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Leukotriene-Induced Hydrolysis of Inositol Lipids in Guinea Pig Lung: Mechanism of Signal Transduction for Leukotriene-D_4 Receptors

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SUMMARY

Addition of leukotriene D_4 (LTD_4) to [^{3}H]*myo*-inositol-labeled guinea pig lung induced rapid breakdown of inositol lipids. Formation of [^{3}H]inositol trisphosphate was rapid, with a peak of 140–160% of the control level, 30 sec post-treatment. Formation of [^{3}H]inositol bisphosphate and [^{3}H]inositol monophosphate ([^{3}H]IP_1) was also rapid in the presence of LiCl. LTD_4-induced [^{3}H]IP_1 formation was concentration dependent, stereoselective, and not inhibited by the cyclooxygenase inhibitor, indomethacin. Agonist analogs of LTD_4 and leukotriene E_4 also induced dose-dependent increases in the synthesis of [^{3}H]IP_1. The rank order potency of the agonist-induced [^{3}H]IP_1 formation was equivalent to those reported for LTD_4 receptor binding, smooth muscle contraction, and thromboxane B_2 biosynthesis. Furthermore, a specific receptor antagonist, SKF 102922, inhibited LTD_4-induced [^{3}H]IP_1 formation in guinea pig lung. These studies suggest that LTD_4 may interact with membrane receptor and activate a phospholipase C, which in turn induces the hydrolysis of inositol lipids. The hydrolysis products, diacylglycerol and inositol trisphosphate, can be regarded as the intracellular messengers for LTD_4 receptors in guinea pig lung. This concept may explain a variety of pharmacological effects of leukotrienes in different types of target cells or tissues.

The leukotrienes LTC_4, LTD_4, and LTE_4 have been identified as the major constituents of slow-reacting substance of anaphylaxis (1). Pharmacological studies have shown that these agents can induce prolonged contraction of smooth muscle via specific receptors (1–5). Using radioligand binding methods, an LTD_4 receptor that is distinctly different from the LTC_4 receptor has been identified and characterized (4). Furthermore, we have shown that LTE_4 binds to the LTD_4 receptors (6) and we have not detected LTE_4-specific receptors in guinea pig lung (7). The LTD_4 receptors were localized primarily on the plasma membrane of smooth muscle cells.[1] The binding of LTD_4 to these receptors is specifically modulated by Na^+ and guanine nucleotides (3, 5, 8). The effects of guanine nucleotides and Na^+ on the LTD_4 receptors are analogous to those in the α_2-adrenergic receptors in platelets (9) or the opiate receptors in the central nervous system (10), suggesting that the LTD_4 receptors may be linked to G_i. This also suggests that LTD_4 can bind to the receptor and inhibit adenylate cyclase and may lead to a decrease of the intracellular concentration of cAMP. However, data accumulated from several laboratories do not support this hypothesis. No evidence has been reported that LTD_4 can inhibit adenylate cyclase (3). Furthermore, when the intracellular concentrations of cAMP and cGMP were quantitated, using a radioimmunoassay, the contraction of guinea pig tracheal smooth muscle induced by LTD_4 was not associated with a decrease of cAMP (11).

Recent evidence suggests that LTD_4 receptor-mediated smooth muscle contraction requires an elevation of the level of intracellular calcium in the target tissue (12). Many smooth muscle contractile agents induce their pharmacological effects via the formation of intracellular messengers such as DAG and IP_3 (13, 14). These two intracellular messengers mobilize calcium and activate C-Kinase, respectively, and result in the

[1] S. Mong, G. Chi-Rosso, M. A. Clark, and S. T. Crooke, submitted for publication.

ABBREVIATIONS: LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; LTD_1, 5(*S*)-hydroxy-6(*R*)-*S*-1-cysteinylglycyl-7(*Z*)-eicosenoic acid; 5*R*,6*S*-LTD, 5-(*R*)-hydroxy-6(*S*)-*S*-*l*-cysteinylglycyl-7-(*Z*)-eicosenoic acid; 5*R*,6*S*-LTE_4, 5(*R*)-hydroxy-6(*S*)-*S*-*l*-cysteinyl-7(*E*),9(*E*),11(*Z*),14(*Z*)-eicosatetraenoic acid; des-amino-LTE_1, 5(*S*)-hydroxy-6(*R*) and 5(*R*)-hydroxy-6(*S*)-carboxyethyl-thio-7(*Z*)-eicosenoic acid; PI, phosphatidylinositol; PIP_2, phosphatidylinositol bisphosphate; IP_1, inositol monophosphate; IP_2, inositol bisphosphate; IP_3, inositol trisphosphate; DAG, diacylglycerol; PMA, phorbol myristate acetate; C-Kinase, protein kinase C; IAP, islet-activating protein; pD_2, negative logarithm of the concentration required for 50% maximal response; αPDD, 4 α-phorbol 12,13-didecanoate; KRH buffer, Krebs-Ringer-Henseleit buffer; TxB_2, thromboxane B_2; SKF 102922, 5-[2-(8-phenyloctyl)phenyl]-4,6-dithianonanedioic acid; FPL 55712, 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxyperoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylic acid; G_i, inhibitory guanine nucleotide-binding protein; G_o, unknown function guanine nucleotide-binding protein; G_x, guanine nucleotide-binding protein of undetermined type.

release of arachidonic acid (15), contraction of smooth muscle (16), and many other pharmacological effects. We have initiated the current study to determine whether LTD_4 and its analogs, via interactions with LTD_4 receptors, induce inositol lipid (PI) hydrolysis in guinea pig lung.

Materials and Methods

LTC_4, LTD_4, LTE_4, the stereoisomers (5*R*,6*S*-LTD_4, 5*R*,6*S*-LTE_4), LTD_1, des-amino-LTE_1 and the receptor antagonist, SKF 102922, were synthesized and supplied by the Department of Medicinal Chemistry, Smith Kline and French Laboratories. The methods employed for the synthesis and purification have been reported previously (17, 18). [^{3}H]LTD_4 (37–42 Ci/mmol) and [^{3}H]*myo*-inositol (35–40 Ci/mmol) were obtained from New England Nuclear Corp. (Boston, MA). Indomethacin, PMA, and Dowex 1 × 8 anion exchange resin were purchased from Sigma Chemical Co. (St. Louis, MO).

Guinea pig lung membrane LTD_4 receptor binding. Guinea pig lung membranes were prepared by Polytron homogenization and differential centrifugation as described previously (2, 3, 6). Binding of [^{3}H]LTD_4 to the receptors was initiated by adding the membrane protein (100 μg/ml) into incubation mixtures that contained 0.5 nM [^{3}H]LTD_4, 20 mM 1,4-piperazinediethanesulfonic acid buffer (pH 6.5), 10 mM $CaCl_2$, 10 mM $MgCl_2$, 5 mM cysteine, 5 mM glycine in a volume of 0.5ml, in triplicate, and incubated at 22° for 30 min. For the determination of the binding affinity of LTD-type agonists and antagonists, varying concentrations of these drugs were included in the incubation mixtures. Total and nonspecific binding of [^{3}H]LTD_4 were determined as the amount of [^{3}H]LTD_4 binding to the membranes in the absence or presence of 500 nM LTD_4, respectively. Receptor-specific binding was defined as the total binding minus the nonspecific binding. The binding affinity of each leukotriene agonist and antagonist was calculated based on the IC_{50} obtained from the displacement isotherms and expressed as K_i. The K_i was defined as: $K_i = IC_{50}/\{1 + ([^3H] - LTD_4/K_d)\}$, where ([^{3}H]-$LTD_4$) was the concentration of the radioligand (0.5 nM) employed; K_d was the dissociation constant (0.2 nM), determined from saturation binding experiments (4); and IC_{50} was the concentration required to compete [^{3}H]LTD_4 receptor binding by 50%.

Incorporation of [^{3}H]*myo*-inositol into guinea pig lung. Young male guinea pigs (body weight 300–400 g) were sacrificed by decapitation and exsanguination. Lungs were perfused with 20 ml of warm and oxygenated (95% O_2/5% CO_2) KRH buffer (118 mM NaCl, 4.6 mM KCl, 1.1. mM $MgSO_4$, 1.8 mM $CaCl_2$, 24.9 mM $NaHCO_3$, 1.0 mM KH_2PO_4, and 11.1 mM glucose) through the pulmonary artery. Lungs were removed, rinsed in KRH buffer, and minced into 1–2-mm^3 blocks with a tissue chopper. KRH buffer was added to the tissue to a final concentration of 0.5 g/ml. [^{3}H]*myo*-Inositol was added to the tissue at a concentration of 10 μCi/ml and incubated at 37° for 80 min in an oxygen-aerated plastic test tube. An aliquot of concentrated LiCl (2.0 M) was added into the test tube to make the final concentration 10 mM. LiCl (10 mM, final concentration) was included in the KRH buffer during washing, resuspension, and incubation. Incubation was continued for 10 min at 37°,then 15 ml of KRH buffer were added. The tissue was then centrifuged, washed, and resuspended three times with 20 ml of KRH buffer to remove the unincorporated [^{3}H]*myo*-inositol. KRH buffer was then added to the tissue suspension (0.5 g/ml) and equilibrated at 37° for 5 min. Three hundred μl of the tissue suspension were aliquoted into test tubes (in triplicate) that contained 3 μl of varying concentrations of LTD_4, LTE_4, or other agonist analogs and incubated for an additional 20 min. For the determination of kinetic effects on [^{3}H]IP_1, [^{3}H]IP_2, and [^{3}H]IP_3 formation, the tissue was incubated with 50 μCi/ml of [^{3}H]*myo*-inositol in KRH buffer (4 g/ml) for 80 min, and the concentration of LiCl was adjusted to 40 mM in the incubation and washing procedures. For the antagonist and other treatments of drugs, SKF 102922 (20 μM), indomethacin (20 μM), PMA (1 μM), or αPDD (1 μM) was added to the tissue together with LiCl (10 mM) and further incubated for 10 min, and then washed twice within 5 min. Each drug was also present in the washing and resuspension (KRH) buffer. LTD_4 was added to these tissue samples and incubated for 20 min to determine the effects of PI hydrolysis.

Measurement of formation of [^{3}H]inositol phosphates. After the [^{3}H]*myo*-inositol-labeled tissue was incubated with LTD_4 or other compounds, 1.35 ml of chloroform:methanol (1:2) were added into each test tube to stop the reaction and extract the lipids. The test tube was maintained at 0° for 20 min, and 450 μl of chloroform were added, followed by 450 μl of H_2O. The test tube was vortexed vigorously for 10 sec and then spun in a Beckman TJ-6 centrifuge at 2000 × *g* for 5 min to separate the aqueous from the organic phase solvents. The inositol phosphates were fractionated into [^{3}H]IP_1, [^{3}H]IP_2, and [^{3}H]IP_3 by anion exchange column chromatography as described by Creba *et al.* (19). Briefly, Dowex anion exchange resin (formate form) was resuspended in H_2O at a ratio of 1:1 (w/v). One ml of the aqueous extract was transferred to a test tube containing 1 ml of Dowex anion exchange resin and 5 ml of H_2O. The contents were then poured into a disposable polystyrene column (0.5 cm × 6 cm). The resin was washed four times with 5 ml of H_2O. The [^{3}H]IP_1 were eluted twice with 1.5 ml of 0.2 M ammonium formate/0.1 M formic acid solution (solution D). The column was then washed four times with 5 ml of solution D. The [^{3}H]IP_2 was eluted twice with 1.5 ml of 0.5 M ammonium formate/0.1 M formic acid solution (solution E). The column was then washed four times with 5 ml of solution E. Finally, the [^{3}H]IP_3 was eluted twice with 1.5 ml of 1.0 M ammonium formate/0.1 M formic acid. Fifteen ml of scintillation cocktail (Aquasol) were added to each vial and the radioactivity in the vial was determined by scintillation spectrometry with efficiency of 20–30%. The [^{3}H]IP_3 fraction probably contains a mixture of inositol-1,3,4-trisphosphate, inositol-1,4,5-trisphosphate (either cyclic or the open-ring form), and inositol-1,3,4,5-tetrakisphosphate (20, 21); the IP_3 fractions quantified in this paper represent a general measurement of all of these inositol polyphosphates.

LTD_4-induced TxB_2 synthesis in guinea pig lung. Minced guinea pig lung was washed, suspended in 1 ml of warm KRH buffer, and incubated with various concentrations of LTD_4 for 20 min at 37°. The concentration of TxB_2 in the culture supernatant was determined by radioimmunoassay as described previously (22, 23).

Results

Kinetic responses of inositol phosphate formation. When [^{3}H]*myo*-inositol-labeled guinea pig lung was incubated with 1 μM LTD_4 at 37°, rapid metabolism of PI was observed. Fig. 1, A–C, shows the kinetics of [^{3}H]IP_1, [^{3}H]IP_2, and [^{3}H]IP_3 formation in the control and LTD_4-stimulated tissue. In the presence of 40 mM LiCl, the basal level(control) of [^{3}H]IP_1 gradually increased. When the tissue was treated with 1 μM LTD_4, rapid accumulation of [^{3}H]IP_1 was observed (Fig. 1A). At the earliest assayable time point, i.e., 2 sec post-treatment with LTD_4, the [^{3}H]IP_1 in the treated samples was not significantly different from the control (basal) samples. Thirty sec after stimulation with LTD_4, formation of [^{3}H]IP_1 was significantly higher than the basal level and increased steadily thereafter. The net increase of [^{3}H]IP_1, induced by LTD_4, was significantly different from the control 30 sec post-stimulation. LTD_4 also induced the net accumulation of [^{3}H]IP_1 when the concentration of LiCl was reduced to 15, 10, or 0 mM during the LTD_4 treatment (incubation) time, although the LTD_4-induced net [^{3}H]IP_1 accumulation was lower (20–25% of that shown in Fig. 1A; results not shown). This observation is consistent with the current notions about the effects of LiCl on PI metabolism (24), i.e., Li^+ inhibits the conversion of inositol-1-phosphate to inositol and causes an accumulation of inositol-1-phosphate. In the presence of Li^+, an agonist can bind to a receptor, stimulate phospholipase C, and result in

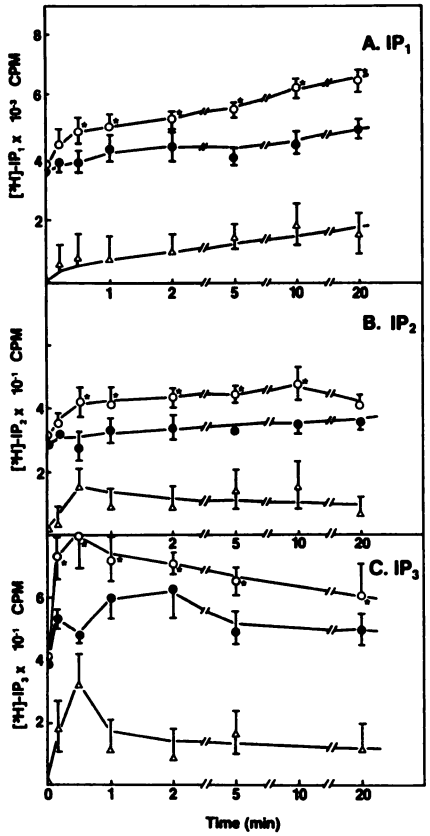

Fig. 1. Kinetic responses of inositol phosphate formation in guinea pig lung. Guinea pig lung was labeled with 50 μCi/ml of [^{3}H]*myo*-inositol in the presence of 40 mM LiCl as described in Materials and Methods. The tissue was treated with1 μM LTD$_4$ (○) or saline as control (●) from 0 to 20 min. The ^{3}H-labeled inositol phosphates were extracted and quantitated by anion exchange column chromatography. Formation of [^{3}H]IP$_1$ (A), [^{3}H]IP$_2$ (B), and [^{3}H]IP$_3$ (C) was determined from triplicate samples of a representative experiment (from three). The differences between the LTD$_4$-stimulated and the control levels of inositol phosphates (△) are defined as the net accumulation induced by LTD$_4$. ★, $p < 0.01$.

hydrolysis of PIP$_2$ (possibly phosphatidylinositol monophosphate and PI), to yield IP$_3$, IP$_2$, and IP$_1$ (13).

The basal and LTD$_4$-stimulated accumulations of [^{3}H]IP$_2$ are shown in Fig. 1B. LTD$_4$-induced [^{3}H]IP$_2$ accumulation was significantly higher than the basal level 30 sec after stimulation. The net accumulation of [^{3}H]-IP$_2$ increased steadily and approached a plateau at 1 min post-stimulation. The basal level and LTD$_4$-stimulated accumulations of [^{3}H]IP$_3$ are shown in Fig. 1C. The LTD$_4$-induced [^{3}H]IP$_3$ accumulation was significantly higher than the basal level as early as 10 and 30 sec after stimulation. The peak accumulation occurred approximately 30 sec after addition of LTD$_4$ and the increase of [^{3}H]IP$_3$ concentration was consistent for 20 min. The kinetic responses of LTD$_4$-induced ^{3}H-inositol phosphates indicate that [^{3}H]IP$_3$ was formed first and followed by [^{3}H]IP$_2$ and [^{3}H]IP$_1$, thus suggesting that [^{3}H]IP$_1$ and [^{3}H]IP$_2$ may be derived from [^{3}H]IP$_3$. These results show that the LTD$_4$-induced PI hydrolysis effect is rapid. Since [^{3}H]IP$_1$ is thought to be the metabolic product of [^{3}H]IP$_2$ and [^{3}H]IP$_3$, we have used the net accumulation of [^{3}H]IP$_1$ as an indicator of LTD$_4$-induced PI hydrolysis.

Pharmacological specificity of [^{3}H]IP$_1$ formation. LTD$_4$ induced dose-dependent increases of [^{3}H]IP$_1$ (Fig. 2). The 50% maximal effective concentration (EC$_{50}$) was 10 ± 5 nM. At a concentration of 1 μM, the effect of [^{3}H]IP$_1$ synthesis was nearly maximal. The less active stereoisomer of LTD$_4$ (5*R*,6*S*-LTD$_4$) also induced a dose-dependent increase in the synthesis of [^{3}H]IP$_1$ with an EC$_{50}$ of 1.5 ± 0.6 μM, approximately 200-fold less effective than LTD$_4$. At a concentration of 30 μM, the effect of 5*R*,6*S*-LTD$_4$ was nearly maximal and was 92 ± 10% of the maximal activity defined by 1 μM LTD$_4$. Several other leukotriene D-type agonist analogs, e.g., LTE$_4$, LTD$_1$, and des-amino-LTE$_1$, were also capable of inducing [^{3}H]IP$_1$ formation in dose-dependent fashion. The EC$_{50}$ values were 130 ± 20, 650 ± 60, and 700 ± 80 nM, respectively. The maximal extents of the [^{3}H]IP$_1$ formation induced by these agonists, however, were 48, 38, and 48% of that induced by LTD$_4$. Table 1 summarizes these results and demonstrates that the PI hydrolysis induced by LTD$_4$ and analogs are highly stereoselective and specific. The rank order of agonist-induced [^{3}H]IP$_1$ for-

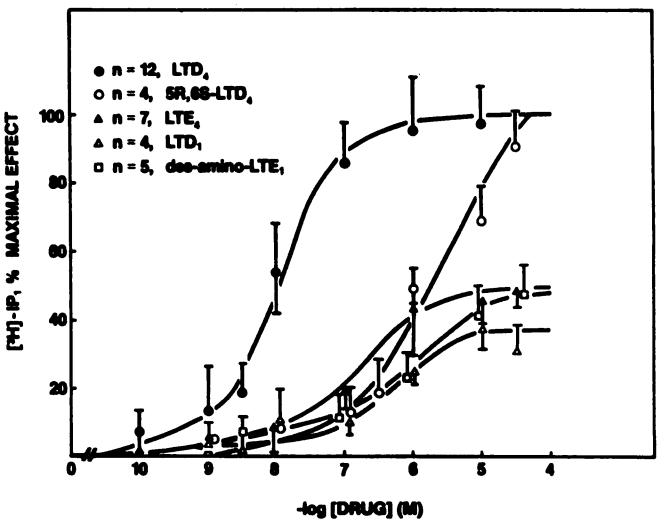

Fig. 2. Dose-dependent increases of [^{3}H]IP$_1$ induced by LTD$_4$ and analogs in guinea pig lung. Guinea pig lung was labeled with 10 μCi/ml of [^{3}H]*myo*-inositol in the presence of 10 mM LiCl as described in Materials and Methods. The tissue was then treated with increasing concentrations of LTD$_4$ (●), 5*R*,6*S*-LTD$_4$ (○), LTE$_4$ (▲), LTD$_1$ (△), and des-amino-LTE$_1$ (□) for 20 min at 37°. The [^{3}H]IP$_1$ was extracted and quantitated. The maximum increase of [^{3}H]IP$_1$ formation, induced by 1 μM LTD$_4$, in each experiment, was defined as 100% of maximal response. The extent of [^{3}H]IP$_1$ formation for each of the agonist analogs was determined as the fraction of the maximal response in each experiment. The results from 4–12 experiments were averaged and shown.

TABLE 1

Comparative properties of the PI hydrolysis and TxB$_2$ biosynthesis responses induced by LTD$_4$ and analogs

	TxB$_2$ biosynthesis activity[a]		PI hydrolysis	
	Potency (pD$_2$)[b]	Intrinsic activity[c]	Potency (pD$_2$)	Intrinsic activity
		%		%
LTD$_4$	8.1	100	8.1	100
5*R*,6*S*-LTD$_4$	6.2	100	6.0	90
5*S*,6*R*-LTE$_4$	6.8	46	6.8	48
5*R*,6*S*-LTE$_4$	ND[d]	4	5.0	<10
5*S*,6*R*-LTD$_1$	6.3	50	6.4	38
des-amino-LTE$_1$	6.0	44	6.1	48

[a] Data taken from a previous publication (23).
[b] pD$_2$, the negative logarithm of the EC$_{50}$ of agonist-induced [^{3}H]IP$_1$ biosynthesis.
[c] The maximal extent of LTD$_4$-induced [^{3}H]IP$_1$ biosynthesis activity is arbitrarily defined as 100%. The maximal extents of other agonist-induced [^{3}H]IP$_1$ biosynthesis activities were taken from the plateau phase of the dose response curves in Fig. 2, as fractions of that induced by 1 μM LTD$_4$.
[d] ND, activity could not be determined.

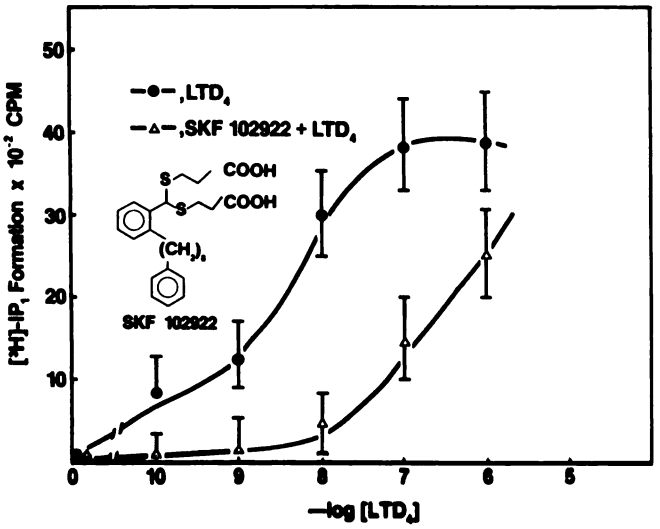

Fig. 3. Inhibition of LTD_4-induced PI hydrolysis by receptor antagonist. Guinea pig lung was labeled with [^{3}H]*myo*-inositol (10 μCi/ml) for 80 min. LiCl (10 mM) was added together with 20 μM SKF 102922 (△) or with KRH buffer (●) as controls and incubated for an additional 10 min. The antagonist-treated tissue was also washed in the presence of 20 μM SKF 102922. The tissue samples were then treated with increasing concentrations of LTD_4 to quantitate the [^{3}H]IP_1 formation.

mation is equivalent to that of LTD_4 receptor-mediated smooth muscle contraction and TxB_2 synthesis effects in guinea pig lung (4, 23). The lower maximal effects on PI hydrolysis by LTE_4, LTD_1, and des-amino-LTE_1 suggest that, when compared with LTD_4 and 5*R*,6*S*-LTD_4, they are partial agonists. It has recently been reported that LTE_4 is a partial agonist in guinea pig tracheal smooth muscle contraction (cyclooxygenase independent) (25) and in TxB_2 synthesis assays (cyclooxygenase dependent) (23).

Results thus far suggest that LTD_4-induced [^{3}H]IP_1 formation is mediated via the LTD receptors in guinea pig lung. To confirm this, a recently synthesized and well characterized LTD_4 receptor antagonist, SKF 102922, was used to study agonist-induced PI hydrolysis. SKF 102922 bound to the LTD_4 receptors with K_i = 220 ± 30 nM (18). SKF 102922 did not induce [^{3}H]IP_1 formation at concentrations from 1 (result not shown) to 20 μM (Fig. 3). The dose response curve of LTD_4-induced [^{3}H]IP_1 formation was shifted to the right with an EC_{50} at 0.5 μM, when tissue was pretreated with 20 μM SKF 102922 (Fig. 3). The antagonist activity [K_B] of SKF 102922 was 310 ± 60 nM ($-\log[K_B]$ = 6.5), close to the receptor binding affinity described above. These results show that the LTD_4 receptor antagonist, SKF 102922, inhibited LTD_4-induced [^{3}H]IP_1 accumulation. Another slow-reacting substance of anaphylaxis antagonist, FPL 55712, also inhibited the LTD_4-induced [^{3}H]IP_1 formation in guinea pig lung, but FPL 55712 was much less potent than SKF 102922 (results not shown). These results demonstrate that specific LTD_4 receptor antagonists can block the agonist-induced PI hydrolysis induced by LTD_4 in guinea pig lung, and that their potencies as antagonists against the PI hydrolysis effect correlate with antagonist activities in receptor binding assays.

Pharmacological regulation of LTD_4 induced [^{3}H]IP_1 formation. When guinea pig lung, smooth muscle cells, or endothelial cells in culture were exposed to LTD_4, many cyclooxygenase metabolites of arachidonic acid were synthesized (12, 20, 22, 23, 26, 27). Many cyclooxygenase metabolites of arachidonic acid can also interact with their target cells to promote hydrolysis of PI and result in [^{3}H]IP_1 formation (28). It is thus possible that the LTD_4-induced [^{3}H]IP_1 formation in guinea pig lung might be due to those cyclooxygenase metabolites. The results shown in Table 2 show that LTD_4-induced [^{3}H]IP_1 formation was not inhibited by indomethacin. This result suggests that LTD_4-induced [^{3}H]IP_1 formation does not require production of cyclooxygenase metabolites of arachidonic acid in guinea pig lung.

PMA can bind to and directly activate C-Kinase (14). In several receptor systems, PMA can activate the cellular C-Kinase and lead to inhibition of the agonist-induced increases of IP_3 and Ca^{2+} mobilization (29). In the guinea pig lung, pretreatment of the tissue with PMA, but not with the biologically inactive analog, αPDD, partially attenuated the LTD_4-induced [^{3}H]IP_1 accumulation (Table 2).

The IAP from *Bordetella pertussis* has been shown specifically to modify the G_i (or G_o) protein (30) by inducing an ADP-ribosylation (15, 31). Many pharmacological responses that are mediated by G_i protein-coupled receptors, such as PI turnover, are sensitive to IAP treatment (15). In the guinea pig lung, however, IAP treatment (1–500 ng/ml for 2 hr at 37°) did not inhibit the incorporation of [^{3}H]*myo*-inositol or the LTD_4-induced accumulation of [^{3}H]IP_1, [^{3}H]IP_2, and [^{3}H]IP_3 (results not shown).

Correlation of PI hydrolysis and other LTD_4 receptor-mediated effects in guinea pig lung. A recent report from our laboratory has demonstrated that both the smooth muscle contractile response (cyclooxygenase independent) in guinea pig trachea and the TxB_2 biosynthesis (cyclooxygenase dependent) response in guinea pig lung were mediated by LTD_4 receptors (23). To analyze further whether the PI hydrolysis effect was mediated via LTD_4 receptors and related to smooth muscle contraction, we have plotted the agonist activities (pD_2) for PI hydrolysis against the smooth muscle contractile activi-

TABLE 2
Pharmacological regulation of PI metabolism induced by LTD_4

	Control		Indomethacin[a]		PMA[b]		αPDD[c]	
	[^{3}H]IP_1	Δ[^{3}H]IP_1	[^{3}H]IP_1	Δ[^{3}H]IP_1	[^{3}H]IP_1	Δ[^{3}H]IP_1	[^{3}H]IP_1	Δ[^{3}H]IP_1
Basal	1236 ± 45		960 ± 165		919 ± 64		1056 ± 170	
LTD_4 (1 μM)	2470 ± 125	1234 ± 134	2119 ± 143	1159 ± 218	1836 ± 52	917 ± 82	2545 ± 210	1489 ± 270
(Stimulation)		(100%)		(94%)		(75%, $p < 0.01$)		(120%, NS)[d]

[a] Tissue was treated with 20 μM indomethacin for 10 min, washed in KRH buffer (containing 20 μM indomethacin) two times, and then treated with KRH buffer (control) or 1 μM LTD_4. Results from a representative experiment (of three) are shown. Numbers indicate cpm.
[b] Tissue was treated (and then washed) with 1 μM PMA as described in Footnote *a* and used for treatment of LTD_4.
[c] Tissue was treated (and then washed) with 1 μM αPDD as described in Footnote *a* and used for treatment of LTD_4.
[d] NS, not significantly different from the control.

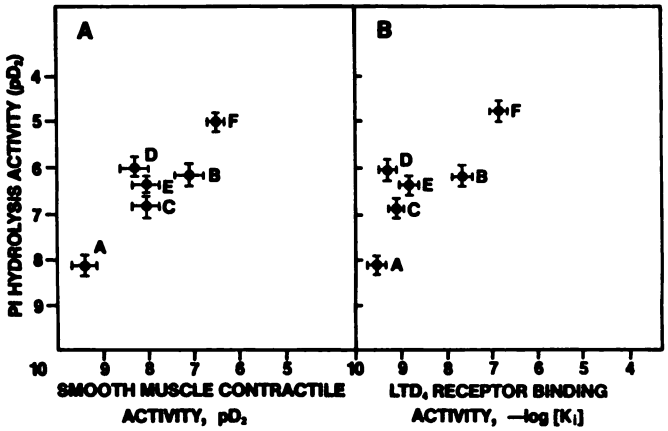

Fig. 4. Correlation of the effects of PI hydrolysis to pharmacological activities of leukotrienes in guinea pig lung. The PI hydrolysis activity and the smooth muscle contraction activity for LTD$_4$ and analogs were quantitated as −log[EC$_{50}$] and expressed as pD$_2$ values. The receptor-binding affinity of LTD$_4$ and analogs was determined previously (4), expressed as −log[K_i] and calculated as described in Materials and Methods and Ref. 34. A. The coefficient of linear correlation between receptor binding and PI hydrolysis was 0.85. B. The coefficient of correlation between smooth muscle contraction and PI hydrolysis was 0.83. The compounds are: *A*, LTD$_4$; *B*, 5*R*,6*S*-LTD$_4$; *C*, LTE$_4$; *D*, des-amino-LTE$_1$; *E*, LTD$_1$, and *F*, 5*R*,6*S*-LTE$_4$.

ties (Fig. 4A), and the LTD$_4$ receptor-binding activities against the PI hydrolysis activity (Fig. 4B) of these analogs. Good correlation of the PI hydrolysis effect to smooth muscle contraction (γ = 0.83) and to receptor binding (γ = 0.85) is demonstrated.

Discussion

The observation that LTD$_4$ binding to membrane receptors is modulated by Na^+ and guanine nucleotides has led many to suggest G$_i$ as one of the transducer proteins for the signal transduction mechanism for LTD$_4$. G$_i$ has been traditionally linked to inhibition of adenylate cyclase (15, 31, 32). In addition, Anderson *et al.* (33) has reported that LTC$_4$ (and possibly LTD$_4$) induces a decrease of cAMP in guinea pig trachea, thus further suggesting that LTD$_4$ binds to G$_i$ protein-regulated membrane receptors, induces an inhibition of the adenylate cyclase, and results in smooth muscle contraction. However, other studies suggest an alternative explanation in guinea pig lung and sheep trachea. For example: (a) LTD$_4$-induced contraction of opossum trachea was not associated with a decrease of intracellular cAMP (11)—rather, it was associated with an increase of intracellular cGMP; (b) LTD$_4$ did not inhibit basal or isoproterenol-stimulated adenylate cyclase activities in guinea pig lung or sheep tracheal smooth muscle membranes (3);[2] and (c) G$_i$, G$_o$ proteins, and possibly other novel G proteins now have been demonstrated to transduce the effects of many types of membrane receptors that are linked to PI hydrolysis in the generation of IP$_3$ and DAG (15).

In the current study we have shown that, for the first time, LTD$_4$ (and analogs) induced rapid accumulation of IP$_1$, IP$_2$, and IP$_3$. The effects on PIP$_2$ hydrolysis were detected as early as 10 sec after stimulation with LTD$_4$. PI hydrolysis occurred earlier than or simultaneous to the onset of smooth muscle contraction (34), arachidonic acid release (22), and cyclooxygenase metabolite formation in smooth muscle cells (22) and guinea pig lung (23) induced by LTD$_4$. Thus, the PI hydrolysis precedes most, if not all, of the measurable pharmacological effects induced by LTD$_4$ in guinea pig lung, suggesting that the LTD$_4$ receptor-mediated cyclooxygenase-dependent and -independent effects occur subsequent to the generation of the PI hydrolysis products.

Our studies on the LTD$_4$ induction of PI hydrolysis have shown several important features. IP$_1$ accumulation induced by LTD$_4$ and analogs was highly stereoselective and specific. The pharmacological specificity of the PI hydrolysis was equivalent to those described earlier for the occupation of LTD$_4$ receptor (4), smooth muscle contraction (1, 35), and TxB$_2$ biosynthesis (23). LTD$_4$-induced PI hydrolysis was independent of cyclooxygenase metabolites of arachidonic acid. The LTD$_4$-specific receptor antagonist SKF 102922 inhibited LTD$_4$-induced PI hydrolysis and, furthermore, the potencies and intrinsic activities of PI hydrolysis induced by LTD$_4$ and analogs were com-

[2] S. Mong, H.-L. Wu, G. K. Hogaboom, M. A. Clark, and S. T. Crooke, unpublished results.

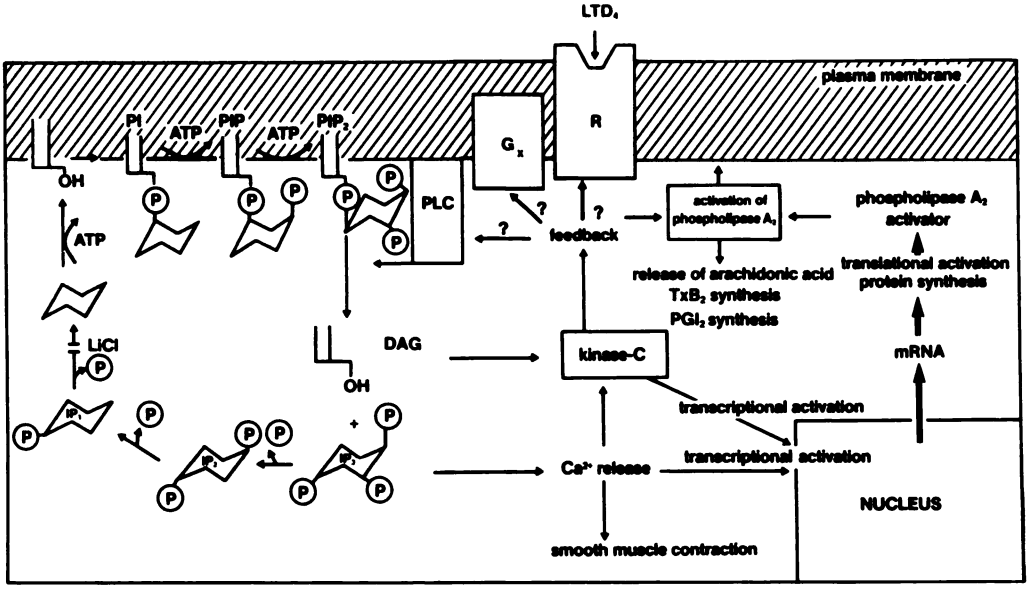

Fig. 5. A model of the cellular mechanism of action of LTD$_4$ and LTE$_4$.

parable to the LTD_4 receptor-mediated TxB_2 biosynthesis and tracheal smooth muscle contractile responses. These data strongly suggest that PI hydrolysis in guinea pig lung is mediated via the LTD_4 receptors. In addition, the correlation of the partial agonist activities of LTE_4 and des-amino-LTE_1 in the TxB_2 synthesis and PI hydrolysis response provide further support for this hypothesis.

LTD_4 receptor-mediated hydrolysis of inositol lipids may yield two major intracellular messengers, DAG and IP_3. DAG can activate C-Kinase in the presence of Ca^{2+}, and IP_3 can increase the intracellular Ca^{2+} concentration. C-Kinase and Ca^{2+}, either independently or acting synergistically, may activate many different biochemical or pharmacological effects in the cell and eventually may result in arachidonic acid release, synthesis of cyclooxygenase metabolites, and smooth muscle contraction. We therefore propose a refined model (Fig. 5) for LTD_4 receptor-mediated events. In this model, we suggest that LTD_4 receptors are coupled via a guanine nucleotide-binding protein of undetermined type (G_x) to a phospholipase C. Agonists bind to the receptors and induce an activation of phospholipase C resulting in the formation of intracellular messengers IP_3 and DAG. IP_3 and DAG may activate many intracellular processes, including Ca^{2+} mobilization and perhaps C-Kinase activation. An event subsequent to PI turnover and Ca^{2+} mobilization is the induction of transcription of a factor (or factors) that activates phospholipase A_2 (22, 27), releasing arachidonic acid in some cells and tissues, resulting in the formation of cyclooxygenase products. The cyclooxygenase products may be the final mediators of many LTD_4 effects. In other cells or tissues, other C-Kinase- or Ca^{2+}-mediated events may be responsible for the pharmacological and physiological effects of LTD_4 and other leukotrienes. This model provides a generalized molecular mechanism of action and is useful to account for the cyclooxygenase-dependent and -independent pharmacological effects of leukotrienes in lung. The detailed mechanisms of leukotrienes in cells, tissues, or animals may vary. Experimental evidence should be provided to confirm this hypothesis in each of the experimental models.

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