Biochemical Pharmacology, Vol. 34, No. 16, pp. 2831–2837, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

IDENTIFICATION OF SPECIFIC BINDING SITES FOR LEUKOTRIENE C_4 IN MEMBRANES FROM HUMAN LUNG*

G. E. ROVATI, D. OLIVA, L. SAUTEBIN, G. C. FOLCO, A. F. WELTON† and S. NICOSIA‡
Institute of Pharmacology and Pharmacognosy, I-20129, Milan, Italy, and †Department of Pharmacology II, Hoffman–La Roche, Nutley, NJ 07110, U.S.A.

(*Received* 14 *September* 1984; *accepted* 26 *February* 1985)

Abstract—Leukotriene C_4 (LTC_4), one of the major components of the slow-reacting substance of anaphylaxis (SRS-A), is a potent constrictor of bronchial smooth muscle in many species including humans. Here we report the identification and characterization of specific binding sites for LTC_4 in membranes from human lung parenchyma. At 4°, ^{3}H-LTC_4 binding is specific, saturable (B_{max} = 32–41 pmoles/mg prot.), rapid (equilibrium being attained within 15 min), reversible and of high affinity ($K_d = 3.6–7 \times 10^{-8}$ M). The binding sites are sensitive to heat and probably possess a protein moiety, being inactivated upon trypsinization. $CaCl_2$ affects both the association and the dissociation rate and dose-dependently enhances the binding of ^{3}H-LTC_4 at equilibrium; maximal enhancement (4-fold) occurred at 10^{-2} M $CaCl_2$. Unlabelled LTC_4 is able to compete with ^{3}H-LTC_4 for its binding sites with an IC_{50} of 7.8×10^{-8} M. The addition of 10^{-2} M $CaCl_2$ increases the potency of LTC_4 in inhibiting the binding (2.2-fold); both the competition curves are monophasic, indicating the existence of a homogeneous class of binding sites. In the presence of $CaCl_2$, LTD_4, LTE_4 and the SRS-A antagonist FPL 55712 can inhibit ^{3}H-LTC_4 specific binding, being, however, less potent than LTC_4 (IC_{50}s = 2.2×10^{-6}, 2.4×10^{-5} and 2.4×10^{-5} M, for LTD_4, LTE_4 and FPL 55712, respectively). FPL 55712 displayed a competitive mechanism; its affinity, however, was lower if absorption to glass was not prevented. The present studies indicate that specific binding sites for ^{3}H-LTC_4 exist in human lung parenchyma, and that a receptor-mediated process might be involved in the bronchoconstriction induced by LTC_4.

The cysteine-containing leukotrienes LTC_4, LTD_4 and LTE_4§ have been identified as the major components of the slow reacting substance of anaphylaxis (SRS-A) [1]. These leukotrienes are generated from arachidonic acid, through the action of a 5-lipoxygenase, during immediate hypersensitivity reactions; they elicit a number of biological effects [2], and in particular they have been demonstrated to be potent bronchoconstrictors in several species [3, 4], including primates [5] and humans [6, 7]. Both in normal and asthmatic humans, inhaled LTC_4 and LTD_4 elicit a sustained and long-lasting airway obstruction [8–10]. Moreover, allergen challenge of lung tissue from asthmatics specifically sensitive to birch pollen triggers a bronchial contraction which is related to the formation of cysteine-containing leukotrienes [11].

Even if their precise mechanism of action has not yet been elucidated, there is increasing evidence that their effects might be mediated through interaction with specific receptors. In fact, they are active in a number of systems at nanomolar concentrations; competitive antagonists for leukotrienes have been found [12, 13]; and, finally, structural determinants with strict requirements have been identified [14, 15].

In this report we describe the identification of specific binding sites for ^{3}H-LTC_4 in a membrane preparation of human lung parenchyma. These binding sites might be related to the receptors which mediate the action of LTC_4 in humans in disease states such as asthma.

MATERIALS AND METHODS

Materials. [14,15-^{3}H]LTC_4 (34–40 Ci/mmole) and [14,15-^{3}H]LTD_4 (40.3 Ci/mmole) were purchased from New England Nuclear (Boston, MA). LTC_4, LTD_4 and LTE_4 were synthesized at Hoffman–La Roche, Nutley, NJ, by the methods previously described [16, 17]. FPL 55712 was also synthesized at Hoffman–La Roche by the technique of Appleton *et al.* [18]. Serine-borate was the equimolar solution of serine and boric acid (both from Sigma Chemical Co., St. Louis, MO) prepared in 50 mM Tris–HCl buffer, pH 7.4. Trypsin and trypsin inhibitor were from Boehringer Mannheim GmbH, and cysteine from Sigma.

Membrane preparation. Macroscopically normal specimens (about 15 g) of human lung, removed at thoracotomy for lung cancer from 6 different individuals, were dissected free of pleura and visible blood vessels. Subpleural parenchymal fragments were prepared and immediately placed in ice-cold Tyrode solution. The fragments were repeatedly washed in 50 mM Tris–HCl buffer (pH 7.4), minced

* Preliminary results were presented to the 9th International Congress of Pharmacology, London, 29 July–3 August 1984 and to the Italian–U.S. meeting on cyclooxygenase and lipooxygenase modulators in lung reactivity, Milan, 5–6 April 1984.

‡ Address reprint requests and correspondence to: Dr. S. Nicosia, Institute of Pharmacology and Pharmacognosy, Via del Sarto 21 I-20129, Milan, Italy.

§ Abbreviations used: LTC_4, LTD_4, LTE_4, leukotriene C_4, D_4 and E_4 respectively; HPLC, high performance liquid chromatography.

and homogenized at 4° in the same buffer (1:24, w:v) with a motor driven glass homogenizer fitted with a Teflon pestle (15 strokes). The homogenate was centrifuged at 770 *g* for 10 min (4°) and the resulting supernatant was centrifuged at 15,000 *g* for 20 min. The pellet was resuspended with 50 mM Tris–HCl and recentrifuged in the same conditions. The final pellet was resuspended in 1/20 of the homogenization volume, stored at −80° up to 3 months, and diluted immediately before use with 50 mM Tris–HCl, pH 7.4 (1:8.4, v:v) containing Serine-borate to give a final concentration of 50 mM in the homogenate.

Binding assay. The standard assay mixture, in a final volume of 250 μl, contained: 50 mM Tris–HCl, pH 7.4; 1–2 nM ^{3}H-LTC$_4$; 20 mM Serine-borate (carried with the homogenate); LTC$_4$, LTD$_4$, LTE$_4$, FPL 55712 and $CaCl_2$ at the indicated concentrations. The incubation was started with the addition of 100 μl of the homogenate (0.04 mg protein/sample) and carried out at 4° for 20 min, unless otherwise indicated. Bound radioactivity was separated from free by vacuum filtration through Whatman GF/C glass fiber filters and rapid washing with two 4 ml aliquots of ice-cold 50 mM Tris–HCl, pH 7.4. Filtration and washing were completed in less than 8 sec. The radioactivity was extracted from the filters by incubation with 10 ml of Aquasol-2 (New England Nuclear) for 90 min at 40° and was then measured in the same scintillation fluid. Specific binding was defined as that which occurred in the presence of 2×10^{-5} M unlabelled LTC$_4$ and represented 80–90% of the total binding. When ^{3}H-LTD$_4$ (10 nM) was used as the radioligand, Serine-borate was omitted, and non-specific binding (40% of total) was measured in the presence of 10^{-4} M LTD$_4$. Data shown are mean ± standard deviation of triplicate determinations in one experiment, performed, however, at least twice.

Computer analysis. The data were fitted by an iterative program (RECEPT) for non-linear regression analysis [19] both to a one-site and a two-site model. The one-site model was then chosen when it yielded the best correlation coefficient, or when the improvement of goodness-of-fit for the two-site model was not statistically significant according to the *F*-test on the sums of squared errors.

Metabolism of ^{3}H-LTC$_4$. ^{3}H-LTC$_4$ (10^{-7} M, 4000 dpm/pmole) was incubated under the standard conditions, for either 20 min, in the presence or absence of Serine-borate, or for 40 min. The incubation was stopped by addition of 4 vol. of cold ethanol. The mixture was allowed to sit at 4° for 1 hr and then centrifuged at 10,000 *g* for 20 min. The supernatant was diluted with distilled water to a final ethanol concentration of 10%, pH was adjusted to 3–3.5 with 1 M citric acid, and the solution was applied to a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA) previously washed according to Powell [20]. The leukotrienes were eluted with methanol/water (70:30, v:v), additioned with tetrabutyl ammonium phosphate (PIC-A, Waters Associates; final concentration: 5×10^{-3} M; pH adjusted to 5.7 with acetic acid). The recovery was approximately 70–80%. The leukotriene fraction was evaporated, resuspended with 200 μl of the eluting system and subjected to reverse phase-HPLC on a Varian 5000 instrument equipped with a U6K injector (Waters Associates) and with an UVIDEC 100 Jasco detector. Instrumental conditions: column (250/4.5 mm) packed with Spherisorb S5-ODS2, 5 μm particles (Phase Separation, U.K.). The mobile phase was the solvent system used for elution from Sep-Pak (1 ml/min); detector: 280 nm. The fractions (1 ml) were collected and the radioactivity measured in 10 ml of Aquasol-2. The recovery from HPLC was 50–70%. Peaks were identified on the basis of the relative elution time on RP-HPLC compared to prostaglandin B_2 added as internal standard at the end of the incubation.

RESULTS

The association time-course (Fig. 1A) indicates that specific binding of ^{3}H-LTC$_4$ was rapid, a plateau being attained within 5 or 15 min, in the absence or presence of 10^{-2} M $CaCl_2$, respectively. The amount bound did not decrease appreciably up to 40 min

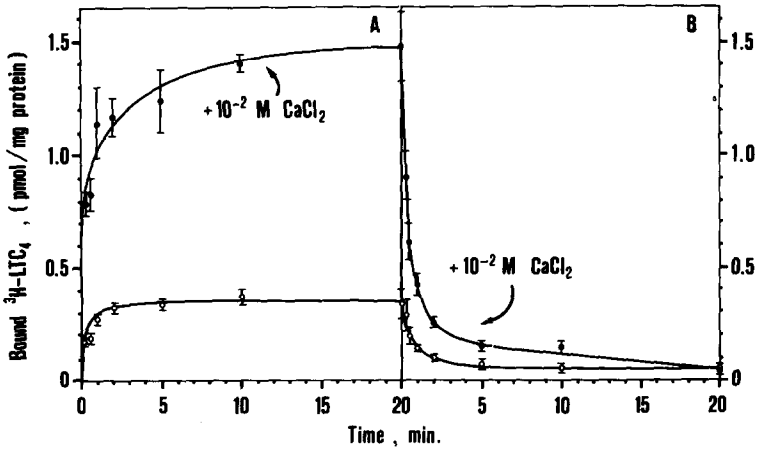

Fig. 1. Association (panel A) and dissociation (panel B) time-course for specific ^{3}H-LTC$_4$ binding in the absence and presence of 10^{-2} M $CaCl_2$. ^{3}H-LTC$_4$ was 2×10^{-9} M. For dissociation studies, 2×10^{-5} M LTC$_4$ was added after 20 min to represent the zero time of the dissociation time-course.

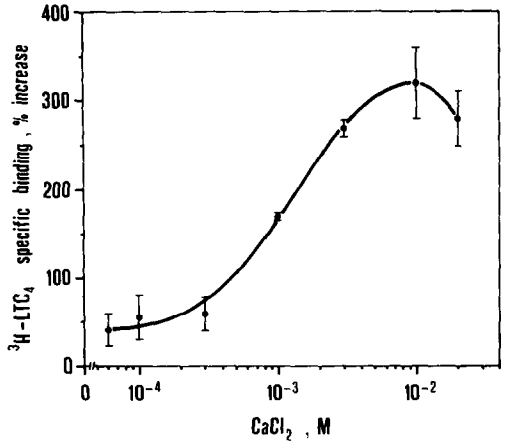

Fig. 2. Dose–response curve for the effect of $CaCl_2$ on ^{3}H-LTC_4 specific binding. ^{3}H-LTC_4 was 10^{-9} M and specific binding in the absence of $CaCl_2$ was 0.28 pmole/mg prot.

(data not shown). The specific binding was rapidly reversible (Fig. 1B) upon addition of excess (10^{-5} M) LTC_4; in fact, more than 90% of the amount bound at equilibrium dissociated in approximately 5 min, in the presence of $CaCl_2$.

It is also apparent from Fig. 1 that $CaCl_2$ affected both the association and the dissociation rate. The resulting effect of $CaCl_2$ on equilibrium binding was to increase the amount of ^{3}H-LTC_4 specifically bound; as shown in Fig. 2, the effect was dose-dependent, and reached a maximum at 10^{-2} M $CaCl_2$. Although such a concentration of Ca^{2+} is somewhat higher than the physiological extracellular levels, nevertheless it was used in most experiments in order to have a higher degree of specific binding.

At equilibrium, unlabelled LTC_4 competed with ^{3}H-LTC_4 for its binding sites, and the inhibition of specific binding was complete at 5×10^{-6} M LTC_4. Computer analysis of the inhibition curve indicated interaction with a homogeneous class of binding sites, with an IC_{50} of 7.8×10^{-8} M (Fig. 3A); in the presence of $CaCl_2$, the potency of LTC_4 in inhibiting the binding was increased by 2.2-fold.

In order to evaluate the specificity of ^{3}H-LTC_4 binding, competition studies were performed also with LTD_4, LTE_4 and FPL 55712 (a widely used antagonist of SRS-A and leukotrienes) [12, 21]. All the compounds were able to compete with ^{3}H-LTC_4, but with an affinity much lower than that of LTC_4; indeed, the IC_{50}s were 2.2×10^{-6}, 2.4×10^{-5} and 2.4×10^{-5} M for LTD_4, LTE_4 and FPL 55712, respectively, in the presence of $CaCl_2$ (Fig. 3B). The potency of LTD_4 was not modified by the addition of 3 mM cysteine (data not shown), which has been reported to be an inhibitor of the conversion of LTD_4 into LTE_4 [22].

It is worth while mentioning that FPL 55712 was effective in inhibiting ^{3}H-LTC_4 binding at the indicated concentrations only when serial dilutions and assays were performed either with Tris–HCl containing 0.02% bovine serum albumin in glass tubes, or with Tris–HCl in polypropylene tubes. In the

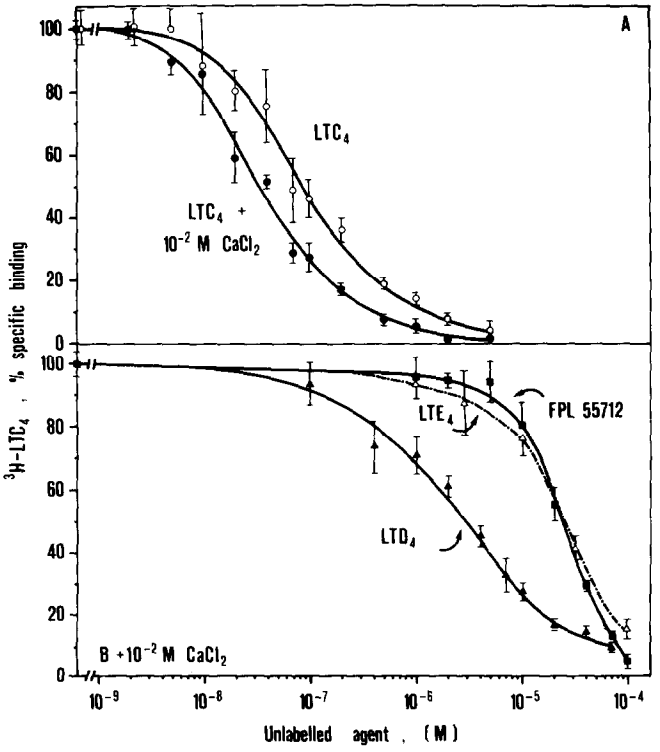

Fig. 3. Competition curves for LTC_4 in the absence and presence of 10^{-2} M $CaCl_2$ (panel A), and LTD_4, LTE_4, and FPL 55712 in the presence of 10^{-2} M $CaCl_2$ (panel B). ^{3}H-LTC_4 was 10^{-9} M and 100% specific binding was 0.30 and 0.80 pmole/mg prot. in the absence and presence of $CaCl_2$, respectively.

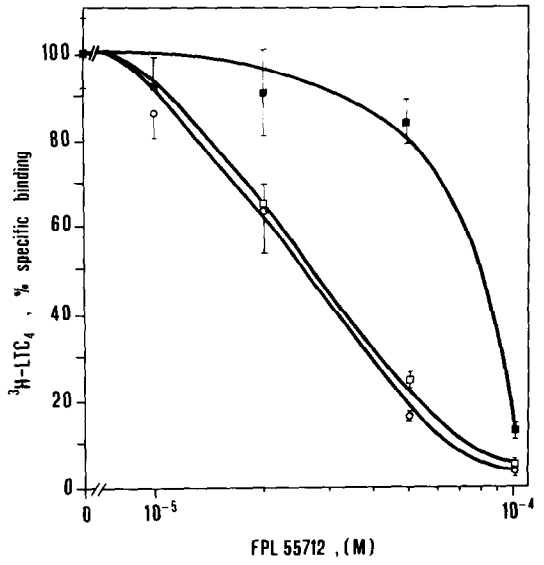

Fig. 4. Effect of bovine serum albumin (BSA) and of glass vs. polypropylene tubes on the inhibition of ^{3}H-LTC$_4$ by FPL 55712. FPL 55712 was diluted and assayed with 50 mM Tris–HCl buffer (pH 7.4) in the absence (■——■) or presence (□——□) of 0.02% BSA in glass tubes, or with Tris–HCl in polypropylene tubes (○——○). BSA at 0.02% did not affect either binding of ^{3}H-LTC$_4$ or its inhibition by LTC$_4$.

absence of these precautions, only much higher concentrations of FPL 55712 were able to inhibit ^{3}H-LTC$_4$ binding (Fig. 4). Bovine serum albumin, at the low concentration used, did not affect LTC$_4$ binding.

Specific binding of LTC$_4$ was saturable, as shown in Fig. 5, and the computer fitting of the binding isotherm was consistent with the existence of a homogeneous class of binding sites (B_{max} = 32–41 pmole/mg prot.); the K_d was 3.6–5.1 × 10^{-8} M, in good agreement with that derived from the competition curve on the basis of the computer calculated IC_{50} ($K_d = IC_{50} - [^3H\text{-}LTC_4]$).

The binding isotherm was shifted to the right by addition of FPL 55712. The shift was approximately parallel, suggesting that the antagonist acted with a competitive mechanism, as confirmed also by the double-reciprocal plot (inset to Fig. 5), where the two intercepts on the y axis were not statistically different [intercept and fiducial limits: 0.0253 (f.l.: 0.0055–0.045) and 0.0207 (f.l.: −0.0019–0.043) in the absence and in the presence of FPL 55712, respectively].

The Scatchard plot of the data was a straight line, both in the presence and in the absence of 10^{-2} M $CaCl_2$ (Fig. 6), indicating the existence of a single class of binding sites, in agreement with the results obtained by the analysis of the competition curves. It is evident that $CaCl_2$ significantly increased the affinity of LTC$_4$ (K_d = 1.9 × 10^{-7} and 3.6 × 10^{-8} M, in the absence and presence of Ca^{2+}, respectively; $P < 0.05$). The apparent decrease in B_{max} caused by $CaCl_2$, on the contrary, was not statistically significant ($P > 0.05$).

The binding sites were sensitive to trypsin and heat. In fact, 0.35 mg/ml trypsin for 30 min at 37° inactivated 85–90% of the specific binding (Table 1, treatment C). When the trypsin inhibitor was added at the end of the preincubation period (treatment D), an apparent increase in binding with respect to preincubation only (treatment B) and to control samples (treatment A) was observed. However, neither increase was statistically significant. Table 1 also shows that boiling for 30 min (treatment E) completely degraded the sites; indeed, less than 1% of the initial specific binding remained.

Conversion of ^{3}H-LTC$_4$ was assayed by RP-HPLC analysis of the incubation mixture. After incubation

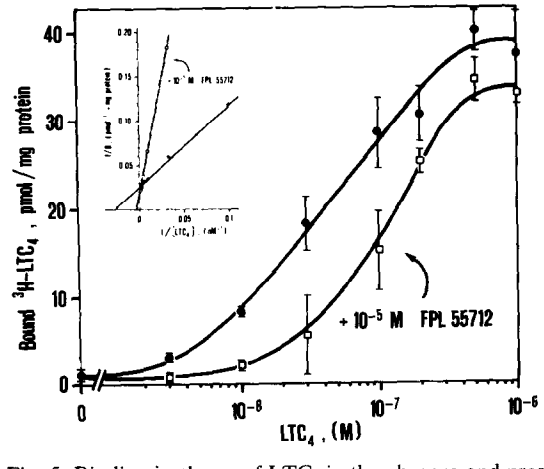

Fig. 5. Binding isotherm of LTC$_4$ in the absence and presence of 10^{-5} M FPL 55712. Each sample contained 10^{-9} M ^{3}H-LTC$_4$ and unlabelled LTC$_4$ up to the concentration shown. The experiment was performed in the presence of 10^{-2} M $CaCl_2$. The inset represents the double reciprocal plot of the data.

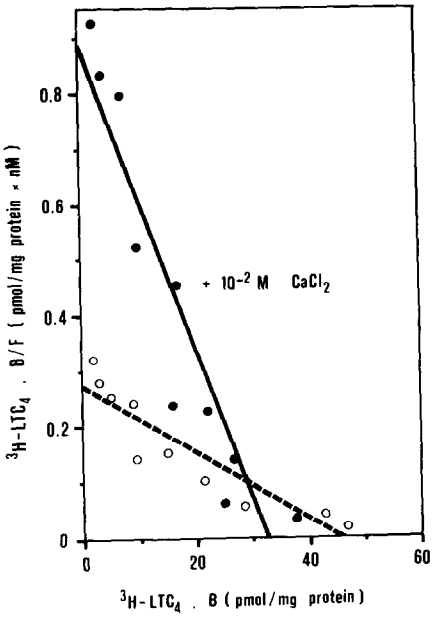

Fig. 6. Scatchard analysis of specific ^{3}H-LTC$_4$ binding in the absence and presence of 10^{-2} M $CaCl_2$.

Table 1. Stability of the binding sites for ^{3}H-LTC_4 to proteases and heat

Treatment	Specific binding (pmole/mg prot., mean ± S.D.)	% of control
(A) None	0.542 ± 0.038	100.0
(B) Preincubation	0.440 ± 0.066	81.2
(C) Preincubation with trypsin (+ trypsin inhibitor)	0.064 ± 0.023	11.8
(C) Preincubation (+ trypsin inhibitor)	0.576 ± 0.173	106.3
(E) Heating	0.003 ± 0.009	0.5

^{3}H-LTC_4 was 2×10^{-9} M and the protein content was 0.040 mg/sample. (A) The membranes were used immediately after thawing and resuspension, as described under Materials and Methods. (B) After thawing, the membranes were preincubated at 37° for 30 min. (C) The membranes were preincubated as in (B), in the presence of 0.35 mg/ml trypsin. Trypsin inhibitor, 0.35 mg/ml, was added at the end of the preincubation period. (D) The membranes were preincubated as in (B), and the trypsin inhibitor was added at the end of the preincubation, as in (C). (E) The membranes were heated at 100° for 30 min. The binding assay was then performed in the standard conditions described under Materials and Methods.

for either 20 (Fig. 7A) or 40 min (data not shown) in the presence of Serine-borate, no ^{3}H-LTD_4 or ^{3}H-LTE_4 were detectable; therefore, less than 3% (detection limit) of the incubated ^{3}H-LTC_4 was metabolized under the standard incubation conditions. That ^{3}H-LTD_4 could actually be detected when γ-glutamyl transpeptidase was not inhibited, was demonstrated by the incubation performed in the absence of Serine-borate (Fig. 7B), where approximately 60–70% conversion of ^{3}H-LTC_4 into ^{3}H-LTD_4 was revealed.

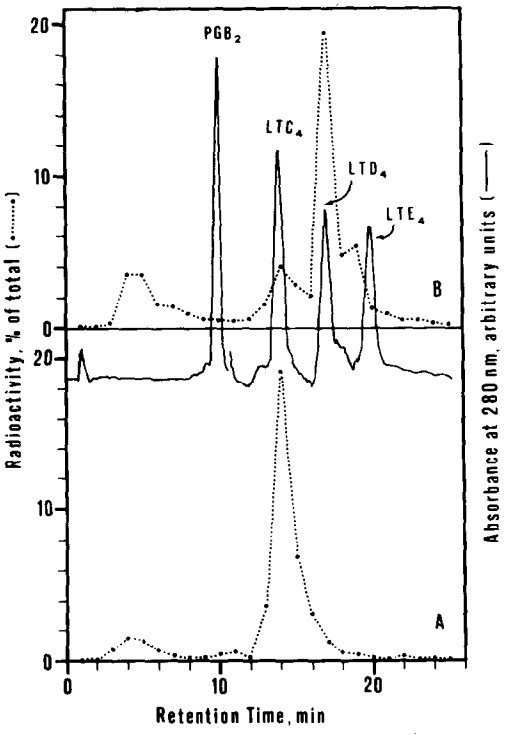

Fig. 7. Reversed phase HPLC analysis of ^{3}H-LTC_4 metabolites. Incubation, extraction and analysis conditions as described under Materials and Methods. The dotted line represents the radioactivity profile expressed as percent of the total amount applied onto the HPLC column. Panel A: 20 min incubation in the presence of 20 mM Serine-borate. Panel B: 20 min incubation in the absence of Serine-borate. The superimposed trace (continuous line) represents the HPL chromatogram of authentic PGB_2, LTC_4, LTD_4 and LTE_4 (330; 70; 60 and 100 ng, respectively). The sensitivity of the absorbance detector was 0.16 until PGB_2 was eluted, and 0.05 afterward.

In further support of the hypothesis that the binding sites identified are specific for LTC_4, a few preliminary experiments have been performed with ^{3}H-LTD_4 as the radioligand. Table 2 indicates that LTD_4 can compete with the labelled homoligand for its binding sites, while LTC_4 is ineffective in this respect at concentrations as high as 10^{-5} M.

DISCUSSION

High affinity binding sites for cysteine-containing leukotrienes have been identified so far in membrane preparations from rat and guinea-pig lung [23–26]. However, it is difficult to assess the real significance of the data obtained in rat, due to the scarcity of reports on the pulmonary effects of leukotrienes in this species; furthermore, the demonstrated difference between the response to leukotrienes of guinea pig and primates (see for instance ref. 27), makes it very difficult to extrapolate to humans the

Table 2. Effect of LTC_4 and LTD_4 on ^{3}H-LTD_4 specific binding

LT (M)	% specific binding (mean ± S.D.) LTD_4	LTC_4
10^{-8}	105 ± 16	103 ± 13
10^{-7}	83 ± 6	117 ± 10
10^{-6}	77 ± 6	130 ± 19
10^{-5}	53 ± 4	96 ± 18

^{3}H-LTD_4 was 10^{-8} M and the protein content was 0.042 mg/sample. 100% specific binding was 1.88 pmole/mg protein.

data on leukotriene binding obtained in lower species.

We report here that ^{3}H-LTC_4 binds to membranes from human lung parenchyma in a saturable, specific and reversible fashion. The binding sites probably possess at least a heat sensitive protein moiety, since they are inactivated upon trypsinization. It is unlikely that binding occurs to γ-glutamyl transpeptidase (the enzyme responsible for LTC_4 biotransformation) [28], since the experiments were performed in the presence of Serine-borate, which is a competitive inhibitor of this enzyme [29]. Indeed, Serine-borate was able to increase the specific binding of ^{3}H-LTC_4 at the concentration employed, while higher concentrations did not further affect binding (data not shown).

It is interesting to note that LTD_4, though being equiactive or more active than LTC_4 in contracting human bronchial smooth muscle [6, 7], showed an affinity a hundred-fold lower than LTC_4 for the binding sites labelled by ^{3}H-LTC_4; this was not due to its conversion to LTE_4, since cysteine, which inhibits the conversion, did not shift the competition curve. Such a lower affinity is actually to be expected if the sites we have identified coincide with the receptors responsible for the contracting effect; in fact, various pieces of evidence indicate that the receptors for LTC_4 and LTD_4 are distinct from one another [30, 31]. Furthermore, practically all the binding studies performed so far in various tissues have evidentiated the existence of specific sites different for the two leukotrienes [23, 24, 26, 32, 33]. Accordingly, also in our hands the sites labelled by LTD_4 display a much lower affinity for LTC_4. We propose therefore that, also in human lung membranes, the sites we have characterized are specific for LTC_4 and therefore display a much lower affinity for LTD_4.

Similarly, the low affinity of FPL 55712 for ^{3}H-LTC_4 binding sites is consistent with the finding that this SRS-A antagonist is much more potent against contractions induced by LTD_4 than by LTC_4 [30, 34]. It should be noted, however, that our present findings (Fig. 4) suggest that FPL 55712 tends to be absorbed to glass; in fact, the use of polypropylene instead of glass tubes, or the inclusion of low concentrations of bovine serum albumin in the buffer, shifted the competition curve to the left. Therefore, the real potency of this antagonist in some systems might need reevaluation. Moreover, FPL 55712 is reportedly credited with numerous pharmacological effects other then LT antagonism [35].

Human lung has been demonstrated to be sensitive to the bronchoconstricting action of cysteine-containing leukotrienes, both *in vivo* and *in vitro* [6–11], and the main site of action of leukotrienes at the airway level seems to be the periphery [30]. It is therefore possible that the binding sites we have identified in human lung parenchyma represent receptors, since they meet most of the necessary requirements such as saturability, specificity, rapidity of association and reversibility.

The identification and characterization of specific binding sites for LTC_4 in human lung could help in clarifying the precise role of leukotrienes in the pathophysiology of asthma. Moreover, our findings might facilitate the study and development of compounds possessing a leukotriene-antagonist activity, as well as of tools with new and useful therapeutic applications in immediate hypersensitivity diseases.

Acknowledgements—We thank Dr. M. Mezzetti (4th Surgery Clinic, University of Milan) for supplying human lung fragments. The skilful technical assistance of Mr. M. Musanti is gratefully acknowledged.

REFERENCES

1. R. C. Murphy, S. Hammarström and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **76**, 4275 (1979).
2. B. Samuelsson, in *Advances in Prostaglandin, Thromboxane, and Leukotriene Research*, Vol. 11 (Eds. B. Samuelsson, R. Paoletti and P. Ramwell), p. 1. Raven Press, New York (1983).
3. P. Hedqvist and S.-E. Dahlén, in *Advances in Prostaglandin, Thromboxane, and Leukotriene Research*, Vol. 11 (Eds. B. Samuelsson, R. Paoletti and P. Ramwell), p. 27. Raven Press, New York (1983).
4. M. N. Samhoun and P. J. Piper, in *Leukotrienes and Other Lipoxygenase Products* (Ed. P. J. Piper), p. 161. Research Studies Press, Chichester (1984).
5. G. Smedegård, P. Hedqvist, S.-E. Dahlén, B. Revenas, S. Hammarstrom and B. Samuelsson, *Nature, Lond.* **295**, 327 (1982).
6. S.-E. Dahlén, P. Hedqvist, S. Hammarström and B. Samuelsson, *Nature, Lond.* **288**, 484 (1980).
7. T. R. Jones, C. Davis and E. E. Daniel, *Can. J. Physiol. Pharmac.* **60**, 638 (1982).
8. M. C. Holroyd, R. E. C. Altounyan, M. Cole, M. Dixon and E. V. Elliott, *Lancet* **ii**, 17 (1981).
9. M. Griffin, J. W. Weiss, A. G. Leitch, E. R. McFadden, Jr., E. J. Corey, K. F. Austen and J. M. Drazen, *New Engl. J. Med.* **308**, 436 (1983).
10. J. W. Weiss, J. M. Drazen, E. R. McFadden, Jr., P. Weller, E. J. Corey, R. A. Lewis and K. F. Austen, *J. Am. med. Ass.* **249**, 2814 (1983).
11. S.-E. Dahlén, G. Hansson, P. Hedqvist, T. Björck, E. Granström and B. Dahlén, *Proc. natn. Acad. Sci. U.S.A.* **80**, 1712 (1983).
12. J. Augstein, J. B. Farmer, T. B. Lee, P. Sheard and M. L. Tattersall, *Nature New Biol.* **245**, 215 (1973).
13. J. G. Gleason, R. D. Krell, B. M. Weichman, F. E. Ali and B. Berkowitz, in *Leukotrienes and Other Lipoxygenase Products* (Eds. B. Samuelsson and R. Paoletti), p. 243. Raven Press, New York (1982).
14. J. M. Drazen, R. A. Lewis, K. F. Austen, M. Todd, F. Brion, A. Marfat and E. J. Corey, *Proc. natn. Acad. Sci. U.S.A.* **78**, 3195 (1981).
15. R. A. Lewis, J. M. Drazen, K. F. Austen, M. Todd, F. Brion, A. Marfat and E. J. Corey, *Proc. natn. Acad. Sci. U.S.A.* **78**, 4579 (1981).
16. M. Rosenberger, C. Newkom and E. R. Ais, *J. Am. Chem. Soc.* **105**, 3656 (1983).
17. N. Cohen, B. L. Banner, R. J. Lopresti, F. Wong, M. Rosenberger, Y.-Y. Liu, E. Thome and A. A. Liebman, *J. Am. chem. Soc.* **105**, 3661 (1983).
18. R. A. Appleton, J. R. Bantick, T. R. Chamberlain, D. N. Hardern, T. B. Lee and A. D. Prutt, *J. med. Chem.* **20**, 371 (1977).
19. F. Benfenati and V. Guardabasso, in *Principles and Methods in Receptor Binding* (Eds. F. Cattabeni and S. Nicosia), p. 41. Plenum Press, New York (1984).
20. W. S. Powell, *Meth. Enzym.* **86**, 467 (1982).
21. A. F. Welton, M. O'Donnell, W. Anderson, H. J. Crowley, A. Medford, B. Simko and B. Yaremko, in *Advances in Prostaglandin, Thromboxane, and Leukotriene Research*, Vol. 12 (Eds. B. Samuelsson, R. Paoletti and P. Ramwell), p. 145. Raven Press, New York (1983).

22. D. E. Sok, J. K. Pai, V. Atrache and C. J. Sih, *Proc. Nat. Acad. Sci. U.S.A.* **77**, 6481 (1980).
23. S.-S. Pong and R. N. DeHaven, *Proc. natn. Acad. Sci. U.S.A.* **80**, 7415 (1983).
24. S.-S. Pong, R. N. DeHaven, F. A. Kuehl, Jr. and R. W. Egan, *J. biol. Chem.* **258**, 9616 (1983).
25. R. F. Bruns, W. J. Thomsen and T. A. Pugsley, *Life Sci.* **33**, 645 (1983).
26. G. K. Hogaboom, S. Mong, H.-L. Wu and S. T. Crooke, *Biochem. biophys. Res. Commun.* **116**, 1136 (1983).
27. S.-E. Dahlén, *Acta physiol. scand. Suppl.* **512**, 1 (1983).
28. H. R. Morris, G. W. Taylor, C. M. Jones, P. J. Piper, M. N. Samhoun and J. R. Tippins, *Proc. natn. Acad. Sci. U.S.A.* **79**, 4838 (1982).
29. L. Örning and S. Hammarström, *Biochem. biophys. Res. Commun.* **106**, 1304 (1982).
30. J. M. Drazen, K. F. Austen, R. A. Lewis, D. A. Clark, G. Goto, A. Marfat and E. J. Corey, *Proc. natn. Acad. Sci. U.S.A.* **77**, 4354 (1980).
31. S. Krilis, R. A. Lewis, J. M. Drazen and K. F. Austen, in *Icosanoids and Ion Transport* (Eds. P. Braquet, S. Nicosia, R. Garay and J. C. Frölich), p. 91, Raven Press, New York (1985).
32. S. Krilis, R. A. Lewis, E. J. Corey and K. F. Austen, *J. clin. Invest.* **72**, 1516 (1983).
33. S. Nicosia, H. J. Crowley, D. Oliva and A. F. Welton, *Prostaglandins* **27**, 483 (1984).
34. D. W. Snyder, M. Barone, M. P. Morrissette, P. R. Bernstein and R. D. Krell, *Pharmacologist* **25**, 205 (1983).
35. A. F. Welton, W. C. Hope, L. D. Tobias and J. C. Hamilton, *Biochem. Pharmac.* **30**, 1378 (1981).