alone (panel A), in the glomerular supernatant (panel B), and [³H]LTC₄ after elution from the glomerular receptor (panel C). The retention times of synthetic leukotriene standards are indicated by the arrows in panel A.

K_d = 35 ± 16 nM and R_max = 14.1 ± 8.0 × 10^8 sites/gglomerulus, or 9.0 ± 3.0 pmol/mg protein. LTD₄, LTE₄, and LTB₄ were found to compete for [³H]LTC₄ binding at concentrations 2.5-3 orders of magnitude higher than LTC₄. Similarly, the sulfidopeptide leukotriene antagonist FPL55712 competed for the glomerular LTC₄-binding sites at concentrations of 10^-5 m or greater (Fig. 4A). Displacement of 50% of specifically bound [³H]LTC₄ by LTD₄ and LTE₄ occurred at concentrations for LTD₄ of 8.0 and 5.6 μM and for LTE₄ of 9.0 and 18 μM in two experiments, and was not reached with either ligand in a third. Similarly, FPL55712 inhibited 50% of specific [³H]LTC₄ binding to glomeruli at 20 and 77 μM in two experiments, whereas, in a third experiment, competition was not demonstrated for concentrations up to 4 μM. Prostaglandin E₂ and reduced glutathione did not achieve 50% inhibition of [³H]LTC₄ binding at concentrations of 1 and 3 mM, respectively (Fig. 4B).

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With the LIGAND program, saturation data were consistently (n = 6) found to best fit a one-site binding model, indicating one population of LTC₄ receptors with a single affinity in isolated glomeruli. The linear Scatchard plot shown for one such experiment with increasing concentrations of [³H]LTC₄ standard when subjected to reverse-phase HPLC with a recovery of 73 ± 5% (mean ± SEM, n = 3) of the applied counts. [³H]LTC₄ incubated in the presence of glomeruli and [³H]LTC₄ previously bound to glomeruli and then dissociated by excess unlabeled LTC₄ each eluted at the retention time of authentic LTC₄ with less than 2% of the radioactivity appearing at the retention times of LTD₄ and LTE₄ standards (Fig. 5). Overall recovery of [³H]LTC₄ in the supernatant and eluate of glomeruli was 74 ± 15% (mean ± SEM, n = 6).
best illustrates that receptors with a single affinity are being detected (Fig. 3). Competitive binding-inhibition studies with increasing concentrations of unlabeled LTC4 allowed analysis over a wider range of LTC4 concentrations (0.5–3000 nM) (Fig. 4) than studies with increasing concentrations of radioabeled LTC4 (0.5–60 nM). Computer analysis of competition data also resolved only one class of receptors with a single affinity, thus ruling out a second, low-affinity site which might have been missed if only increasing concentrations of [3H]LTC4 had been used. Equilibrium-binding parameters were derived directly by LIGAND analysis and were similar for saturation and competition studies with equilibrium Kd = 25 ± 7 nM (mean ± SEM) and 35 ± 16 nM, respectively, and Rn = 8.0 ± 1.5 pmol/mg protein and 9.0 ± 3.0 pmol/mg protein, respectively. These values are likely to represent true Kd and Rn values for the glomerular LTC4 receptor under these conditions, as LTC4 was shown to remain intact during the binding studies (Fig. 5).

The Kd for the glomerular LTC4 receptor is about one-half order of magnitude greater than that of LTC4 receptors in guinea pig ileal smooth muscle segments, Kd = 7.6 nM (Krilis et al., 1984), and of cultured DDT cells, Kd = 5.0 nM (Krilis et al., 1983), indicating a lower affinity of glomerular receptors for LTC4. It is unlikely that this difference is due to variations in assay conditions, as the temperature, pH, and length of incubation were the same, and calcium was used as the divalent cation in all three of these studies, albeit at a higher concentration of 5 mM with glomeruli, as compared to 1 mM in the other studies. NaCl, at concentrations of 125 mM, has been reported to inhibit LTD4 binding to a subcellular fraction of guinea pig lung (Pong and De Haven, 1983), but not LTC4 binding to a subcellular fraction of rat lung (Pong et al., 1983). NaCl at this concentration was required to preserve isotonicity in the studies with intact glomeruli, and would be present during any response of cell membrane receptors in vivo. The LTC4 receptor affinity (Kd = 41 nM) previously reported for rat lung particulate fractions (Pong et al., 1983) more closely resembles that found in isolated rat glomeruli, suggesting that LTC4 receptors may exhibit a degree of heterogeneity in different tissues or, perhaps, across species.

Competitive binding-inhibition studies demonstrated a high degree of specificity of the glomerular LTC4-binding site for LTC4, as structurally related sulfidopeptide leukotrienes LTD4 and LTE4 were able to compete for the receptor only at concentrations 2.5–3 orders of magnitude greater than LTC4 (Fig. 4). The fact that LTD4 and LTE4—and LTE5, which does not contain a thiopeptide domain—all displaced [3H]LTC4 with similar potencies, indicates that these other naturally occurring leukotrienes interact with the glomerular LTC4 receptor in a rather nonspecific manner. FPL55712, an agent previously shown to partially inhibit LTD4 but not LTC4 constrictor effects in lung parenchymal strips (Drazen et al., 1980), is a very weak competitive inhibitor of [3H]LTC4 binding in glomeruli with only a 10-fold greater affinity than arachidonic acid, which displaced 50% of specifically bound [3H]LTC4 at a concentration of 0.56 nM. The high degree of specificity of LTC4 for glomerular receptors is very similar to the findings of DDT cells (Krilis et al., 1983), guinea pig ileum (Krilis et al., 1984), and rat lung particulate fractions (Pong et al., 1983), where LTD4, LTE4, LTB4, and FPL55712 were also relatively ineffective in displacing [3H]LTC4.

Whole rat glomeruli consist of at least three cell types, endothelial cells, contractile smooth muscle-like mesangial cells, and epithelial cells; the exact cell type(s) expressing the LTC4 receptor is not known. Mesangial cell contraction is known to occur in response to vasopressin and angiotensin II (Au-siello et al., 1980), and glomerular mesangial and epithelial cells release several prostaglandins in response to stimulation with the calcium ionophore A23187 (Kreisberg et al., 1982). Knowledge of functional responses of single populations of vascular cells to LTC4 is presently limited to the report that cultured endothelial cells from human umbilical veins are induced by LTC4 to produce prostacyclin (Cramer et al., 1983). The responses of microvascular beds to LTC4, i.e., arteriolar constriction and augmented venular permeability (Drazen et al., 1980; Dahlen et al., 1981), should relate to an effect on smooth muscle and endothelial cells, respectively.

The demonstration of specific LTC4 receptors in isolated renal glomeruli and previous indications of direct renal vasoconstrictor effects of LTC4 (Rosenthal and Pace-AscaI, 1983; Badr et al., 1984) raise the question of the circumstances under which LTC4 might be expected within the microenvironment of the renal glomerulus. Evaluations of lipoxygenase activities from isolated rat glomeruli (Jim et al., 1982) and from human and murine glomeruli (Baud et al., 1983; Sraer et al., 1983) have shown production of 12-HETE, 15-HETE, and smaller quantities of 8- or 9-HETE, but not of 5-HETE. However, infusion of either the calcium ionophore A23187 into isolated, perfused rat kidneys or antigen into the same preparation from rats previously sensitized with ovalbumin released LTC4 and LTD4 into the renal venous effluent (Pirotsky et al., 1984). The bioavailability of these particular 5-lipoxygenase products from their undefined renal source or from infiltrating inflammatory cells such as macrophages (Holdsworth et al., 1978; Lewis and Austen, 1984) or eosinophils (Galpin et al., 1978; Weller et al., 1983) would establish relevance for the effects of LTC4 infusion on renal glomerular function. The present report defining the existence of specific renal glomerular receptors for LTC4 therefore provides an additional and critical indication that this compound could act as a local mediator in certain renal inflammatory diseases that adversely affect local microvascular function.
References


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