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 ($K_d = 0.5 \pm 0.3 \text{ nM}$) 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 ($K_d = 0.7 \pm 0.1 \text{ nM}$) that were regulated by GTP$\gamma$S. 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 $K_i$ 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.

Lipoxygenase (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 $^3$H-LXA4 binding in neutrophils (4). Interactions between LXA4 and LTB4 were also noted with human endothelial cells, where a putative LTB4 receptor binds $^3$H-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 LTB4 receptor antagonist (SKF104353), LXA4 specific

Abbreviations 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; GTP$\gamma$S, 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-glycynyl)-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, lipoxygenase; LTD4, 9-oxy-11o~,15S-dihydroxy-5-cis-13-trans-prostadienoic acid; PT, Pertussis toxin holotoxin.
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/LXA₄+r).

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), [3H]FMLP (53.6 Ci/mmol), and [3H]-arachidonate (100 Ci/mmol) were also from NEN, DuPont Co. N-(p-amylcinnamoyl)anthranilic acid and synthetic LXA₄, LXB₄, LTD₄, 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 (DBPS) and cell culture reagents were from Whittaker M. A. Bioproducts (Walkersville, MD), and plasticware was from Marsh Biomedical Products, Inc. (Rochester, NY). GTPγS, FMPL, and 4-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 Böyum 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 disisopropyl fluorophosphate (5 mM) for 30 min at 37°C, neutrophils were adjusted to 4 × 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 sequentially 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 adenosine, 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 pINF114, pINF154) and formyl peptide receptor (FPR) (16, 17). The sequences of cDNA for pINF114 (17) and pINF154 (16) and deduced amino acid sequences have been reported. 48 h after transfection (10 μg DNA/dish), cells were detached using PBS + containing EDTA (5 mM) (3 min, 20°C) and centrifuged (200 g, 10 min) after addition of complete αMEM (2:1 vol/vol). CHO cell permeabilization was achieved with two cycles of freezing (dry ice-acetone bath) followed by thawing at room temperature. All cell preparations were resuspended in PBS + 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 (2-5 × 10⁶ cell/0.5-ml aliquots), and filtration through microspin filter units (0.45 μm cellulose acetate; PGC Sciences, 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 homolog or hetero-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 (Pharmacia-Wallac Oy, Tarkku, Finland). Results obtained were analyzed 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 pINF114 or a mock vector. Adherent cells were detached from culture plates using PBS + (5 mM EDTA). Cells were washed with PBS and resuspended (10⁶ cells/ml in ice-cold buffer (containing 138 mM KCl, 25 mM Tris-HCl, 1 mM EDTA, 1 mM MgCl₂, 0.4 mg/ml creatine kinase, 5 mM phosphocreatine, 0.8 mM 5'-adenylylimidodiphosphate [AMP-PNP], 0.1 mM ATP, 0.1 μM GTP, and 0.5 μM [γ-32P]GTP). Cells in suspension were transferred to electroporation cuvettes kept in ice and were permeabilized with one discharge of 875 V/cm from a 250 μF capacitor (cat. no. 165-2098; Bio-Radiations, Bio-Rad Laboratories, Richmond, 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 for 3 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 subtracting the amount of free [32P] at time zero from values at designated intervals and calculating individual slopes. The rates of GTP hydrolysis in pINF114 CHO cells were equivalent to mock transfected cells with vehicle and PGE₂, whereas, with LXA₄, the rates in pINF114 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 1H-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 [1H]-arachidonate/mL. Esterification of [1H]-arachidonate represented 71.0 ± 12.5% of added material. Phospholipid class distribution was resolved by two-dimensional TLC (3) (with ~81.1% of esterified [1H]-arachidonate in the total phospholipid fractions), and the individual classes were phosphatidylserine/phosphatidylinositol, 27%; phosphatidylcholine, 24.2%; and phosphatidylethanolamine, 29.9% of the esterified label. Next, cells were washed twice and resuspended in PBS + (5 mM EDTA). After resuspension in PBS + (2 × 10⁶ cells/ml), cells transfected with either FPR- or pINF114-carrying vectors were incubated at 37°C with selected putative ligand. LXA₄-induced (10⁻⁹ M) [1H]-arachidonate release from pINF114 transfected CHO cells was 2.4-11.3% of incorporated [1H]-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 pINF114 transfected CHO...
cells exposed to vehicle (EtOH 0.1%) were ~1.0% of the esterified 
3H-arachidonate. In parallel determinations, cells were exposed to 
two commonly used phospholipase A2 inhibitors, 4-p-bromophenyl-
acyl bromide or N-(p-aminomethyl) anthranilic acid, 10 min be-
fore agonist additions. At indicated time intervals, aliquots (1 ml) 
were layered on a cushion of silicon and centrifuged (30 s, 12,000 g). 
The radio-label content of individual supernatants (750-μl aliquots) 
was determined (21).

Northern Blot Analysis. Multiple human tissue Northern blots 
(Clontech, Palo Alto, CA) containing ~2.0 μg/lane poly(A+) 
RNA were probed using a plNF114 open reading frame that had 
been labeled with [α-32P]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 32P-labeled human 
actin (Clontech) probe using identical conditions.

Constructs. FLAG-FPR and FLAG-plNF114 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 [11,12-3H]LXA4 in human neutrophils 
gives a Kd of 0.5 ± 0.3 nM and is distributed in plasma membra-
ne (~42%), granule- (34.5%), and nuclear- (23.3%) 
enriched fractions. 3H-LXA4 binding with intact neutrophils 
and plasma membrane is modulated by GTP stable analogs 
(4). To determine whether the interaction of 3H-LXA4 with 
different subcellular fractions gives similar characteristics, 
[11,12-3H]LXA4 specific binding with granule membrane 
was compared with that observed with plasma mem-
brane-enriched fractions. Isothermic binding was performed 
at 4°C with 3H-LXA4 (0.1–15 nM) in the presence or ab-
ence of a 3 log excess of unlabeled LXA4. Results from 
Scatchard analyses show that 3H-LXA4 binding to neutrophil 
granule membrane-enriched fractions with comparable Kd 
(0.8 nM) but larger Bmax (4.1 × 10^{-11} M) than with plasma 
membranes (Kd 0.7 nM, Bmax 2.1 × 10^{-11} M) (Fig. 1). In 
addition, exposure of granule membrane fractions to GTPγS, 
a stable analog of GTP, reduced the Bmax (2.7 × 10^{-11} M) 
to values similar to those observed for 3H-LXA4 specific 
binding with plasma membranes (Fig. 1). These findings sug-
gest that 3H-LXA4 binding to intact neutrophils and 
granule membrane-associated fractions involves one class 
of binding sites. Therefore, a higher abundance of LXA4 re-
ceptors is likely for total cellular copies than that calculated 
solely on the basis of cell surface receptor expression (~1,800/
cell in neutrophils, cf. 4). This granule membrane–associated 
fraction of LXA4 binding sites may represent a reserve store 
as documented for other neutrophil receptors (23).

Since LXA4 specific and functional receptors present in 
neutrophils are induced upon differentiation in HL-60 cells 
(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–coupled receptor family 
and also display general sequence homology to the FMLP

Figure 1. Scatchard plots of 3H-LXA4 binding with isolated neutro-
ophil granule and plasma membranes: modulation by GTPγS. Neutrophil 
subcellular fractions were obtained as in the Materials and Methods. In-
creasing concentrations of 3H-LXA4 (0.3–15 nM) with or without a 3 
log order of magnitude excess of unlabeled LXA4 were added to isolated 
granule membranes or plasma membranes (50 μg protein/determination), 
and specific binding was determined (4°C, 10 min). Parallel experiments 
with granule membranes were performed after incubation with GTPγS 
(20 μM, 3 min). Results represent the average values of duplicate determi-
nations obtained with neutrophils from two separate donors.
B<sub>max</sub> values obtained with permeabilized cells were about five- to sevenfold higher than those with intact cells (2.3 x 10<sup>-11</sup> M vs. 4.9 x 10<sup>-11</sup> M for FMLP and 3.6 x 10<sup>-10</sup> M versus 5.2 x 10<sup>-11</sup> M for LXA<sub>4</sub>) (see Fig. 2, bottom). Next, plNF114 cDNA was modified at the corresponding NH<sub>2</sub>-terminus sequence to contain a FLAG peptide-encoding sequence (22). After transfection, FLAG-plNF114-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 plNF114 construct associated with plasma membranes as ~35%, organelle pellet ~52%, and nuclei ~13%.

Given the higher B<sub>max</sub> obtained with permeabilized cell suspensions and the finding that >50% of plNF114-encoded protein was associated with intracellular fractions, permeabilized transfected CHO cells were used to further charac-

**Figure 3.** Scatchard plot of 3H-LXA<sub>4</sub> binding to plNF114-transfected CHO cells. plNF114-transfected CHO cells (10<sup>7</sup> cells/0.5 ml) were permeabilized and incubated (4°C, 10 min) with an increasing concentration of 3H-LXA<sub>4</sub> (0.3-15 nM) with or without 3 log order excess of unlabeled LXA<sub>4</sub> (n = 8). Results are from computer-assisted analysis of isothermic binding data analyzed using the Ligand program.

Table 1. Eicosanoid Competition of 3H-LXA<sub>4</sub> Binding with plNF114-transfected CHO Cells

<table>
<thead>
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<th>Compound</th>
<th>K&lt;sub&gt;i&lt;/sub&gt; (nM)</th>
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<tr>
<td>LXA&lt;sub&gt;4&lt;/sub&gt;</td>
<td>5.6</td>
</tr>
<tr>
<td>LXB&lt;sub&gt;4&lt;/sub&gt;</td>
<td>NS*</td>
</tr>
<tr>
<td>LTD&lt;sub&gt;4&lt;/sub&gt;</td>
<td>79.9</td>
</tr>
<tr>
<td>LTB&lt;sub&gt;4&lt;/sub&gt;</td>
<td>NS*</td>
</tr>
<tr>
<td>PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>NS*</td>
</tr>
</tbody>
</table>

After transfection with plNF114 (48 h), intact CHO cells were harvested in PBS<sup>−</sup> (5 mM EDTA), washed twice in PBS<sup>+</sup> and adjusted to 4-10 x 10<sup>7</sup> cells/ml. Aliquots (200 µl) were added to microcentrifuge tubes containing 800 µl of PBS<sup>+</sup> and 3H-LXA<sub>4</sub> (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 = 1.013). Samples were centrifuged and pellet radioactivity measured by scintillation counting. K<sub>i</sub> 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 initial 30 s after ligand addition (10^-7 M) to electroporpermeabilized, pNF114-transfected CHO cells (+ SEM, n = 3). (*) Significantly higher rates are from one experiment (d = 2) representative of three separate experiments.

Figure 4. Structure-function relationship of ligand-induced GTPase activity in pNF114-transfected CHO cells. Rates of [γ-32P]GTP hydrolysis were determined by calculating the linear regression of 32p release in the initial 30 s after ligand addition (10^-7 M) to electroporpermeabilized, pNF114-transfected CHO cells (+ SEM, n = 3). (*) Significantly higher results are from one experiment (d = 2) representative of three separate experiments.

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 [■]; pNF114 transfect [□]) or FMLP (5 x 10^-7 M, PPR; transfect [●]). 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 transfected 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.

Does pNF114 Transduce LXA4 Signals? As shown in Fig. 4, after transfection of CHO cells with pNF114, 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). LXA4 and PGE2 gave significantly lower levels of GTPase activity than LXA4. LTD4 also stimulated GTPase activity in pNF114 transfectants when activity associated with mock transfectedants assayed in parallel was subtracted (Fig. 4, inset). These results suggest that the structure-function relationship for stimulating GTPase activity is similar to that obtained for 3H-LXA4 binding competition (Table 1).

LXA4 stimulates arachidonate release (2, 3), and CHO cells can mobilize arachidonate in response to ligand-specific stimulation of transfected receptors (21). Next, pNF114-CHO and mock transfected CHO cells were labeled with 3H-arachidonate and exposed to LXA4 (10^-9 M) to determine whether this receptor can stimulate release. A specific, LXA4-dependent release of esterified 3H-arachidonate was obtained with pNF114 transfected CHO cells (Fig. 5). Time course experiments showed maximal release in the 3–5 min interval with a subsequent decline. A similar profile was also observed for FMLP-induced release of esterified 3H-arachidonate with PPR transfected CHO cells, reported for purposes of direct comparison (Fig. 5 and its inset). Both LXA4 (10^-9 M) and FMLP (10^-7 M)-induced arachidonate release proved sensitive to phospholipase inhibitors N-(p-amyl-cinnamoyl)anthranilic acid (100 μM) (n = 3) and 4-p-bromophenacyl bromide (n = 1; 50 μM) (data not shown). Selective inhibition of LXA4-induced release of 3H-arachidonate from pNF114 transfected CHO cells was observed when labeled cells were exposed to PT (4–6 h, 37°C) before agonist addition (Table 2). To ensure that PT treatment did not simply impact in the agonist's temporal response, time course of 3H-arachidonate release was monitored at 0-, 5-, and 10-min intervals (data not shown). Thus, CHO cells transfected with pNF114 specifically bind and give responses with LXA4.

Preliminary studies on the distribution of mRNA for this receptor showed that the most abundant levels for the spe-
Table 2. Impact of PT Treatment on LXA₄-induced Arachidonate Release in Transfected CHO Cells

<table>
<thead>
<tr>
<th>PT (ng/ml)</th>
<th>Mock CHO cells</th>
<th>pINF114 CHO cells</th>
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<tr>
<td></td>
<td>A₂₁₃₆₇ (2.5 µM)</td>
<td>LXA₄ (10⁻⁹ M)</td>
</tr>
<tr>
<td>0</td>
<td>100.0⁺</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>100.0</td>
<td>0.0</td>
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<tr>
<td>100</td>
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After ³H-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, ³H-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 A₂₁₃₆₇ (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 ³H-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 ³H-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 crosshybridize 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.
other receptors in addition to the product of pINF114 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 pINF114 encoded receptor.

pINF114 and pINF154 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 pINF114 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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