Identification of a Human cDNA Encoding a Functional High Affinity Lipoxin A₄ Receptor

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Summary

Lipoxin A₄ (LXA₄) triggers selective responses with human neutrophils that are pertussis toxin sensitive and binds to high affinity receptors (Kd = 0.5 ± 0.3 nM) that are modulated by stable analogues of guanosine 5'-triphosphate (GTP). Here, we characterized [11,12-3H]LXA₄ specific binding with neutrophil granule and plasma membranes, which each display high affinity binding sites (Kd = 0.7 ± 0.1 nM) that were regulated by GTPγS. Since functional LXA₄ receptors are inducible in HL-60 cells, we tested orphan cDNAs encoding 7-transmembrane region receptors cloned from these cells for their ability to bind and signal with LXA₄. Chinese hamster ovary (CHO) cells transfected with the orphan receptor cDNA (plNF114) displayed specific 3H-LXA₄ high affinity binding (1.7 nM). When displacement of LXA₄ binding with plNF114-transfected CHO cells was tested with other eicosanoids, including LXB₄, leukotriene D₄ (LTD₄), LTB₄, or prostaglandin E₂, only LTD₄ competed with LXA₄, giving a Kᵢ of 80 nM. In transfected CHO cells, LXA₄ also stimulated GTPase activity and provoked the release of esterified arachidonate, which proved to be pertussis toxin sensitive. These results indicate that plNF114 cDNA encodes a 7-transmembrane region-containing protein that displays high affinity for 3H-LXA₄ and transmits LXA₄-induced signals. Together, they suggest that the encoded protein is a candidate for a LXA₄ receptor in myeloid cells.

1 Lipoxigenase (LO)-derived eicosanoids are important lipid mediators (1). The 5-LO-derived products include leukotriene B₄ (LTB₄), a potent stimulus for phagocytic cells, and peptido-leukotrienes C₄, D₄, and E₄, which are potent bronchoconstrictors and are associated with the pathogenesis of asthma (1). Lipoxins (LX) are a newer class of bioactive LO-derived products that are generated by the interactions of either 5- and 12-LO and/or 15- and 5-LO followed by subsequent reactions (for a review see reference 2). The LXs are functionally distinct from leukotrienes and other eicosanoids and are primarily generated in human tissues during cell–cell interactions that are exemplified by leukocyte–platelet interactions (2). LXA₄ displays intriguing biological responses in several tissues (2), and with neutrophils they involve G protein–mediated signal transduction events (3–5). A LXA₄ receptor is induced in HL-60 cells upon differentiation, and it activates phospholipase D (5). LXA₄-induced lipid remodeling events are similar to those of other leukocyte stimuli (i.e., LTB₄ and FMLP), but specifically differ by triggering only selective responses (in the nanomolar range) without initiating aggregation or degranulation (3). In addition, LXA₄ modulates and inhibits neutrophil responses elicited by receptor-mediated stimuli including FMLP (6–8) and LTB₄ in vivo (9). Thus, LXA₄ has a selective profile of action of interest in multicellular responses.

Albeit some structural similarities between LXA₄ and LTB₄ exist (e.g., are identical between C1-C5), LXA₄ receptor interactions in neutrophils involve binding sites that are not recognized by LTB₄ (3, 4). Peptido-leukotrienes, the actions of which are antagonized by LXA₄ in vivo and in vitro (10–12), compete with 3H-LXA₄ binding in neutrophils (4). Interactions between LXA₄ and LTD₄ were also noted with human endothelial cells, where a putative LTD₄ receptor binds 3H-LXA₄ with an affinity ~20-fold lower than that observed with neutrophils (5). With the exception of endothelial and mesangial cells, where LXA₄ is blocked by a LTD₄ receptor antagonist (SKF104353), LXA₄ specific

References

1 Abberations used in this paper: AMP-PNP, 5'-adenylylimidodiphosphate; CHO, Chinese hamster ovary cells; DPBS/PBS, Dulbecco's PBS; PBS 2- without divalent cations; FPR, formyl peptide receptor; GTPγS, guanosine 5'-O-(3-thiotriphosphate); leukotriene B₄ (LTB₄), 5S,12R-dihydroxy-6,14-cis-8,10-trans-dihydroxyeicosatetraenoic acid; leukotriene D₄ (LTD₄), 5S, hydroxy-6R-(S-cysteinyl-glycyl)-7,9-trans-11,14-cis-eicosatetraenoic acid; lipoxin A₄ (LXA₄), 5S,6R,15S-trihydroxy-7,9,13-trans-11-cis-eicosatetraenoic acid; lipoxin B₄ (LXB₄), 5S,14R,15S-trihydroxy-6,10,12-trans-8-cis-eicosatetraenoic acid; LO, lipoxigenase; PGE₂, 9-oxo-11α,15S-dihydroxy-5-cis-13-trans-prostaglandino acid; PT, Pertussis toxin holotoxin.

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binding is not observed with other common cell types including red cells, platelets, or lymphocytic cell lines (5, 10). Thus, in addition to binding with specific leukocyte LXA₄ receptors, it appears that LXA₄ can also interact with LTD₄ receptor sites which, in certain tissues, may represent a subset or subtype of LTD₄ receptors (i.e., LTD₄/LXA₄r).

These results, and the finding that functional LXA₄ receptors are inducible in promyelocytic lineages (HL-60 cells) (5), as is the case for other receptors (13-15), prompted us to investigate whether orphan 7-transmembrane receptor cDNAs recently isolated from myeloid lineages (16, 17) could encode for LXA₄ receptors or binding proteins.

Materials and Methods

Materials. Tritiated LXA₄ ([11,12-3H]-LXA₄) (40 Ci/mmol) was obtained from a custom catalytic hydrogenation of 11,12-acetylenic LXA₄ methyl ester performed by New England Nuclear (NEN), DuPont Co. (Boston, MA) and purified as in references 4 and 5. [γ-32P]GTP (30 Ci/mmol), [H]-FMLP (53.6 Ci/mmol), and [H]-arachidonate (100 Ci/mmol) were also from NEN, DuPont Co. N-(p-aminocinnamoyl)anthranilic acid and synthetic LXA₄, LXB₄, LTD₄ and LTB₄, prostaglandin E₂ (PGE₂), and LTβ, were obtained from Cascade Biochem Ltd. (Reading, Berkshire, England). Pertussis toxin (holotoxin) (PT) was purchased from List Biological Laboratories, Inc. (Campbell, CA). Dulbecco’s PBS (DPBS) and cell culture reagents were from Whittaker M. A. Bioproducts (Walkersville, MD), and plasticware was from Marsh Biomedical Products, Inc. (Rochester, NY). GTPγS, FMLP, and 4-p-bromophenacyl bromide were from Sigma Chemical Co. (St. Louis, MO), and silicon oil was from Huls America (Bristol, PA).

Neutrophil Isolation and Subcellular Fractionation. Human neutrophils were obtained by the modified Boyum method (18) from fresh heparinized blood after venipuncture of healthy normal volunteers. Cell suspensions in PBS were monitored for cell number and viability. To obtain plasma membrane and granule-enriched fractions, neutrophil fractionation was carried out as in reference 4. Briefly, after suspension in HBSS containing 0.1% albumin and disopropyl fluorophosphate (5 mM) for 30 min at 37°C, neutrophils were adjusted to 4 x 10⁶ cells/ml of HBSS with added MgCl₂ (2.5 mM). Next, cells kept at 4°C throughout the procedure were sonicated four times using an ice-cold probe (100 W, 15 s). Crude sonicates were supplemented with EDTA (2.5 mM final) and centrifugally centrifuged at 150 (10 min), 18,000 (30 min), and 100,000 g (1 h). Granule- and plasma membrane–enriched fractions were recovered in the 18,000 and 100,000 g pellets, respectively, as indicated by the monitoring of marker enzymes (cf. 4).

Expression of Orphan Receptors in Chinese Hamster Ovary Cells. Cells were grown in 100-mm petri dishes incubated in a 5% CO₂ atmosphere at 37°C in αMEM supplemented with adenine, deoxyadenosine, and thymidine in addition to serum and antibiotics. Chinese hamster ovary (CHO) cells were transfected using the DEAE-dextran procedure (19) for transient expression of plasmid DNAs for orphan receptors (i.e., denoted pLibN14, pLibF154) and formyl peptide receptor (FPR) (16, 17). The sequences of cDNA for pLibN14 (17) and pLibF154 (16) and deduced amino acid sequences have been reported. 48 h after transfection (10 μg DNA/dish), cells were harvested and centrifuged at 100 g (1 min) and frozen (dry ice-acetone bath) fol-

lowed by thawing at room temperature. All cell preparations were resuspended in PBS²⁻ before being used in binding assays.

Ligand Binding Assays. [3H]LXA₄ binding was performed as in (4, 5). Briefly, centrifugation at high speed (30 s, 12,000 g) through silicon oil was used with intact cell suspensions (2 x 10⁶ cells/0.5 ml aliquots), and filtration through microspin filter units (0.45 μm cellulose acetate; PGC Scientifics, Gaithersburg, MD) was used with both subcellular fractions and permeabilized cell suspensions. Incubations were performed at 4°C for the indicated duration in the presence or absence of excess unlabeled homolo-
genic ligands (1-3 log excess) to determine total and specific binding. Both pellets and filters were next resuspended in scintillation cocktail and radioactivity determined by a Wallac 1409 β-counter (Pharmacal-Wallac Oy, Tarkku, Finland). Results obtained were ana-
yzed with the Ligand program ( Biosoft Elsevier).

GTPase Assays. GTPase activity was determined in transfected CHO cells by a modification of the method described by Cassel and Selinger (20). CHO cells were harvested 72 h after transfection with pLibN14 or a mock vector. Adherent cells were detached from culture plates using PBS²⁻ (5 mM EDTA). Cells were washed with PBS and resuspended (10⁶ cells/ml) in ice-cold buffer (containing 138 mM KCl), 25 mM Tris-HClI, 1 mM EGTA, and 1 mM MgCl₂, 0.4 mg/ml creatine kinase, 5 mM phosphocreatine, 0.8 mM N-5'-adenylylimidodiphosphate [AMP-PNP], 0.1 mM ATP, 0.1 μM GTP, and 0.5 μM [γ-32P]GTP. Cells in suspension were transfected to electroporation cuvettes kept in ice and were per-
memabilized with one discharge of 875 V/cm from a 250 μF capacitor (cat. no. 165-2098; Bio-Radiations, Bio-Rad Laboratories, Rich-
don, CA). Uptake of 32P by cells was monitored and averaged <12% of total [γ-32P]GTP in solution. Samples were transferred to a 30°C water bath and incubated for the indicated times in the presence of agonists. Aliquots (100 μl) were removed at indicated times and added to 750 μl of ice-cold NaH₂PO₄, 10 mM, pH 2.0, containing 5% (wt/vol) charcoal and 0.1% Triton X-100, and vortexed. After incubating on ice for at least 15 min, samples were centrifuged at 3,000 g for 5 min at 3,000 g, and radioactivity in 400 μl of supernatant was determined by liquid scintillation counting. The rate of agonist-dependent GTPase activity was determined by sub-
tracting the amount of free 32P, at time zero from values at design-
ated intervals and calculating individual slopes. The rates of GTP hydrolysis in pLibN14 CHO cells were equivalent to mock trans-
fected cells with vehicle and PGE₂, whereas, with LXA₄, the rates in pLibN14 cells were more than three times higher. Vehicle added to mock transfected CHO cells typically gave values of 0.06 pmol/min/10⁶ cells.

Release of Esterified H-Arachidonate from CHO Cells. 48 h after transfection, CHO cells were incubated for 3 h at 37°C in complete αMEM (5 ml/dish) containing 0.1 μCi of [H]-arachidonate/ml. Esterification of [H]-arachidonate represented 71.0 ± 12.5% of added material. Phospholipid class distribution was resolved by two-dimensional TLC (3) (with ~81.1% of esterified [H]-arachidonate in the total phospholipid fractions), and the individual classes were phosphatidylethanolamine/phosphatidylinositol, 27%; phosphatidylcholine, 24.2%; and phosphatidylethanolamine, 29.9% of the es-
terified label. Next, cells were washed twice and resuspended in PBS²⁻ (5 mM EDTA). After resuspension in PBS²⁻ (2 x 10⁶ cells/ml), cells transfected with either FPR- or pLibN14-carrying vectors were incubated at 37°C with selected putative ligand. LXA₄-induced (10⁻⁹ M) [H]-arachidonate release from pLibN14 transfected CHO cells was 2.4-11.3% of incorporated [H]-arachid-
one. This was equivalent to 24.4-39.7% of the maximal release

obtained with mock and pLibN14 transfected CHO cells was 2.4-11.3% of incorporated [H]-arachidonate. This was equivalent to 24.4-39.7% of the maximal release obtained using the calcium ionophore A23187 (2.5 μM). Background values obtained with mock and pLibN14 transfected CHO
cells exposed to vehicle (EtOH 0.1%) were \( \sim 1.0 \% \) of the esterified \(^3\text{H}\)-arachidonate. In parallel determinations, cells were exposed to two commonly used phospholipase A\(_2\) inhibitors, 4\,p-bromophenacyl bromide or \( N\,-(\text{p-amylcinnamoyl})\text{anthranilic acid, 10 min before agonist additions. At indicated time intervals, aliquots (1 ml) were layered on a cushion of silicon and centrifuged (30 s, 12,000 g). The radiolabel content of individual supernatants (750-\mu l aliquots) was determined (21).

Northern Blot Analysis. Multiple human tissue Northern blots (Clontech, Palo Alto, CA) containing \( \sim 2.0 \mu g/\text{lane poly(A} {+}\text{)} RNA were probed using a \( \text{pLNI14}\) open reading frame that had been labeled with \( {\alpha}\text{-\text{p}}\text{P}\text{dCTP by random priming, as described (13). Hybridization was done at 42°C for 18 h. After washing (as described in legend to Fig. 6), the blot was exposed to X-Omat AR5 film at \(-70°C\) overnight with an intensifying screen, after which they were stripped and reprobed with a \( \text{p}\text{P}\text{-labeled human actin (Clontech) probe using identical conditions.}

Constructs. FLAG-FPR and FLAG-pINF114 were constructed employing an octapeptide (DYKDDDDK) encoding sequence termed FLAG as in (22). All constructs were inserted into the EcoRI site of pRe/CMV that had been mutated so as to have a single EcoRI site located within the cloning site (22).

Results

Specific binding of [11,12-\(^3\text{H}\)]LXA\(_4\) in human neutrophils gives a \( K_a \) of 0.5 \pm 0.3 nM and is distributed in plasma membrane- (\( \sim 42\% \)), granule- (34.5%), and nuclear- (23.3%) enriched fractions. \(^3\text{H}\)-LXA\(_4\) binding with intact neutrophils and plasma membrane is modulated by GTP stable analogs (4). To determine whether the interaction of \(^3\text{H}\)-LXA\(_4\) with different subcellular fractions gives similar characteristics, [11,12-\(^3\text{H}\)]LXA\(_4\) specific binding with granule membrane was compared with that observed with plasma membrane-enriched fractions. Isothermic binding was performed at 4°C with [11,12-\(^3\text{H}\)]LXA\(_4\) (0.1-15 nM) in the presence or absence of a 3 log excess of unlabeled LXA\(_4\). Results from Scatchard analyses show that [11,12-\(^3\text{H}\)]LXA\(_4\) binds neutrophil granule membrane-enriched fractions with comparable \( K_a \) (0.8 nM) but larger \( B_{\text{max}} \) (4.1 \times 10^{-11} M) than with plasma membranes (\( K_a \) 0.7 nM, \( B_{\text{max}} \) 2.1 \times 10^{-11} M) (Fig. 1). In addition, exposure of granule membrane fractions to GTPyS, a stable analog of GTP, reduced the \( B_{\text{max}} \) (2.7 \times 10^{-11} M) to values similar to those observed for [11,12-\(^3\text{H}\)]LXA\(_4\) specific binding with plasma membranes (Fig. 1). These findings suggest that [11,12-\(^3\text{H}\)]LXA\(_4\) binding to intact neutrophils and granule membrane-associated fractions involves one class of binding sites. Therefore, a higher abundance of LXA\(_4\) receptors is likely for total cellular copies than that calculated solely on the basis of cell surface receptor expression (\( \sim 1,800/\text{cell in} \) neutrophils, cf. 4). This granule membrane-associated fraction of LXA\(_4\) binding sites may represent a reserve store as documented for other neutrophil receptors (23).

Since LXA\(_4\) specific and functional receptors present in neutrophils are induced upon differentiation in HL-60 cell (5), we assessed orphan cDNAs recently cloned from libraries derived from differentiated myeloid lineages. Several orphan receptor cDNAs have been isolated that are members of the 7-transmembrane domain G protein-associated receptor family and also display general sequence homology to the FMLP receptor (16, 17). High affinity ligands have not been identified for these sequence-related putative receptors, and they were coined FMLP homologous orphan receptors (16) or related FMLP receptors (RFP, 17). CHO cells were transfected with these cDNAs and tested for their ability to bind [11,12-\(^3\text{H}\)]LXA\(_4\) (48 h after transfection). As shown in Fig. 2 (left), CHO cells transfected with pLNI14 display specific binding with LXA\(_4\) \( (K_a \sim 5 \text{nM} \) and, for purposes of direct comparison, those transfected with FPR \( (right) \) displayed high affinity binding for its ligand FMLP \( (K_a \sim 5 \text{nM}) \). Nonspecific binding was comparable with both ligands (Fig. 2). [11,12-\(^3\text{H}\)]LXA\(_4\) did not display high affinity binding with several other related orphan 7 transmembrane region receptors transfected in CHO cells including pLNI154 (see 16, 17) (data not shown). Albeit a low abundance of \( \sim 2,000 \) LXA\(_4\) receptors per cell was observed with intact cells (Fig. 2), the receptor abundance may reflect, in part, transfection efficiency that is usually 20-30% with DEAE-dextran transfection. Also, posttranscriptional modification and/or intracellular trafficking could lead to partitioning among cellular membranes other than the plasma membrane, which may give lower receptor \( B_{\text{max}} \) with transfected cells. To test this hypothesis, we examined permeabilized cells after transfection for specific binding with both ligands. Experiments with [11,12-\(^3\text{H}\)]FMLP \( (n = 4) \) and [11,12-\(^3\text{H}\)]LXA\(_4\) \( (n = 7) \) indicate that \( K_a \) values were unaltered after permeabilization of transfected cells (intact 5.6 nM versus permeabilized 5.0 nM for FMLP; and intact 6.5 nM versus permeabilized 7.3 nM for LXA\(_4\)). In contrast, the
B$_{\text{max}}$ values obtained with permeabilized cells were about five- to sevenfold higher than those with intact cells (2.3 x 10$^{-10}$ M vs. 4.9 x 10$^{-11}$ M for FMLP and 3.6 x 10$^{-10}$ M vs. 5.2 x 10$^{-11}$ M for LXA$_4$) (see Fig. 2, bottom). Next, pNF114 cDNA was modified at the corresponding NH$_2$-terminus sequence to contain a FLAG peptide-encoding sequence (22). After transfection, FLAG-pNF114-expressing cells were harvested (48 h) and fractionated, and individual enriched fractions for nuclei, organelle, and plasma membrane were electrophoresed by polyacrylamide gel. Western blot analysis with a mouse mAb recognizing the FLAG peptide portion showed the distribution of pNF114 construct associated with plasma membranes as ~35%, organelle pellet ~52%, and nuclei ~13%.

Given the higher B$_{\text{max}}$ obtained with permeabilized cell suspensions and the finding that >50% of pNF114-encoded protein was associated with intracellular fractions, permeabilized transfected CHO cells were used to further charac-

Table 1. Eicosanoid Competition of $^3$H-LXA$_4$ Binding with pNF114-transfected CHO Cells

| Compound | $K_i$ (nM) |
|----------|------------|
| LXA$_4$  | 5.6        |
| LXB$_4$  | NS*        |
| LTD$_4$  | 79.9       |
| LTB$_4$  | NS*        |
| PGE$_2$  | NS*        |

After transfection with pNF114 (48 h), intact CHO cells were harvested in PBS$^+$ (5 mM EDTA), washed twice in PBS$^+$ and adjusted to 4-10 x 10$^7$ cells/ml. Aliquots (200 µl) were added to microcentrifuge tubes containing 800 µl of PBS$^+$ and $^3$H-LXA$_4$ (0.3 nM final) alone, or in the presence of increasing concentrations of indicated compounds (3-300 nM). All solutions were kept at 4°C. Cells were incubated for 5 min followed by layering aliquots (0.5 ml) from each experimental point on top of a silicon oil cushion (density = 0.1013). Samples were centrifuged and pellet radioactivity measured by scintillation counting. $K_i$ values reported are obtained from evaluating displacement curves via the Ligand program. Results are the means of three separate experiments.

* Ligand program analysis of data failed linear regression for competition in the concentration range tested (3–300 nM).
were determined by calculating the linear regression of 32p release in the
Figure 4. Structure-function relationship of ligand-induced GTPase ac-
plNF114-transfected CHO cells (+ SEM, n = 3). (*) Significantly higher
results are from one experiment (d = 2) representative of three separate
initial 30 s after ligand addition (10^-7 M) to electropermeabilized,
tivity in plNFl14-transfected CHO cells. Rates of [3,-32p]GTP hydrolysis
activity of transfected CHO cells minus activity obtained with mock trans-
binding of 3H-LXA4 (1-15 nM, 5 min at 4~ to plNF114
letted CHO cells, expressed as percent LXA4-indueed GTPase activity;
selective in that neither LTB4 nor LXB4 competes for LXA4
among the compounds examined, only
stimulating GTPase activity is similar to that obtained for
stimulating 3H-LXA4 binding activity. Time course of label association at 4°C gave results similar to those
obtained with neutrophils, and equilibrium binding was obtained within 5 min (data not shown). Analysis of isothermic binding of 3H-LXA4 (1-15 nM, 5 min at 4°C) to plNF114 transfected CHO cells gave a Kd of 1.7 ± 0.8 nM and a Bmax of 3.8 ± 0.5 x 10^-10 M (Fig. 3). These values are in agreement
with the Kd values obtained for both neutrophil and retinoic acid differentiated HL-60 cells (5).
LXA4 binding with neutrophils and HL-60 cells is stereo-
selective in that neither LTB4 nor LXB4 competes for LXA4 binding (4). Structural requirements for 3H-LXA4 binding with plNF114-transfected CHO cells were examined with LXB4, LTD4, LTb4, or PGE2. Only LTD4 proved effective in displacing tritiated LXA4 binding with plNF114 trans-
fected CHO cells (Ki ~80 nM, Table 1). Results are consistent with those obtained with neutrophils (4, 5) where,
among the compounds examined, only LTD4 competed with 3H-LXA4 binding (Ki ~70 nM). These findings indicated that specific LXA4 binding with plNF114-transfected CHO cells is selective, prompting the assessment of transmembrane signaling.

Does plNF114 Transduce LXA4 Signals? As shown in Fig.
4, after transfection of CHO cells with plNF114, LXA4 specifically induced GTPase activity. The maximum rate
triggered by LXA4 was reached within 30 s and proved to be concentration dependent (data not shown). LXB4 and PGE2 gave significantly lower levels of GTPase activity than LXA4. LTD4 also stimulated GTPase activity in plNF114 transfectants when activity associated with mock transfectants assayed in parallel was subtracted (Fig. 4, inset). These results suggest that the structure-function relationship for
Figure 5. Time course of LXA4-induced release of 3H-arachidonate from transfected CHO cells. After labeling with 3H-C20:4 (37°C, 3 h), transfected CHO cells were kept at 37°C and exposed to either LXA4 (10^-9 M; mock transfected [●]; plNF114 transfect [○]) or FMLP (5 x 10^-7 M, PPR transfact [□]). At the indicated intervals, aliquots (2 x 10^6 cells) were layered onto a cushion of silicon oil and centrifuged. The 3H-arachidonic acid content released into the supernatants was determined. Parallel determinations were performed with ligands and vehicle in trans-
ected and mock transfected CHO cells. Values obtained with vehicle alone (EtOH, 0.1% final) in mock and transfected CHO cells were subtracted from those obtained with respective ligands. Results are representative of three separate experiments with duplicate determinations. (Inset) The profile as percentage of maximal release. Results are the mean ± SEM of three separate experiments.
Table 2. Impact of PT Treatment on LXA4-induced Arachidonate Release in Transfected CHO Cells

| PT (ng/ml) | Mock CHO cells | pINF114 CHO cells |
|------------|----------------|-------------------|
| A<sub>23187</sub> (2.5 μM) | LXA4 (10<sup>-9</sup> M) | A<sub>23187</sub> (2.5 μM) | LXA4 - (percent inhibition) (10<sup>-9</sup> M) |
| 0          | 100.0*         | 0.0               | 100.0* | 36.5 - (0.0%) |
| 10         | 100.0          | 0.0               | 100.0  | 3.7 - (89.9%) |
| 100        | 100.0          | 0.0               | 100.0  | 0.0 - (100.0%) |

After <sup>3</sup>H-arachidonate labeling (37°C, 3 h), cells were exposed to increasing concentrations (0–10–100 ng/ml) of PT (37°C, 6 h). At 5 min after additions, <sup>3</sup>H-arachidonate release was measured as described in the legend to Fig. 5. Results are the average of duplicate determinations from a representative of four separate experiments.

| Data are reported as percentages obtained with each addition relative to A<sub>23187</sub> (2.5 μM) stimulation after subtraction of values obtained with vehicle alone.

Discussion

The present results establish that the interactions of LXA4 with cells expressing pINF114, a previously isolated orphan receptor of the 7-transmembrane region class (16, 17), meet the criteria commensurate with ligand–receptor interactions and transmembrane signaling (25). Namely, transfected cells display specific binding with <sup>3</sup>H-LXA4 that is displaced with excess unlabeled ligand (Fig. 2). Binding was selective for LXA4 in that other eicosanoids including LXB4, LTB4, and PGE2 did not displace LXA4 specific binding (Table 1). CHO cells possess the components required for transmembrane signaling with activation of phospholipases after expressing rogue 7-transmembrane spanning receptors and addition of appropriate ligands (21, 26). In the present experiments, CHO cells transfected with pINF114 transduced signal in response to LXA4, by both activating GTPase (Fig. 4) and releasing arachidonic acid (Fig. 5). Thus, the product encoded by pINF114 specifically binds <sup>3</sup>H-LXA4 and transduces signals with LXA4, indicating that pINF114 is a candidate for a functional LXA4 receptor. This does not, however, preclude the existence of other LXA4 binding sites or species of interest (i.e., ~1.8–2.0 kb) were associated with human lung, followed by placenta (Fig. 6), tissues known to have a relatively high degree of phagocytic cell infiltrates. Other bands were observed that cross-hybridized with this receptor (Fig. 6). The 1.0-kb band is unknown. The 1.4-kb band corresponds to the FMLP receptor size (24). The 2.4–2.6-kb band is also FMLP-like, but distinct from LXA4 receptor. Bands of higher molecular size may represent presplicing forms of these receptors. Both the FMLP and the LXA4 receptor genes possess a 5.0-kb intron (24) that is spliced to generate the open reading frame.

Figure 6. Tissue distribution of LXA4 receptor mRNA. Multiple human tissue blot was probed with <sup>32</sup>P-pINF114 and <sup>32</sup>P-actin. (A) pINF114 probe. (B) Actin probe. After hybridization, blot was washed at room temperature (2× SSC, 0.1% SDS) for 40 min followed by a second (40 min) wash in 0.1× SSC, 0.1% SDS at 50°C.
other receptors in addition to the product of plNF114 that can transduce LXA4 signals. To date, neither LTD4 receptors nor other receptors for LO-derived products have been cloned. LTD4 did displace 3H-LXA4 from transfected CHO cells (Ki, 79.9 nM) and gave ~65–70% of the GTPase activity when compared with equimolar amounts of LXA4 (inset, Fig. 3). LTD4 competes for 3H-LXA4 binding (5), and, in certain tissues, LXA4 and peptide-leukotrienes appear to share a common site of action (5, 10–12). Thus, the present findings suggest that certain types or subclasses of peptide-leukotriene receptors may be structurally related to the plNF114 encoded receptor.

plNF114 and plNF154 were originally sequenced as FMLP-related receptors and coined "related formyl peptide receptors" (RFP) (16, 17). FPR transduces signal with FMLP (22), and FMLP clearly activates leukocytes; however, the endogenous ligands for these receptors have been questioned earlier (23). The present results indicate that at least one FPR receptor-related sequence is a receptor for a lipid-derived ligand. In this regard, it is of interest that the plNF114 gene has been mapped to chromosome 19, as have the genes for the complement component 5a receptor (16) and the recently identified thromboxane A2 receptor gene (28). The present results will now permit further analysis of LX site(s) of action, the mechanism underlying LXA4 responses such as inhibition of neutrophil function (6–9), and elucidation of components involved in LX signal transduction.

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