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IDENTIFICATION AND CHARACTERIZATION OF LEUKOTRIENE D_4 RECEPTORS IN ADULT AND FETAL HUMAN LUNG

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Abstract—Leukotriene D_4 (LTD_4) receptors were identified and characterized in adult and fetal human lung membranes. Macroscopically normal adult lung tissue was selected from seventeen surgical biopsy specimens, and twenty-seven fetal lung samples were obtained from therapeutic abortions. Binding assays were performed using pooled adult or fetal human lung membranes at 30° under conditions which prevented metabolism of [^{3}H]LTD_4. Specific binding reached equilibrium within 30 min, remained constant for 60 min, was enhanced by Mg^{2+}, and was inhibited by Na^+ and guanyl-5′-yl-imidodiphosphate. Computer-assisted analyses of saturation binding data showed a single class of binding sites with similar apparent K_d (0.15 ± 0.09 and 0.12 ± 0.003 nM) and B_{max} (68 ± 29 and 62 ± 14 fmoles/mg protein) values for adult and fetal samples respectively. Competition binding studies with [^{3}H]LTD_4 showed the same rank order potency for adult and fetal lung receptors (5*S*,6*R*-LTD_4 > 5*S*,6*R*-LTD_1 > 5*R*,6*S*-LTD_4 > 5*S*,6*R*-LTE_4 > FPL 55712). A comparison of the receptor binding affinities of these compounds with their smooth muscle contractile agonist (pD_2) and antagonist ($-\log[K_B]$) activities in guinea pig lung and trachea showed a good correlation ($r = 0.88$), suggesting that the saturable, high-affinity, stereoselective [^{3}H]LTD_4 specific binding sites identified in human lung may be physiologically relevant receptor moieties.

Leukotrienes C_4, D_4 and E_4 have been identified as the principal active components of the slow reacting substance of anaphylaxis [1–4]. In humans, leukotrienes have been shown to contract respiratory tissues *in vivo* [5–8] and *in vitro* [9–12], to decrease cardiac contractility *in vitro* [13], and to induce cutaneous vasodilation and vascular permeability *in vivo* [14–16].

The physiological actions of the leukotrienes are presumed to be mediated through interaction of these compounds with specific receptors [11, 13, 17–19]. In support of this assumption, our laboratory [20–24] and others [25–30] have provided direct evidence for the presence of heterogeneous populations of specific, high-affinity and saturable leukotriene binding sites in guinea pig lung [20–22, 24–26], heart [23], ileum [29] and uterus [28]; rat lung [27]; and a hamster smooth muscle cell line [30].

Recently, we identified and characterized specific binding sites for LTC_4 in human fetal lung membranes [31]. Our preliminary studies also suggested the presence of LTD_4 specific binding sites in these membranes and in membranes prepared from adult lung biopsy specimens [31]. The objectives of the current study were to optimize conditions for studying LTD_4 specific binding sites in adult and fetal human lung; to characterize the kinetics, saturability, affinity and density of LTD_4 binding to these specific sites; to examine their regulation by cations and guanine nucleotides; and to compare the receptor binding activities of a series of leukotriene D analogs with their contractile activities in guinea pig airway tissues.

MATERIALS AND METHODS

Materials. 5*S*,6*R*-LTD_4, 5*R*,6*S*-LTD_4, 5*S*,6*R*-LTD_1, and 5*S*,6*R*-LTE_4 were synthesized as previously described [32] and were provided by Dr. John Gleason (Department of Medicinal Chemistry, Smith Kline & French Laboratories). FPL 55712 was also synthesized in-house. [^{3}H]LTC_4 (35.7 Ci/mmole) and [^{3}H]LTD_4 (35.7 Ci/mmole) were obtained from the New England Nuclear Corp., and high performance liquid chromatography (HPLC) analysis in our laboratory showed these radioligands to be 90–95% pure. Other chemicals were the highest grades available from the Sigma Chemical Co.

Preparation of membranes from adult and fetal human lung. Adult and fetal human lung samples were obtained after informed consent. Adult lung tissue was obtained by surgical resection of tumors from seventeen patients at the University of Pennsylvania Hospital, Philadelphia, PA. Macroscopically normal tissue was excised from the tumor mass and frozen in liquid nitrogen within 30 min after surgery. Twenty-seven fetal lung samples from therapeutic abortions performed during the second trimester of pregnancy were obtained from the University of Minnesota and VA Medical Centers, Minneapolis, MN. Aborted fetuses were placed in

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normal saline, and the age was determined by measuring cranium size. All fetuses appeared normal. Lung tissue was removed, placed in 0.9% saline, and frozen in liquid nitrogen within 1–2 hr after abortion. Adult and fetal lung samples were stored at −70° prior to use. Crude membrane fractions were prepared as previously described [31]. Crude membrane pellets were resuspended in 26 ml of 20 mM Tris–HCl (pH 7.5) containing protease inhibitors (PI) and 10% sucrose, and were layered onto a 10-ml cushion of 40% sucrose in 20 mM Tris–HCl plus PI. Following centrifugation at 25,000 rpm for 60 min at 6° using the SW 28 rotor in the Beckman L8-80 ultracentrifuge, the membranes were removed from the interface above the sucrose cushion and were then pelleted again by ultracentrifugation at 60,000 rpm for 30 min at 6°. The resulting pellet was resuspended in 20 mM Tris–HCl (pH 7.5) including PI, and was stored at 0°. Membrane suspensions were utilized in binding studies within 48 hr.

Radioligand binding assays. [^{3}H]LTD_4 binding assays were performed in triplicate (unless otherwise noted) at 30° in 20 mM Tris–HCl (pH 7.5) buffer containing 20 mM serine-borate, 10 mM cysteine, 10 mM glycine, and 5 mM $MgCl_2$. Cysteine (100 mM) was prepared immediately prior to use. All assays (0.5 ml) contained pooled adult or fetal human lung membrane protein and [^{3}H]LTC_4 or [^{3}H]LTD_4 in concentrations as noted in the figure legends. For [^{3}H]LTD_4 time course experiments, samples were incubated for 0–60 min, and saturation and competition binding experiments were performed after equilibrium was reached (30-min incubation). These [^{3}H]LTD_4 binding conditions differ from the [^{3}H]LTC_4 binding conditions we have published previously for human lung [31]. [^{3}H]LTC_4 binding assays were performed in triplicate at 4° in 20 mM Tris–HCl (pH 7.5) buffer containing 80 mM serine-borate, 10 mM cysteine, 10 mM glycine, 5 mm $MgCl_2$, 3 mM $CaCl_2$, 1 mM NaCl and 1 mM dithiothreitol (DTT). It was necessary to perform the [^{3}H]LTC_4 binding assays at lower temperature in the presence of a higher concentration of serine-borate to prevent the rapid metabolism of LTC_4 by human lung membranes [31]. NaCl, $CaCl_2$ and DTT improved specific binding in the [^{3}H]LTC_4, but not in the [^{3}H]LTD_4 binding assays. Free radioligand was separated from membrane bound ligand by a vacuum filtration technique, as previously described [33]. Radioactivity remaining on the filter was determined by liquid scintillation spectrometry. Total and non-specific binding of [^{3}H]LTC_4 or [^{3}H]LTD_4 were determined as the mean (± standard deviations) of assays performed in the absence or presence of a 1000-fold excess of unlabeled LTC_4 or LTD_4. Specific binding was calculated as the difference between total and non-specific binding. Saturation binding data were subjected to computer-assisted non-linear least squares curve fitting, as previously described [34], and were further analyzed by the method of Scatchard [35].

Metabolism of [^{3}H]LTD_4 by adult and fetal human lung membranes. Bioconversion of [^{3}H]LTD_4 was determined by HPLC. Fetal human lung membranes (250 μg/ml protein) were incubated with [^{3}H]LTD_4 (0.5 mM) for 30 min at 30° under standard binding conditions in a volume of 1 ml. Following incubation, the reaction mixture was poured into a 24-mm borosilicate glass filter holder containing a Whatman GF/C filter paper disc and was processed as previously described [33]. Aliquots (230 μl) of the filtrate and methanol-extracted membrane-bound material were mixed separately with LTC_4, LTD_4 and LTE_4 standards and injected for HPLC analysis. A reverse phase C_{18} (RP-18 10 μm) LiChrosorb column (0.46 × 25 cm) was used with acetonitrile/10 mM phosphate buffer (pH 6.7) as mobile phase at a flow rate of 2 ml/min for the separation of LTC_4, LTD_4 and LTE_4. A 25–35% acetonitrile gradient was used in the first 10 min and 35% was used during the last 10 min. The radioactivity of 0.5-ml fractions was determined by liquid scintillation spectrometry.

Guinea pig lung myotonic studies. These studies were performed as previously described [36].

RESULTS

Characteristics of [^{3}H]LTD_4 binding. Preliminary experiments were conducted to establish optimum conditions for [^{3}H]LTD_4 binding (results not shown). To minimize leukotriene bioconversion, serine-borate (20 mM), cysteine (10 mM) and glycine (10 mM) were utilized to inhibit endogenous γ-glutamyl transpeptidase [37] and other peptidase activities [38, 39] in the adult and fetal human lung membranes. Under standard binding conditions [20 mM Tris–HCl (pH 7.5), 20 mM serine-borate, 10 mM cysteine, 10 mM glycine, 5 mM $MgCl_2$ and assay at 30°], greater than 85% of the total membrane-bound radioactivity, as analyzed by HPLC, co-eluted with the unlabeled LTD_4 standard. Less than 2 and 4% of the membrane-bound radioactivity eluted with the LTC_4 and LTE_4 standards respectively (data not shown). Approximately 11% of the radioactivity eluted within the first 6 min, representing hydrophilic degradative products of [^{3}H]LTD_4 that were also present in membrane-free incubation mixtures. The chromatogram of the filtrate was very similar to the profile of the membrane bound radioactivity (data not shown). These results showed that the bulk of the radioactivity is not metabolized under standard binding conditions and thus reflects [^{3}H]LTD_4 specific binding.

As shown in Fig. 1, under the standard conditions, specific binding of [^{3}H]LTD_4 to pooled fetal lung membranes represented 45–50% of total binding, reached equilibrium within 30 min, and remained stable for at least an additional 30 min. Similar data was observed in two previous experiments using crude membranes (40,000 *g* pellet) from adult and fetal human lung (results not shown).

Specific binding of [^{3}H]LTD_4 to adult or fetal human lung membranes was enhanced approximately 2-fold by the addition of 5 mM Mg^{2+} (data not shown). A comparison of the Na^+ and guanyl-5′-yl-imidodiphosphate (GppNHp) sensitivity of [^{3}H]-LTC_4 versus [^{3}H]LTD_4 specific binding in fetal human lung purified membranes (Fig. 2, A and B) showed that [^{3}H]LTD_4 binding to the LTD_4 sites was much more sensitive to Na^+ (50% inhibition [IC_{50}] at 75–100 mM) than [^{3}H]LTC_4 specific binding (IC_{50} at 500 mM). Similarly, [^{3}H]LTD_4 specific binding

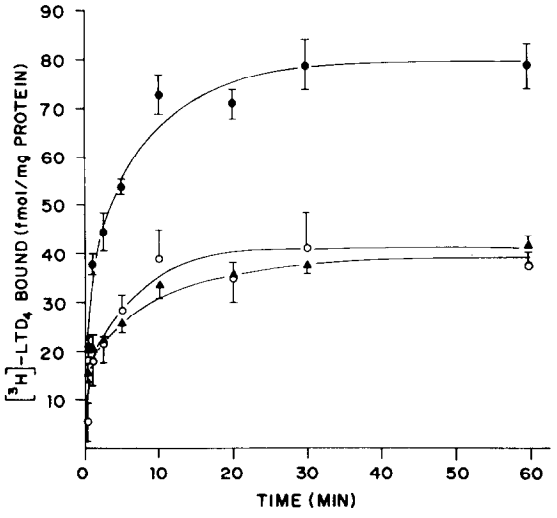

Fig. 1. Binding of $[^3H]LTD_4$ to fetal human lung membranes. Membranes (250 μg/ml protein) were incubated with 0.5 nM $[^3H]LTD_4$ in a total volume of 0.5 ml for the designated times in the presence of standard buffer conditions at 30° as described in Methods. Non-specific (▲) and total (●) binding were determined in the presence or absence of a 1000-fold excess of LTD_4, and specific (○) binding was calculated as the difference between total and non-specific binding. The error bars represent the standard deviation of triplicate replicates from this experiment.

was much more sensitive to GppNHp (IC_{50} of 50 μM) than $[^3H]LTC_4$ specific binding (only 10–15% inhibition at 500 μM). Non-specific binding of $[^3H]LTC_4$ or $[^3H]LTD_4$ was not affected significantly by Na^+ or GppNHp. These results are supported by two previous experiments which examined Na^+ and GppNHp sensitivity in crude membrane preparations (40,000 *g* pellet) from human fetal lung (results not shown). These results are similar to those observed for the LTD_4 receptor in guinea pig lung membranes [21, 24, 26]. It was not possible to study Na^+ or GppNHp sensitivity of $[^3H]LTC_4$ or $[^3H]$-LTD_4 specific binding in adult human lung membranes, since the tissue supply was limited.

Saturation analyses. Binding of $[^3H]LTD_4$ to adult and fetal human lung membranes (250 μg/ml protein) was studied, using optimal defined conditions, at several concentrations of $[^3H]LTD_4$ (0.05 to 1.0 nM) to determine the saturability, dissociation constant (K_d) and density (B_{max}) of the specific binding sites. Representative experiments using pooled adult or fetal human lung membranes are shown in Fig. 3, A and B. Computer-assisted analyses of saturation binding data [34] showed a single class of binding sites with similar apparent K_d (0.15 ± 0.09 and 0.12 ± 0.003 nM) and B_{max} (68 ± 29 and 62 ± 14 fmoles/mg protein) values for adult and fetal human lung respectively (average of six determinations from two experiments for adult lung and nine determinations from three experiments for fetal lung). Further analyses of saturation binding data by the method of Scatchard [35] showed a linear plot. The apparent K_d values are very similar to those reported for the LTD_4 receptor in guinea pig lung, but the apparent B_{max} values are 20-fold less than those reported in guinea pig lung [22, 24].

Pharmacological specificity of $[^3H]LTD_4$ binding. To investigate the pharmacological specificity of $[^3H]LTD_4$ specific binding, radioligand binding studies were performed in the presence or absence of unlabeled leukotriene agonists and the antagonist FPL 55712 (Fig. 4, A and B and Table 1). Natural form 5*S*,6*R*-LTD_4 competed with $[^3H]LTD_4$ in a highly effective, concentration-dependent manner, with inhibition constants (K_i values) of 0.5 and 0.15 nM for adult and fetal human lung respectively. The structural analogs of LTD_4, 5*S*,6*R*-LTD_1 (K_i = 29.3 and 19.2 nM) and unnatural form 5*R*,6*S*-LTD_4 (K_i = 32.8 and 21.6 nM), showed very similar values for adult and fetal samples respectively. 5*S*,6*R*-LTE_4

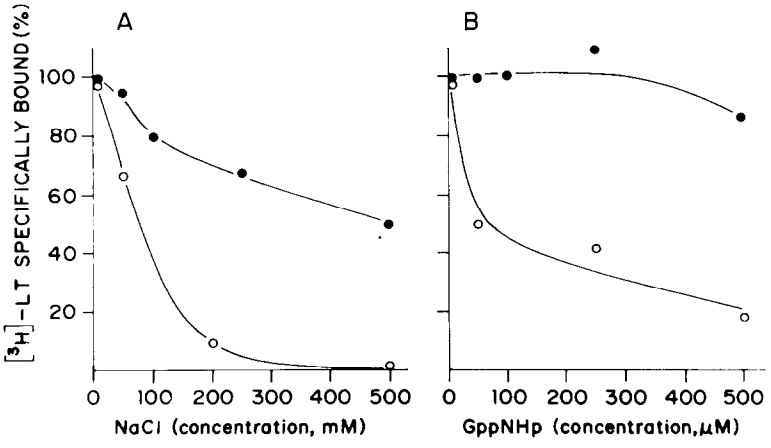

Fig. 2. Effects of Na^+ and GppNHp on $[^3H]LTC_4$ and $[^3H]LTD_4$ specific binding. Fetal human lung membrane protein (25 μg/ml for LTC_4, 250 μg/ml for LTD_4) was incubated with 2 nM $[^3H]LTC_4$ (●) or 0.5 nM $[^3H]LTD_4$ (○) in the presence of various concentrations of NaCl (A) or GppNHp (B) in a volume of 0.5 ml under standard conditions for LTC_4 or LTD_4 binding (see Methods). Specific binding was calculated as described in Methods. The level for $[^3H]LTC_4$ or $[^3H]LTD_4$ specific binding was taken as 100% when no exogenous Na^+ or GppNHp was added to the incubation mixtures. The standard deviation of each point taken from triplicate determinations in these experiments was 5–10%.

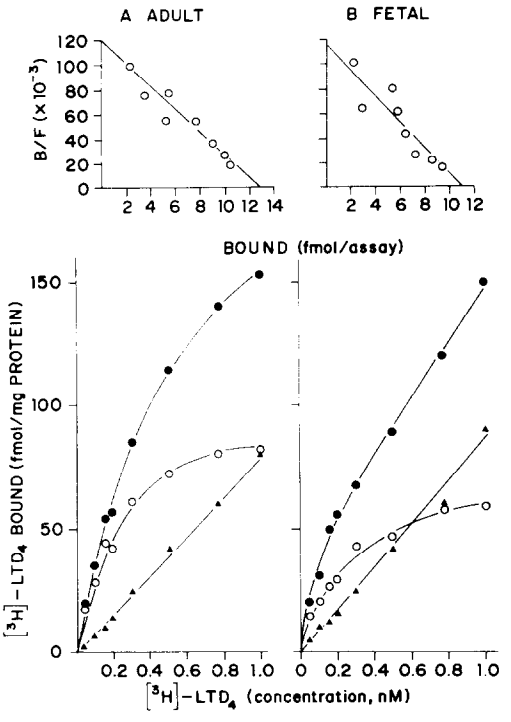

Fig. 3. Saturation experiments and Scatchard analyses of [^{3}H]LTD$_4$ binding to adult and fetal human lung. Membrane protein (250 μg/ml) from adult (A) or fetal (B) samples was incubated with [^{3}H]LTD$_4$ (from 0.05 to 1.0 nM) in a total volume of 0.5 ml for 30 min at 30° under standard incubation conditions (see Methods). Non-specific binding was determined in the presence of a 1000-fold excess of LTD$_4$. Total (●), non-specific (▲) and specific (○) binding were determined from triplicate incubation mixtures of representative experiments. The K_d and B_{max} values were calculated by computer-assisted analysis [34] and are quoted in the text as the average of two or three separate experiments. Saturation binding data were further plotted according to the method of Scatchard (top panels). B/F, the ratio of specifically bound ligand (B) to the concentration of free (F) radioligand. The standard deviation of each point in these representative experiments and in two (six determinations) or three (nine determinations) separate experiments was 5–10%.

Table 1. Human lung competition binding studies

Competitor	IC_{50} (nM)		K_i (nM)	
	Fetal	Adult	Fetal	Adult
5*S*,6*R*-LTD$_4$	0.8	2.2	0.15	0.5
5*R*,6*S*-LTD$_4$	112.2	141.3	21.6	32.8
5*S*,6*R*-LTD$_1$	100.0	125.9	19.2	29.3
5*S*,6*R*-LTE$_4$	223.9	199.5	43.1	46.4
FPL 55712	6309.6	5623.4	1213.4	1307.8

Competition experiments were performed as described in the legend to Fig. 4. Apparent K_i values were calculated using the equation $K_i = IC_{50}/(1 + [LTD_4]/K_d)$ [40] and are reported as the average value for two or three experiments using pooled adult or fetal human lung membranes. [LTD$_4$] is the concentration of [^{3}H]LTD$_4$ (0.5 nM) in the assay tube, and K_d is the apparent dissociation constant (0.15 and 0.12 for adult and fetal membranes respectively). Standard deviation of each value was usually 5–10% of the mean.

was also an effective competitor (K_i = 46.4 and 43.1 nM) in both membrane preparations. FPL 55712, an antagonist of LTD$_4$-induced smooth muscle contraction [41], was a weak competitor (K_i = 1.3 and 1.2 μM), compared with the agonists, in both adult and fetal human lung. These results showed the same rank order potency of the competitors in adult and fetal human lung and indicated that [^{3}H]LTD$_4$ specific binding is stereoselective, since the natural 5*S*,6*R* form of LTD$_4$ was a 65- and 140-fold more potent competitor than the unnatural 5*R*,6*S* form in adult and fetal human lung respectively.

Radioligand binding studies were also performed with unlabeled LTC$_4$ (data not shown). In two separate experiments, LTC$_4$ (at 10, 30 and 100 nM) caused an approximately 2-fold increase in filter-bound [^{3}H]LTD$_4$. At higher concentrations (300 nM and 1 μM), LTC$_4$ competed for [^{3}H]LTD$_4$ binding sites, and it was possible to estimate apparent K_i values of 200 and 100 nM for adult and fetal human lung respectively. Although the increase in [^{3}H]-LTD$_4$ binding at the lower concentrations cannot be explained and is under investigation, it is clear from

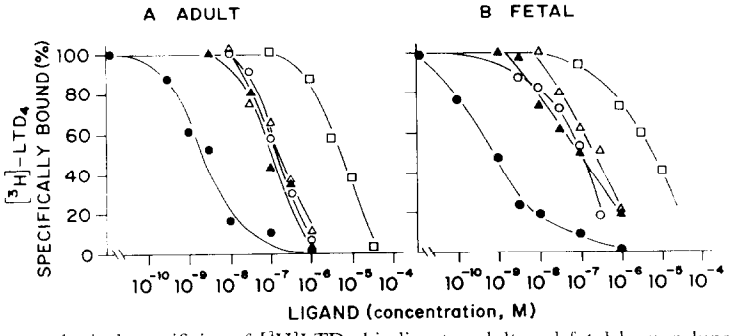

Fig. 4. Pharmacological specificity of [^{3}H]LTD$_4$ binding to adult and fetal human lung membranes. Membrane protein (250 μg/ml) from adult (A) or fetal (B) samples was incubated with 0.5 nM [^{3}H]-LTD$_4$ and increasing concentrations of unlabeled 5*S*,6*R*-LTD$_4$ (●), 5*R*,6*S*-LTD$_4$ (○), 5*S*,6*R*-LTD$_1$ (▲), 5*S*,6*R*-LTE$_4$ (△) and FPL 55712 (□) in a total volume of 0.5 ml for 30 min under conditions described in Methods. In panel A, each point represents the mean of four determinations from two separate experiments; in panel B, each point represents the mean of nine determinations from three separate experiments. The standard deviation was 5–12% of these values.

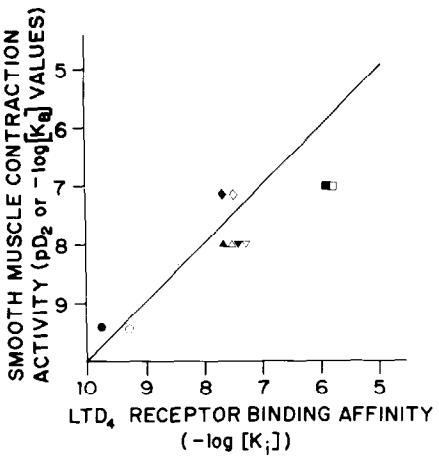

Fig. 5. Correlation of smooth muscle contraction and radioligand competition activities of leukotriene analogs. Agonist-induced smooth muscle contraction activities were determined from organ-bath systems using guinea pig lung parenchyma, and pD_2 values were calculated as described previously [36]. The K_B value for the antagonist FPL 55712 was determined by pretreating guinea pig tracheal tissue with 100 μM FPL 55712 [42]. Apparent K_i values were calculated as described in the legend to Table 1. Key: leukotriene D analogs 5*S*,6*R*-LTD_4 (●○), 5*R*,6*S*-LTD_4 (◆◇) and 5*S*,6*R*-LTD_1 (▲△); 5*S*,6*R*-LTE_4 (▼▽); and the antagonist FPL 55712 (■□). Open symbols represent adult human lung K_i values; closed symbols are fetal human lung K_i values (Table 1).

the apparent K_i values that LTD_4 (Table 1) and LTC_4 bind to different sites within human lung.

Correlation of [^{3}H]LTD_4 specific binding and smooth muscle contraction. The smooth muscle contractile activities of several leukotriene agonists in guinea pig lung parenchyma (pD_2) and the antagonist FPL 55712 in tracheal tissue ($-\log[K_B]$) were taken from previous results [36]. Comparison of the pD_2 and $-\log[K_B]$ values of the leukotriene analogs with their apparent K_i values derived from radioligand binding studies (Table 1) are illustrated in Fig. 5. The contractile activities of the leukotriene D and E agonists and the antagonist FPL 55712 showed good correlation with the binding affinities determined from [^{3}H]LTD_4 competition studies (coefficient of correlation, $r = 0.88$). These results suggested that the specific binding sites identified in the radioligand competition binding studies are physiologically relevant receptors.

DISCUSSION

The potent contractile activities of the leukotrienes on isolated human airway smooth muscle [9–12] have caused investigators to speculate on the role of leukotrienes in the pathogenesis of human lung disease [8, 43–46]. Inhibition by FPL 55712 of leukotriene-induced, but not histamine-induced, contractions in human lung [9, 10] led to the hypothesis that specific leukotriene receptors existed in this tissue. However, additional studies were needed to define the sites and determine mechanisms of action.

In the present study, we have identified and characterized [^{3}H]LTD_4 receptors in adult and fetal human lung. Under binding conditions which inhibit leukotriene metabolism, the bulk of the membrane-bound radioactivity, as analyzed by HPLC, was in the form of [^{3}H]LTD_4, indicating that the radioactivity bound under the standard conditions is an accurate measurement of [^{3}H]LTD_4 binding. Binding of [^{3}H]LTD_4 to adult and fetal human lung membranes was saturable and stereospecific.

It is difficult to demonstrate the reversibility of [^{3}H]LTD_4 binding in this system. Given the apparent association rate ($K_{obs} = 0.15\ min^{-1}$ or 0.6 fmole/min) and apparent K_d values from the present study, it is possible to calculate a theoretical dissociation rate of $0.023\ min^{-1}$ or 0.09 fmole/min, thus requiring 3.3 hr for complete dissociation. Results from our laboratory [23] have shown that the majority of [^{3}H]LTD_4 is either metabolized or degraded after 3 hr of incubation. Therefore, the dissociation rate of [^{3}H]LTD_4 from the receptor in human lung cannot be determined accurately. There is circumstantial evidence to suggest that the binding of [^{3}H]LTD_4 to human lung membranes is reversible. Previous results from our laboratory [24] and others [26] have shown that guanine nucleotides can increase both the association and dissociation rates in guinea pig lung membrane preparations. The sensitivity to GppNHp shown in the present study suggests that [^{3}H]LTD_4 binding to human lung membranes is reversible.

Further characterization of this binding allowed us to address a number of important questions. First, were these LTD_4 receptors developmentally regulated? Second, were human lung LTD_4 receptors similar to LTD_4 receptors in guinea pig lung? Third, were leukotriene receptors in human lung heterogeneous? Finally, did radioligand binding studies correlate with a physiological function of leukotrienes?

Since the kinetics, affinity, density and pharmacological specificity of the [^{3}H]LTD_4 binding sites were similar in both adult and fetal human lung, there is no evidence at present to suggest developmental regulation. In comparing human and guinea pig [^{3}H]LTD_4 binding data, the kinetics, sensitivity to Na^+ and GppNHp, and affinities (K_d) of [^{3}H]LTD_4 binding to the receptors are very similar, but the B_{max} value for the guinea pig LTD_4 receptor is 20-fold greater than that observed in adult or fetal human lung [22, 24]. These data suggest that the LTD_4 receptors are similar in guinea pigs and humans, but species variation in the density of LTD_4 receptors exists in both guinea pig and human lung.

We have previously identified [^{3}H]LTC_4 specific binding sites in human fetal lung [31]. The affinity and density of the [^{3}H]LTC_4 ($K_d = 26$ nM, $B_{max} = 84$ pmoles/mg protein) and [^{3}H]LTD_4 specific binding sites in adult ($K_d = 0.15$ nM, $B_{max} = 68$ fmoles/mg) and fetal ($K_d = 0.12$ nM, $B_{max} = 62$ fmoles/mg) human lung are distinctly different. Furthermore, results from the present study showed that the [^{3}H]LTC_4 and [^{3}H]LTD_4 binding sites in fetal human lung differed in their sensitivity to Na^+ and GppNHp. Therefore, we conclude that heterogeneous populations of leukotriene binding sites exist in human lung. Similar results have been reported for guinea pig lung [22].

Two lines of evidence suggest that the [^{3}H]LTD_4 specific binding sites identified in the current study are receptors. First, regulation by cations and guanine nucleotides is characteristic of several well-documented receptors [47–53]. The sensitivity to Na^+ and GppNHp demonstrated in the current study suggests that the [^{3}H]LTD_4 specific binding sites may be coupled to adenylate cyclase or other mediators via guanine nucleotide binding proteins.

Second, an important criterion for distinguishing pharmacologically relevant receptors is the correlation of binding data with the rank order potency observed in a biological response. We have shown good correlation ($r = 0.88$) of the myotonic activities in guinea pig lung and trachea of several compounds (LTD_4 and LTE_4 agonists and the antagonists FPL 55712) with the radioligand binding activities of these compounds in adult and fetal human lung. Ideally, these myotonic studies should be conducted in human lung tissue, since correlation of human lung LTD_4 receptor binding activity to guinea pig lung myotonic activity might be regarded as coincidental. Previous studies using human lung parenchyma have been difficult to perform, necessitating the use of human airway tissue [10]. Since human lung parenchyma is difficult to acquire, the amount of airway tissue needed to perform these myotonic studies is prohibitive. In the absence of myotonic data from human lung, the data obtained from this study indicated that the guinea pig lung myotonic activity of LTD_4 and LTE_4 analogs correlated well with the human lung receptor binding activity. We feel that guinea pig lung is a good model system since our radioligand binding experiments suggest that the LTD_4 receptors in both guinea pig and human lung are quite similar.

In conclusion, we have identified and characterized [^{3}H]LTD_4 receptors in adult and fetal human lung. These receptors do not appear to be developmentally regulated, nor do they differ substantially from LTD_4 receptors in guinea pig lung, except for the density. The experimental data presented support the existence of heterogeneous populations of leukotriene specific binding sites in human lung, but confirmation of this will require development of a new class of leukotriene antagonists which can distinguish binding sites for LTC_4 and LTD_4 or solubilization and purification of the individual receptor macromolecules.

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