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

Barbara J. Ballermann, Robert A. Lewis, E.J. Corey, K. Frank Austen, and Barry M. Brenner
From the Department of Medicine, Harvard Medical School, the Departments of Medicine and Rheumatology/Immunology, Brigham and Women's Hospital, Boston, Massachusetts; and the Department of Chemistry, Harvard University, Cambridge, Massachusetts

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, 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-dihydroxy-6,14-cis-8,10-trans-eicosatetraenoic acid (LTB₄), or by a glutathione-S-transferase to 5,6-dihydroxy-6R-S-glutathionyl-7,9-trans-11,14-cis-eicosatetraenoic acid (LTC₄). From LTC₄, 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-5-cysteylnyl-7,9-trans-11,14-cis-eicosatetraenoic acid (LTE₄), respectively (Samuelsson, 1983). LTC₄ and LTD₄ are potent spasmsogens for some nonvascular smooth muscles in vitro, as exemplified by the response of guinea pig ileum and both upper and lower guinea pig airways (Drzen 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 (Drzen et al., 1980) or topical application to the hamster cheek pouch (Dahlgren 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 C\(_4\), D\(_4\), E\(_4\), and B\(_4\) 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. \[^{3}H\]LT\(_C\) (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-hydroxylpropyl-piperazine-N'-2-ethane sulfonic acid (HEPES), reduced glutathione, sodium tetraborate, arachidonic acid (Sigma Chemical Corp.), and prostandiol E\(_2\) (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 the 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 CaCl\(_2\). 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 1 and 2 x 10\(^4\) glomeruli were usually obtained from each rat, representing 1.8-3.6 mg of protein, as determined by the method of Lowry et al. (1951).

To determine the influence of any retained blood products on LTC\(_C\) binding, rats were anesthetized with ether, and the aorta was cannulated below the renal arteries and cross-clamped between the renal arteries. The left kidney was immediately perfused with 50 ml of ice-cold saline to produce complete blanching, while the right kidney continued to be perfused by the systemic circulation. Both kidneys then were removed, and their glomeruli were isolated separately. The binding of \[^{3}H\]LT\(_C\) to rat ileal segments was also examined, using conditions identical to those utilized to define receptor binding to guinea pig ileal segments (Krilis et al., 1984).

Druggand 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 \[^{3}H\]LT\(_C\) in 0.5 ml of assay buffer consisting of 20 mM HEPES, pH 7.4, 125 mM NaCl, 5 mM CaCl\(_2\), and 1 mM serine-borate complex at 4°C for 60 minutes on a rotary shaking platform. Serine-borate complex was included to prevent LT\(_C\) metabolism by peptide cleavage (Tate and Meister, 1978; Örning 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. Titrated experiments (n = 2), each done in replicate, at 10-second intervals, demonstrated that total and nonspecific, and thus also specific \[^{3}H\]LT\(_C\) 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 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 LT\(_C\). 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 \[^{3}H\]LT\(_C\) concentrations used.

The time course of specific \[^{3}H\]LT\(_C\) binding to glomeruli was assessed in six separate experiments for concentrations of \[^{3}H\]LT\(_C\) of 0.3, 0.6, 1.0, 3.3, 6.7, and 8.5 nM. Glomeruli were incubated with these concentrations of \[^{3}H\]LT\(_C\) with and without 3 μM unlabeled LT\(_C\) 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 LT\(_C\) after 60 minutes of incubation with \[^{3}H\]LT\(_C\) and determining remaining bound radioactivity as a function of time.

Specific \[^{3}H\]LT\(_C\) binding, as a function of increasing glomerular concentration, was assessed at fixed concentrations of \[^{3}H\]LT\(_C\). In four separate experiments in which the \[^{3}H\]LT\(_C\) 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 \[^{3}H\]LT\(_C\) 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 LT\(_C\) binding site was determined by boiling glomeruli for 15 minutes before assessing specific and nonspecific \[^{3}H\]LT\(_C\) binding.

To determine the saturaibility, affinity, and density of LT\(_C\)-binding sites, 250-300 glomeruli were incubated with increasing concentrations of \[^{3}H\]LT\(_C\) (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 \[^{3}H\]LT\(_C\). 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 \[^{3}H\]LT\(_C\) in a volume of 0.5 ml in the presence of increasing concentrations of unlabeled LT\(_C\), 0.1-3000 nM). Data for competition by unlabeled LT\(_C\) 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 [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 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 [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 solution for reverse-phase-HPLC, namely, methanol:assay buffer at 5:1, vol/vol, pH 5.6. Each sample was injected individually with 200–500 ng of unlabeld LTC₄ standard onto an Altex Cis 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 aqua-regia, 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. Non-specific 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 non-perfused 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 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 [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.
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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 (Kᵤ) 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 Kᵤ = 25 ± 7 nM (mean ± SEM) and glomerular LTC₄ receptor density = 7.8 ± 2.0 ± 10⁵ 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

![Figure 3. Total (●), nonspecific (▲), and specific (○) binding (B) of increasing concentrations of [³H]LTC₄ to isolated glomeruli. All points are means of duplicate determinations. The right hand panel shows Scatchard transformation of these data. B/F, bound/free.](http://circres.ahajournals.org/)

![Figure 4. Competitive inhibition of [³H]LTC₄ binding to glomeruli. B₀ refers to binding of [³H]LTC₄ in the absence of a competing substance, and B/B₀ is the fraction of [³H]LTC₄ that remains bound in the presence of a competing ligand. Panel A, competition by unlabeled LTC₄ (●) enclosed in the shaded area. Competition by LTD₄ (▲),LTE₄ (○), LTB₄ (▲), and FPL55712 (△). Panel B, competition by unlabeled LTC₄ (●), arachidonic acid (▲), prostaglandin E₂ (△), and reduced glutathione (▲).](http://circres.ahajournals.org/)
Binding of \[^3H\]LTC\textsubscript{4} Degradation during and after Receptor

Specific \[^3H\]LTC\textsubscript{4} binding to isolated rat renal glomeruli 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\textsubscript{4} binding to isolated renal glomeruli was rapidly reversible when excess unlabeled LTC\textsubscript{4} was added at equilibrium, showing that the association of \[^3H\]LTC\textsubscript{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\textsubscript{4} binding was to glomerular tissue and not to retained blood elements, since specific \[^3H\]LTC\textsubscript{4} binding was identical in glomeruli isolated from saline-perfused and nonperfused kidneys within the same animal. Specific \[^3H\]LTC\textsubscript{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\textsubscript{4} binding to isolated glomeruli increased linearly with increasing glomerular concentration (Fig. 2), and was saturable with increasing \[^3H\]LTC\textsubscript{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\textsubscript{4} receptors (Krilis et al., 1984), rat intestinal tissue, which does not respond to LTC\textsubscript{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\textsubscript{4} receptors with a single affinity in isolated glomeruli. The linear Scatchard plot shown for one such experiment with increasing concentrations of \[^3H\]LTC\textsubscript{4}}
best illustrates that receptors with a single affinity are being detected (Fig. 3). Competitive binding-inhibition studies with increasing concentrations of unlabeled LTC\textsubscript{4} allowed analysis over a wider range of LTC\textsubscript{4} concentrations (0.5–3000 nM) (Fig. 4) than studies with increasing concentrations of radiolabeled LTC\textsubscript{4} (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 \textsuperscript{3}H\textsubscript{LTC}\textsubscript{4} had been used. Equilibrium-binding parameters were derived directly by LIGAND analysis and were similar for saturation and competition studies with equilibrium \(K_d = 25 \pm 7 \text{ nM} \) (mean ± SEM) and 35 ± 16 nM, respectively, and \(R_o = 8.0 \pm 1.5 \text{ pmol/mg protein} \) and 9.0 ± 3.0 pmol/mg protein, respectively. These values are likely to represent true \(K_d \) and \(R_o \) values for the glomerular LTC\textsubscript{4} receptor under these conditions, as LTC\textsubscript{4} was shown to remain intact during the binding studies (Fig. 5).

The \(K_d \) for the glomerular LTC\textsubscript{4} receptor is about one-half order of magnitude greater than that of LTC\textsubscript{4} receptors in guinea pig ileal smooth muscle segments, \(K_d = 7.6 \text{ nM} \) (Krillis et al., 1984), and of cultured DDT; cells, \(K_d = 5.0 \text{ nM} \) (Krillis et al., 1983), indicating a lower affinity of glomerular receptors for LTC\textsubscript{4}. 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. Na\textsuperscript{+}, at concentrations of 125 mM, has been reported to inhibit LTD\textsubscript{4} binding to a subcellular fraction of guinea pig lung (Pong and DeHaven, 1983), but not LTC\textsubscript{4} 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 LTC\textsubscript{4} receptor affinity (\(K_d = 41 \text{ nM} \)) previously reported for rat lung particulate fractions (Pong et al., 1983) more closely resembles that of the receptor found in isolated rat glomeruli, suggesting that LTC\textsubscript{4} 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 LTC\textsubscript{4}-binding site for LTC\textsubscript{4} as structurally related sulfidopeptide leukotrienes LTD\textsubscript{4} and LTE\textsubscript{4} were able to compete for the receptor only at concentrations 2.5–3 orders of magnitude greater than LTC\textsubscript{4} (Fig. 4). The fact that LTD\textsubscript{4} and LTE\textsubscript{4}—and LTE\textsubscript{4}, which does not contain a thiopeptide domain—all displaced \textsuperscript{3}H\textsubscript{LTC}\textsubscript{4} with similar potencies, indicates that these other naturally occurring leukotrienes interact with the glomerular LTC\textsubscript{4} receptor in a rather nonspecific manner. FPL55712, an agent previously shown to partially inhibit LTD\textsubscript{4} but not LTC\textsubscript{4} constrictor effects in lung parenchymal strips (Drazen et al., 1980), is a very weak competitive inhibitor of \textsuperscript{3}H\textsubscript{LTC}\textsubscript{4} binding in glomeruli with only a 10-fold greater affinity than arachidonic acid, which displaced 50% of specifically bound \textsuperscript{3}H\textsubscript{LTC}\textsubscript{4} at a concentration of 0.56 nM. The high degree of specificity of LTC\textsubscript{4} for glomerular receptors is very similar to the findings of DDT; cells (Krillis et al., 1983), guinea pig ileum (Krillis et al., 1984), and rat lung particulate fractions (Pong et al., 1983), where LTD\textsubscript{4}, LTE\textsubscript{4}, LT\textsubscript{B}\textsubscript{4}, and FPL55712 were also relatively ineffective in displacing \textsuperscript{3}H\textsubscript{LTC}\textsubscript{4}.

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 LTC\textsubscript{4} 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 LTC\textsubscript{4} is presently limited to the report that cultured endothelial cells from human umbilical veins are induced by LTC\textsubscript{4} to produce prosta-cyclin (Cramer et al., 1983). The responses of microvascular beds to LTC\textsubscript{4}, 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 LTC\textsubscript{4} receptors in isolated renal glomeruli and previous indications of direct renal vasoconstrictor effects of LTC\textsubscript{4} (Rosenthal and Pace-Asciak, 1983; Badr et al., 1984) raise the question of the circumstances under which LTC\textsubscript{4} 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 LTC\textsubscript{4} and LTD\textsubscript{4} into the renal venous effluent (Pirozsky 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 LTC\textsubscript{4} infusion on renal glomerular function. The present report defining the existence of specific renal glomerular receptors for LTC\textsubscript{4} 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.


INDEX TERMS: Sulfidopeptide leukotrienes • Inflammatory mediator • Glomerular function • Glomerulonephritis

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Reprint requests: Barbara J. Ballermann, M.D., Laboratory of Immunology and Kidney and Electrolyte Physiology, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115.

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B J Ballermann, R A Lewis, E J Corey, K F Austen and B M Brenner

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