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 DDT₁ nonvascular 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)

THE oxidative metabolism of arachidonic acid via 5-lipoxygenase forms 5-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid (5-HPETE), which is enzymatically converted to 5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid (LTA₄). LTA₄ is processed by an epoxide hydrolase to 5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid (LTA₄H₂). From LTA₄, sequential cleavage of glutamic acid by γ-glutamyl transpeptidase and of glycine by a dipeptidase forms 5-hydroxy-6R-S-cysteinylglycyl-7,9-trans-11,14-cis-eicosatetraenoic acid (LTD₄) and 5-hydroxy-6R-S-cysteinyl-7,9-trans-11,14-cis-eicosatetraenoic acid (LTE₄), respectively (Samuelsson, 1983). LTC₄ and LTD₄ are potent spasmogens for some nonvascular smooth muscles in vitro, as exemplified by the response of guinea pig ileum and both upper and lower guinea pig airways (Drazen et al., 1980; Lewis et al., 1981). LTC₄ and LTD₄ are likewise effective as airway smooth muscle constrictors when inhaled as aerosols by guinea pigs (Leitch et al., 1983) and humans (Weiss et al., 1982). Vascular activities of the sulfidopeptide leukotrienes have been demonstrated upon intradermal injection into guinea pigs (Drazen et al., 1980) or topical application to the hamster cheek pouch (Dahlén et al., 1981), where LTC₄ and LTD₄ cause constriction of arterioles, followed by leakage of plasma from postcapillary venules. Intracoronary injection of LTC₄ in sheep (Michelassi et al., 1982) or bolus administration intravenously of LTC₄ or LTD₄ in ventilated rats (Pfeffer et al., 1983) causes coronary artery constriction and a reduction in cardiac output. Infusion of LTC₄ and LTD₄ into the renal artery of isolated, perfused rat kidneys causes a marked, dose-dependent increase in renal vascular resistance (Rosenthal and Pace-Asciak, 1983). When infused systemically into the intact, anesthetized rat, LTC₄ produces immediate vasoconstriction of the peripheral, coronary, and renal circulations, followed by a significant reduction of plasma volume, presumably on the basis of enhanced vascular permeability and activation of the renin-angiotensin system (Badr et al., 1984).

Specific receptors for LTC₄ have been characterized in nonvascular smooth muscle cells and tissues (Krilis et al., 1983, 1984), and in crude membrane fractions of rat lung homogenate (Pong et al., 1983). In view of the reported vascular effects of LTC₄, and specifically those on renal vascular resistance and glomerular filtration rate (Badr et al., 1984), receptors for LTC₄ are now demonstrated in renal glomeruli isolated from normal rats.
Methods

Leukotrienes C₄, D₄, E₂, and B₄ were prepared synthetically (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. [³²P]-LTC₃ (35.7–40 Ci/μmol) was supplied by New England Nuclear, and FPLS5712 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 sieves with pore sizes of 180, 150, and 90 μm, respectively. The glomeruli were retained on the 90-μm sieve and were washed extensively; the 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 largely intact, devoid of Bowman’s capsule and afferent and efferent arterioles, and routinely of better than 98% purity as assessed by light microscopy. Between 500 and 1000 glomeruli were incubated in duplicate or triplicate with constant concentrations of [³²P]-LTC₃ 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 LTC₃ metabolism by peptide cleavage (Tate and Meister, 1978; Örnh and Hammström, 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 [³²P]-LTC₃ 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: 4:3 (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 counter 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 LTC₃. 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 [³²P]-LTC₃ concentrations used.

The time course of specific [³²P]-LTC₃ binding to glomeruli was assessed in six separate experiments for concentrations of [³²P]-LTC₃ of 0.3, 0.6, 1.0, 3.3, 6.7, and 8.5 nM. Glomeruli were incubated with these concentrations of [³²P]-LTC₃ with and without 3 μM unlabeled LTC₃ 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 LTC₃ after 60 minutes of incubation with [³²P]-LTC₃ and determining remaining bound radioactivity as a function of time.

Specific [³²P]-LTC₃ binding was found as a function of increasing glomerular concentration was assessed at fixed concentrations of [³²P]-LTC₃ in four separate experiments in which the [³²P]-LTC₃ 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 Ca++ on [³²P]-LTC₃ 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 LTC₃-binding site was determined by boiling glomeruli for 15 minutes before assessing specific and nonspecific [³²P]-LTC₃ binding.

To determine the saturability, affinity, and density of LTC₃-binding sites, 250–300 glomeruli were incubated with increasing concentrations of [³²P]-LTC₃ (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 [³²P]-LTC₃. 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 [³²P]-LTC₃ in a volume of 0.5 ml in the presence of increasing concentrations of unlabeled LTC₃ (0.1–3000 nM). Data for competition by unlabeled LTC₃ were analyzed with the LIGAND program to determine the number of distinct sites, binding affinity, and receptor density.
of LTD₄ (0.01–100 μM), LTE₄ (0.01–100 μM), LTB₄ (0.01–100 μM), FPI 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 [³H]LTC₄ binding was also determined.

Bioconversion and/or oxidative inactivation of [³H]LTC₄ was examined by incubating [³H]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 [³H]LTC₄; the glomeruli were then digested 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 [³H]LTC₄ to glomeruli was determined in the routine fashion. Solutions of [³H]LTC₄ incubated without glomeruli, the glomerular [³H]LTC₄ supernatant, and the glomerular eluate each were adjusted to the solvent for reverse-phase-HPLC, namely, methanol:assay buffer:acetic acid (65:34:9, vol/vol/vol, pH 5.6). Each sample was injected individually with 200–500 ng of unlabeled LTC₄ standard onto an Altex C₁₈ 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 and LTE₄, 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 ± so.

Results

Time-course and Reversibility of [³H]LTC₄ Binding at 4°C

Specific binding of [³H]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 [³H]LTC₄ binding which was 86 ± 4% (mean ± SEM) complete 60 minutes later. A representative time-course of [³H]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 [³H]LTC₄ Binding

Specific [³H]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 [³H]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 [³H]LTC₄ binding to glomerular tissue, and not to retained blood products.

Specific [³H]LTC₄ binding in 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 [³H]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 [3H]LTC4 to Rat Ileum Smooth Muscle

With a radioligand concentration of 2.22 nM, rat ileal longitudinal smooth muscle strips bound 37 ± 4 fmol [3H]LTC4/mg protein in the absence of unlabeled LTC4, and 79 ± 11 fmol/mg protein in the presence of 0.3 μM unlabeled LTC4. In glomeruli from the same rats, which were processed concurrently, total [3H]LTC4 binding was 568 ± 69 fmol/mg protein in the absence of unlabeled LTC4, and 252 ± 23 fmol/mg in the presence of 0.3 μM unlabeled LTC4. Thus, specific binding of radiolabeled LTC4 was not observed in rat ileal smooth muscle, a tissue known not to contract in response to LTC4 (Goldenberg and Subers, 1982).

Equilibrium Binding Parameters Derived from [3H]LTC4 Binding as a Function of Increasing Concentrations of Radioligand

Specific [3H]LTC4 binding to isolated glomeruli with increasing concentrations of [3H]LTC4 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 LTC4-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 (R0) 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 LTC4 receptor density = 7.8 ± 2.0 ± 10^8 sites/glomerulus or 8.0 ± 1.5 pmol/mg protein.

Competitive Binding Inhibition Studies

Incubation of glomeruli with 0.5–1 nM [3H]LTC4 in the presence of increasing concentrations of unlabeled LTC4 (0.1–3000 nM) resulted in dose-dependent displacement of [3H]LTC4 from its binding site (n = 5), as illustrated in Figure 4A. Because increasing the concentration of unlabeled LTC4 above 3 μM did not result in further inhibition of [3H]LTC4 binding, [3H]LTC4 binding in the presence of 3 μM unlabeled LTC4 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
Binding Degradation during and after Receptor 4B). respectfully (Fig. at concentrations of 1 and 3 occurred at concentrations for LTD 4 and LTE 4 by LTD 4 specifically bound [%]LTC binding at concentrations 2.5-3 orders of magnitude for 90 minutes co-eluted with the synthetic LTC 3 binding. [3H]LTC after elution from the glomerular supernatant (panel B), and [3H]LTC after elution from the glomerular receptor (panel C). The retention times of synthetic leukotriene standards are indicated by the arrows in panel A.

Kd = 35 ± 16 nM and R0 = 14.1 ± 8.0 x 10^8 sites/gglomerulus, or 9.0 ± 3.0 pmol/mg protein. LTD 4 , LTE 4 , and LTB 4 were found to compete for [3H]LTC binding at concentrations 2.5-3 orders of magnitude higher than LTC 4 . Similarly, the sulfidopeptide leukotriene antagonist FPL55712 competed for the glomerular LTC 4 -binding sites at concentrations of 10^-5 M or greater (Fig. 3A). Displacement of 50% of specifically bound [3H]LTC 4 by LTD 4 and LTE 4 occurred at concentrations for LTD 4 of 8.0 and 5.6 μM and for LTE 4 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]-LTC 4 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 E 2 and reduced glutathione did not achieve 50% inhibition of [3H]LTC 4 binding at concentrations of 1 and 3 mM, respectively (Fig. 4B).

[3H]LTC 4 Degradation during and after Receptor Binding

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

Discussion

Radioligand-binding techniques were used to identify and characterize specific binding sites for LTC 4 in isolated rat renal glomeruli. Association of [3H]LTC 4 at 4°C with the glomerular binding site was time-dependent and reached equilibrium at 60 minutes of incubation for concentrations of [3H]LTC 4 ranging from 0.3 nM to 8.5 nM, and was stable for at least 60 minutes thereafter (Fig. 1). Specific [3H]LTC 4 binding to isolated renal glomeruli was rapidly reversible when excess unlabeled LTC 4 was added at equilibrium, showing that the association of [3H]LTC 4 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]LTC 4 binding was to glomerular tissue and not to retained blood elements, since specific [3H]LTC 4 binding was identical in glomeruli isolated from saline-perfused and nonperfused kidneys within the same animal. Specific [3H]LTC 4 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]LTC 4 binding to isolated glomeruli increased linearly with increasing glomerular concentration (Fig. 2), and was saturable with increasing [3H]LTC 4 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 LTC 4 receptors (Krilis et al., 1984), rat intestinal tissue, which does not respond to LTC 4 (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 LTC 4 receptors with a single affinity in isolated glomeruli. The linear Scatchard plot shown for one such experiment with increasing concentrations of [3H]LTC 4

![Graph](http://circres.ahajournals.org/)

**FIGURE 5.** RP-HPLC profile of [3H]LTC 4 after 90 minutes of incubation of 1.1 nM LTC 4 in assay buffer alone (panel A), in the glomerular supernatant (panel B), and [3H]LTC 4 after elution from the glomerular receptor (panel C). The retention times of synthetic leukotriene standards are indicated by the arrows in panel A.
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 radiolabeled 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 Kₐ = 25 ± 7 nM (mean ± SEM) and 35 ± 16 nM, respectively, and R_max = 8.0 ± 1.5 pmol/mg protein and 9.0 ± 3.0 pmol/mg protein, respectively. These values are likely to represent true Kₐ and R_max values for the glomerular LTC₄ receptor under these conditions, as LTC₄ was shown to remain intact during the binding studies (Fig. 5).

The Kₐ 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, Kₐ = 7.6 nM (Krilis et al., 1984), and of cultured DDT; cells, Kₐ = 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 DeHaven, 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 (Kₐ = 41 nM) previously reported for rat lung particulate fractions (Pong et al., 1983) more closely resembles that 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 LTBr—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 mM. 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, LTBr, 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 (Austin 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-Asciak, 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., 1980; Dahlen et al., 1981), should relate to an effect on renal glomerular function. The present report defining the existence of specific renal glo-
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References


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