GPR92 as a New $G_{12/13}$- and $G_q$-coupled Lysophosphatidic Acid Receptor That Increases cAMP, LPA$_5^*$

The signaling effects of lysophospholipids such as lysophosphatidic acid (LPA) are mediated by G protein-coupled receptors (GPCRs). There are currently four LPA receptors known as LPA$_{1-4}$. Genetic deletion studies have identified essential biological functions for LPA receptors in mice. However, these studies have also revealed phenotypes consistent with the existence of as yet unidentified receptors. Toward identifying new LPA receptors, we have screened collections of GPCR cDNAs using reverse transfection and cell-based assays. Here we report an interim result of one screen to identify receptors that produced LPA-dependent changes in cell shape: the orphan receptor GPR92 has properties of a new LPA receptor. Sequence analyses of human GPR92 and its mouse homolog have ~35% amino acid identity with LPA$_4$/GPR23. The same cell-based approaches that were used to identify and/or characterize LPA$_{1-4}$, particularly heterologous expression in B103 cells or RH7777 cells, were utilized and compared with known LPA receptors. Retroviral-mediated expression of epitope-tagged receptors was further combined with G protein minigenes and pharmacological intervention, along with calcium imaging and whole-cell patch clamp electrophysiology. LPA-dependent receptor internalization following exposure to LPA but not related lysosphospholipids was observed. Furthermore, LPA induced concentration-dependent activation of $G_{12/13}$ and $G_q$ and increased cAMP levels. Specific $[^3H]$LPA binding was detected in cell membranes heterologously expressing GPR92 but not control membranes. Northern blot and reverse transcriptase-PCR studies indicated a broad low level of expression in many tissues including dorsal root ganglia, as well as embryonic stem cells. These results support GPR92 as a fifth LPA receptor, LPA$_5$, which likely has distinct physiological functions in view of its expression pattern.

Lyosphosphatidic acid (LPA)$^2$ is a lysophospholipid whose functions are mediated by at least four G protein-coupled receptors (GPCRs) referred to as LPA$_{1-4}$ (1–4). These receptors couple to multiple G proteins, particularly $G_{12/13}$, $G_i$, and $G_q$, and possibly $G_s$. In turn, activated downstream pathways are even more diverse and include stimulation of phospholipase C and D, inhibition of adenyl cyclase (5, 6), and stimulation of small GTPases, mitogen-activated protein kinases, and phosphoinositide 3-kinase (7, 8). Receptor-mediated actions of LPA have important influences on cell survival, cytoskeletal remodeling, cell migration, and cell proliferation (5, 9–11).

LPA signaling via GPCRs has physiological consequences that have been revealed through gene deletion studies of most of the LPA receptors (12, 13). This approach has identified roles in normal organismal development, nervous system development, influences on brain architecture and function (12, 14, 15), female fertility and implantation (16), and the initiation of neuropathic pain (17). While essential roles for individual receptors have been observed, it has been somewhat surprising that more profound effects like early embryonic lethality have not been seen from single or even double receptor knock-outs (13), particularly in view of deletion studies for a key LPA biosynthetic enzyme, autotaxin, that produces complete embryonic lethality (18).

To identify possible new LPA receptors, we have used reverse transfection (19) in an unbiased screening approach. Reverse transfection utilizes a strategy whereby collections of cDNAs are arrayed on slides or in tissue culture wells followed by placement of a single, suitable cell line that can 1) take up and express the cDNA in question and 2) provide a reliable readout. During the initial phases of one screen to identify LPA-dependent changes in the actin cytoskeleton, a positive response was observed for the orphan GPCR known as GPR92. Here we report the initial characterization of this novel LPA receptor and describe downstream signaling pathways mediated by LPA activation of GPR92.

**EXPERIMENTAL PROCEDURES**

**Materials**—LPA (1-oleoyl-2-hydroxy-sn-glycero-3-phospho-ethanolamine) was purchased from Avanti Polar-Lipids (Alabaster, AL). Y27632 and pertussis toxin (PTX) were obtained from Calbiochem (San Diego, CA). B103 neuroblastoma cells and RH7777 hepatoma cells were from laboratory stocks as reported previously. Retrovirus expression vector (LZR5S-EGFP) and Phoenix retrovirus producer cell lines were provided by Dr. Garry P. Nolan (Stanford University, Stanford, CA). The initial GPCR collection was obtained through the IMAGE consortium and Dr. Mike Brownstein (National Institutes of Health/NIMH; image.lnl.gov) and combined with additional cDNA clones produced by standard PCR
and cDNA library screening approaches. [3H]LPA (1-oleoyl-[9,10-
3H]LPA, 47 Ci/mmol) was obtained from PerkinElmer Life Scien-
ces. Ki66425 (3-[4-(4-[1-(2-chlorophenyl)ethoxy]carbo-
nylamino)-3-methyl-5-isoxazolyl]benzylsulfonyl)propanoic acid) was a gift from Kirin Brewery Co. (Takasaki, Japan).

Protein Sequence Alignment and Construction of the Phyloge-
netic Tree—Protein sequences of GPCRs were obtained from GenBankTM and Swiss-Prot. The amino acid alignment and phylogenetic tree were assembled using the computer programs ClutalW and TreeTop.

Cell Culture and Stable Transfection—B103 cells and RH7777 cells were maintained in Dulbecco’s modified Eagle’s medium with 10% heat-inactivated fetal bovine serum (Hyclone, Logan, UT) and antibiotics. For experiments, cells were serum-starved overnight before examination. To produce stable cell lines, B103 cells were transfected with linearized HA-
tagged GPR92-pcDNA3.1 (Invitrogen) using Effectene transfection reagent (Qiagen, Valencia, CA) and stable transfectants selected using 1 mg/ml of genetin (Invitrogen).

Construction of Retroviral Vectors and Production of Retrovi-
ruses for LPA, GPR92, and G Protein Minigenes—Retrovirus for mouse LPA1 was produced as described (20). For GPR92, an epitope HA tag was introduced onto the 5’ end of the of a full-length human GPR92 cDNA by PCR using the following primers: 5’-GAATTCACCATATGACATGTTCTCCAGATACGTTCCTCAACC-3’ (forward) and 5’-GAATTTTCAGGGCCGGAATCTGC-3’ (reverse). The cDNA was amplified using the Expand High Fidelity PCR system (Roche Applied Science, Mannheim, Ger-
many). PCR products were then gel-purified using QiaexII gel extraction kit (Qiagen, Valencia, CA) and subcloned into the pGEM-T Easy T vector (Promega, Madison, WI). The cDNA insert was sequenced at the Scripps Research Institute sequencing core facility and validated sequences subsequently cloned into the EcoRI site of the LZRS-EGFP Moloney murine leukemia retroviral vector. Retrovirus was produced by transfection of the Phoenix ecotropic packaging cell line (21) using FuGENE 6 transfection reagent (Roche Diagnostics). Retroviral supernatant was harvested 48 h post-transfection, filtered through a 0.45-µm filter, aliquoted, and frozen. For G protein minigenes, Gq/11 (MGLQNLKEYNLV), Gs (MGQRMHLQK- YELL), G12 (MGLQENLDIMLMQ), and G13 (MGLHDLNKQ-
MLMQ) were constructed as described (22) and subcloned into the above retroviral vector.

Reverse Transfection of GPCR Collection—Reverse transfection method was performed as published (19). Briefly, for each cDNA, 0.3 µg of DNA (encoding GPCR and EGFP in a 10 to 1 ratio) was mixed with 5 µl of Effectene (Qiagen), then mixed with 7.5 µl of 0.05% gelatin (Sigma, Type B, St. Louis, MO) and used to coat the bottom of a multiwell plate. After drying, B103 cells were seeded for reverse transfection and EGFP-positive cells analyzed for LPA-dependent neurite retraction using a fluorescence microscope (Axio Imager D1, Carl Zeiss, Thornwood, NY).

Reverse Transcriptase-Polymerase Chain Reaction—RNA was extracted from cultured cells or tissues using TRIzol rea-
gent (Invitrogen) as described in the manual. Total RNA (5 µg) was reverse-transcribed for 50 min at 42 °C using Superscript II reverse transcriptase (Invitrogen). An equal aliquot of cDNA was used to amplify the different receptor transcripts using the following conditions: 94 °C for 30 s, 60 °C for 45 s, and 72 °C for 45 s for a total of 30–35 cycles. The primers were used as follows: GPR92, 5’-AGGAAGAGCAACCAGGAC-
CAG-3’ (forward for B103 and RH7777), 5’-AGGAAGAGCA-
ACGGATCACAG-3’ (forward for mouse tissues), and 5’-ACCACCATATGCAACAGTGGT-3’ (reverse for both cell lines and tissues); β-actin, 5’-TGGAATCCTGTGGCATCC-
TGAAC-3’ (forward) and 5’-TAAGAGCGCTCAGTAA-
CAGTCCG-3’ (reverse). A hot start of 5 min at 94 °C and a final incubation of 10 min at 72 °C were carried out for all PCR reac-
tions. The PCR products were separated by electrophoresis on a 1.2% agarose gel.

LPA Binding Assay—LPA binding was performed as described previously (23). Cells were harvested, homogenized in ice-cold binding buffer (20 mM Tris-HCl, pH 7.5) containing 1 mM EDTA and protease inhibitor mixture (Roche Diag-

nosis) and centrifuged at 2,000 rpm for 10 min at 4 °C. The super-
natant was centrifuged further at 15,000 rpm for 30 min at 4 °C. The membrane preparation (30 µg) was incubated with 20 nm [3H]LPA (1-oleoyl-[9,10-[3H]LPA, 47 Ci/mmol) in LPA binding buffer containing 0.1% fatty acid-free BSA (Sigma) and 0.5 mM CuSO4 for 30 min at room temperature, and the bound [3H]LPA was collected onto a Unifilter 96-GF/B (PerkinElmer Life Sciences). The filter was then rinsed 10 times with binding buffer containing 1% normal BSA and dried for 30 min at 50 °C. After the addition of 30 µl of MicroScint-O (PerkinElmer Life Sciences) to each well of the filter, radioactivity was measured using a microplate liquid scintillation counter. Total and non-
specific binding was evaluated in the absence and presence of 10 µM unlabeled LPA, respectively.

Membrane Fractionation and Western Blotting—Cells were homogenized on ice, using a Dounce homogenizer, in homogenization buffer (20 mM Tris buffer, pH 7.5) containing 1 mM EGTA, 1 mM EDTA, and protease inhibitor mixture (Roche Diagnostics). The homogenized sample was first cleared at 2,000 rpm for 5 min at 4 °C, and the supernatant was further centrifuged at 15,000 rpm for 90 min. The pellets were resuspended on ice in the homogenization buffer contain-
ing 1% Triton X-100. The extracted pellets were cen-
trifuged at 15,000 rpm for 20 min, and the obtained superna-
tant membrane fraction was separated under reducing, denaturing conditions on a 4–12% SDS-polyacrylamide gel (Invitrogen) and transferred to a polyvinylidene difluoride membrane (Millipore, Woburn, MA). The HA-tagged pro-
teins were detected with an anti-HA antibody (Roche Diag-
nostics), horseradish peroxidase-conjugated anti-mouse secondary antibody, and visualized with ECL Plus (Amer-
sham Biosciences).

Immunofluorescence and Receptor Internalization Assay—Cells were plated on poly-1-lysine-coated glass coverslips in 24-well plates. After stimulation with LPA, cells were fixed with 4% paraformaldehyde/PBS, permeabilized with 0.1% Triton

X-100/PBS for 15 min, and blocked with 3% BSA/PBS for 30 min. F-actin was detected with 25 µg/ml rhodamine-phalloidin (Sigma) in PBS, 1% BSA for 40 min. Samples were visualized on a fluorescence microscope (Carl Zeiss). For the internalization
assay, serum-starved cells (12–16 h) were treated with BSA or LPA, or sphingosine 1-phosphate (S1P), and then fixed with 4% paraformaldehyde in PBS for 1 h and permeabilized with 0.1% (w/v) Triton X-100 with 3% BSA in PBS. HA-tagged receptor was detected using an anti-HA antibody and Cy3-conjugated anti-mouse IgG antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). The cells were analyzed using standard and confocal fluorescence microscopy (Carl Zeiss).

cAMP Measurements—B103 cells were serum-starved on 24-well plates with or without PTX (200 ng/ml) overnight. After treatment with 0.5 mM 3-isobutyl-1-methylxanthine for 20 min, cells were stimulated for 30 min with or without forskolin (5 μM) using a range of LPA concentrations. The cells were lysed with 0.1N HCl, and then cellular cAMP levels were measured using an enzyme-linked immunosorbent assay-based detection kit (Cayman Chemicals, Ann Arbor, MI) according to the manufacturer’s directions.

Imaging of Intracellular Calcium Levels—Cells were plated on glass coverslips, loaded with Fura-2 acetoxyethyl ester (2.5 μM), and incubated for 30–60 min at 37 °C in 1.5 μM pluronic acid (Molecular Probes, Eugene, OR) in Opti-MEM (Invitrogen). Cells were then washed with Opti-MEM. Coverslips were placed in a laminar flow perfusion chamber (Warner Instrument Corp., Hamden, CT) and constantly perfused with Opti-MEM, LPA (1 μM) or ionomycin (1 μg/ml, Calbiochem) was bath-applied. Images of Fura-2-loaded cells with the excitation wavelength alternating between 340 and 380 nm were captured with a cooled CCD camera. Following subtraction of background fluorescence, the ratio of fluorescence intensity at the two wavelengths was calculated. Ratio levels in groups of 20–40 individual cells were analyzed using MetaFluor (Universal Imaging Corp., West Chester, PA).

Electrophysiology—Whole cell patch clamp technique was used to record from B103 cells, 2–3 days

FIGURE 1. Predicted amino acid alignment of the human and mouse LPA4/GPR23 and GPR92 proteins and phylogenetic tree of the human LPA and S1P receptors. A, alignment of the amino acid sequences created using the ClustalW multiple sequence alignment program, “*” means identical, “:” and “.” means conserved and semiconserved substitution, respectively. B, phylogenetic tree showing protein sequence relationship of the human LPA and S1P receptor families. The x axis values are a ruler that indicates the lengths of the horizontal branches. The amino acid sequence divergence between any pair of sequences is equal to the sum of the lengths of the horizontal branches. Shorter lengths mean more closely related genes.
after retrovirus infection. Extracellular solution contained (in mM): 145 NaCl, 2.5 KCl, 1.5 CaCl₂, 1.5 MgSO₄, 10 HEPES, 10 dextrose, pH 7.4 (with NaOH). Compounds were added to the bath by gravity perfusion at room temperature. No effect of vehicle was observed in these experiments. Recording electrodes were fabricated from borosilicate capillary tubing (B150-86-10; Sutter Instrument, Novato, CA). The electrodes were coated with dental periphery wax (Miles Laboratories, South Bend, IN) and had resistances of 2–4 MΩ when containing intracellular saline (in mM): 100 potassium gluconate, 25 KCl, 0.483 CaCl₂, 1.0 BAPTA-K₄, 10 hemi-Na-HEPES, pH 7.4. Whole cell access was achieved by applying short, high voltage pulses (zap), and data were accepted if the access resistance was greater than 15 MΩ (“resistive” whole cell configuration). If the access resistance was less than 15 MΩ, only half of the GPR92-infected cells responded to LPA. The average voltage error was 8 ± 2 mV (n = 15) at the potentials reported. Current signals were detected and filtered at 10 KHz with a MultiClamp 700A amplifier (Molecular Devices (previously Axon Instruments), Union City, CA), digitally recorded with a DigiData 1322A laboratory interface (Axon Instruments) and PC-compatible computer system, and stored for off-line analysis. Data acquisition and analysis were performed with PClamp9 software (Axon Instruments). The total membrane capacitance (C_m) was determined as the difference between the maximum current after a 30 mV hyperpolarizing voltage ramp from −20 mV generated at a rate of 1.2 mV/ms and one-tenth of the difference between the current at the end of the ramp and the steady state current at the final potential (20 s sampling rate) (24) was comparable with the C_m determined on-line. Stimulus-induced changes in whole cell currents were monitored using a voltage-ramp protocol. Usually, cells were held at −50 mV and V_m was stepped to −120 mV for 60 ms and ramped to +120 mV (at a rate of 1.2 mV/ms) at 0.5 Hz. All currents are normalized to the C_m during stimulus application (current density).

K16425 (10 μM) was bath-applied for 3 min prior to LPA exposure.

**Northern Blot Analysis**—A Northern blot containing 2 μg/lane of adult mouse poly(A)⁺ RNA from several mouse tissues was...
RESULTS

Comparison of amino acid sequences showed that GPR92 has ~35% amino acid identity with the LPA₄/GPR23 receptor. Protein sequence alignment of mouse and human GPR92 demonstrated 80% identity (Fig. 1A). Phylogenetic analysis indicated that GPR92 was more related to the fourth receptor LPA₄/GPR23 when compared with the other less similar LPA and S1P receptors (Fig. 1B).

The initial identification of GPR92 as a cytoskeleton-influencing LPA receptor in a reverse transfection screen was reassessed by retroviral mediated GPR92 expression. To ascertain appropriate expression of receptor protein, human GPR92 was epitope-tagged with HA at the extracellular N terminus (Fig. 2A), and the construct was introduced into a replication- incompetent Moloney leukemia retroviral vector to allow virus production and transduction of single-copy GPR92 expression.
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following cell infection. Two cell lines used throughout this study, B103 and RH7777 (23), were examined for GPR92 gene expression by PCR using intron-spanning primers for non-coding exon 1 and coding exon 2 (Fig. 2B). B103 cells do not express endogenous GPR92 mRNA, and it was therefore used in all subsequent analyses. Surprisingly, GPR92 mRNA was detected in RH7777 cells, further revealing two bands consistent with alternative splicing in a 5'-non-coding region of GPR92. However, no LPA-dependent responses have been observed in naïve cells from either B103 or RH7777.

HA-tagged GPR92 virus-infected versus control virus-infected cells were examined by Western blot analysis. Cell membrane fractions identified a single band of the expected molecular mass (Fig. 2C). Consistent with this result, tagged receptor could be identified on the surface of GPR92 expressing B103 cells (Fig. 2D) using Cy3 anti HA immunolabeling and visualized with confocal microscopy. No other known LPA receptors were up-regulated in GPR92 infected cell lines, as assessed by PCR (data not shown). GPCRs typically undergo internalization upon treatment with a full agonist (25), and heterologously expressed GPR92 was therefore assessed for LPA-dependent receptor internalization versus a distinct lysophospholipid, S1P. Cell surface GPR92, revealed by Cy3 immunolabeling, was internalized following LPA but not S1P exposure (Fig. 2D). To assess the ability of GPR92 to bind LPA, isolated membranes from B103 cells stably expressing GPR92 or control vector were tested for specific [3H]LPA binding. Membranes derived from GPR92-expressing cells, but not control vector infected cells, revealed a statistically significant increase in specific [3H]LPA binding (Fig. 2F).

Positive controls in which LPA1 had been heterologously expressed produced neurite retraction in B103 cells and stress fiber formation in RH7777 cells (Fig. 3A). GPR92 was similarly assessed. When GPR92- or LPA1-expressing cells were pretreated with 5 μM Ki16425 (LPA1 and LPA3 receptor antagonist) for 30 min, only LPA1-mediated cellular responses were blocked, while GPR92-mediated cellular responses still remained (Fig. 3, A and C). GPR92-mediated neurite retraction of B103 cells was dependent on LPA concentration (Fig. 3B). Ki16425 strongly inhibited neurite retraction of LPA1-expressing B103 cells but did not affect GPR92-expressing cells (Fig. 3).

It is well established that G12/13 couples to LPA receptors and activates downstream RhoA to induce actin stress fiber formation as well as other RhoA-dependent cellular effects (26–28), while Gq/11-mediated signaling can also activate RhoA (28, 29). To determine which G proteins couple to GPR92, several approaches were utilized, including standard pharmacological intervention and the use of “minigenes” for defined G proteins (22) (Fig. 3D). We first focused on pathways that could produce changes in the cytoskeleton: G12/13-mediated Rac activation (30) and Gq/11-mediated Rho activation. Only blockade of G12/13 (using G12 and G13 minigenes) and downstream Rho (using Rho kinase inhibitor, Y27632) could block neurite retraction (Fig. 3D). Neither PTX nor a dominant negative Rac (RacN17, data not shown) abolished LPA-induced cytoskeletal alterations in GPR92-infected cells.

We next assessed whether non-cytoskeletal changes were altered with these and other G proteins (Figs. 4–6). All lysophospholipid GPCRs influence cAMP levels (15). LPA increased intracellular cAMP levels in GPR92-expressing B103 cells either in the absence (Fig. 4A) or presence (Fig. 4B) of forskolin (5 μM). Intracellular cAMP levels can be regulated by G protein βγ subunits as well as G12/13 subunit (31, 32). We found that use of a G12/13 minigene did not block cAMP accumulation by LPA in B103 cells (Fig. 4C). These results suggest that GPR92-mediated intracellular cAMP production might involve βγ subunits, under the employed assay conditions. Similarly, many lysophospholipid receptors also couple to Gq or G13 to raise intracellular calcium levels (2, 15). In GPR92-expressing B103 cells, LPA significantly enhanced intracellular calcium levels (Fig. 5A), with a response latency consistent with GPCR-mediated mechanisms. This intracellular calcium

![FIGURE 4. Effects of LPA on cAMP accumulation in GPR92-expressing B103 cells. A, effects of LPA on basal cAMP levels. GPR92-expressing B103 cells were stimulated with increasing concentrations of LPA in the presence of 0.5 mM 3-isobutyl-1-methylxanthine. The basal cAMP content in GPR92-expressing cells ( ), PTX-treated cells (200 ng/ml) ( ), and empty vector transfected cells ( ), PTX-untreated cells ( ), PTX-treated cells ( ) are indicated. The increase in cAMP levels following PTX treatment likely results from blockade of other basally active, G12/13-coupled receptors in B103 cells. B, effects of forskolin on cAMP accumulation. Cells were stimulated with LPA in the presence of 5 μM forskolin in the same manner as in A. C, GPR92-expressing B103 cells were infected with a control retrovirus or G12/13 minigene retrovirus and then cAMP assay was performed.](image-url)
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The expression pattern of GPR92 was assessed by Northern blot analysis on poly(A)⁺ mRNA from adult mouse tissues probed with a fragment of the GPR92 mouse cDNA (Fig. 7A). Consistent with our PCR results of these and other tissues (Fig. 7B), GPR92 mRNA is expressed broadly at low levels, such as embryonic brain, and comparatively high levels in small intestine, and moderate levels in skin, spleen, stomach, thymus, lung, and liver. Two bands, 3.3 and 2.8 kb, were also observed by Northern analysis, consistent with RT-PCR data (Fig. 2B), along with a larger band (6 kb) that may represent unspliced mRNA. RT-PCR also detected signals in small subpopulations of cells that could not be assessed by Northern blot, most notably the dorsal root ganglia (DRG) as well as embryonic stem cells (ES) (Fig. 7B).

**DISCUSSION**

Lyosphospholipid signaling mediated by GPCRs accounts for an expanding repertoire of organismal influences. The identification of LPA₄ as a receptor with lower homology to other LPA GPCRs (Fig. 1) suggests that other dissimilar LPA GPCRs could well exist, as speculated previously (15). This perhaps surprising heterogeneity of LPA receptors is nonetheless consistent with previously identified heterogeneity in another class of lipid receptors, leukotriene

patch clamp technique to determine whether LPA could influence ion channel function in B103 cells expressing GPR92. LPA (1 μM) activated an apparent nonselective cation conductance (Fig. 6A) in GPR92-infected cells (“92”) but not cells infected with vector alone (“empty”) (Fig. 6B). Preincubation of GPR92 expressing cells with the LPA₁₉ antagonist Ki16425 (10 μM) had no statistically significant effect on either the percentage of LPA responsive cells (Fig. 6B) or LPA-induced current density measured at either −120 (downward bars) or +120 (upward bars) mV (Fig. 6C). Currents induced by GPR92 receptor activation were outwardly rectifying (current density was greater at +120 mV versus −120 mV) and were concentration-dependent (0.1 versus 1 μM; Fig. 6C).

Increase was significantly blocked by expression of a Gq minigene (18 of 27 cells were completely blocked, Fig. 5C). However, PTX had no effect on calcium levels (Fig. 5D). These results indicate that GPR92 couples to Gq.

To further investigate intracellular signaling pathways modulated by GPR92 receptor activation, we used the whole cell

*increased intracellular calcium in B103 cells heterologously expressing GPR92. LPA was 34 ± 3% (n = 19), the percentage of cells responding to LPA (1 μM) for cells infected with GPR92 (bars indicated by “92”) and S003 vector (bar indicated by “empty”) in the presence (“+”) or absence (“−”) of the Ki16425 compound (10 μM) is shown. The number of cells tested is shown above each bar. C, the LPA-induced current at −120 mV (negative-going bars) and +120 mV (positive-going bars) is shown normalized to cell capacitance (picoampere/picofarad). The response to 1 μM LPA was significantly larger than the response to 100 nM LPA (p < 0.05) and not significantly different from the response in the presence of 10 μM Ki16425 for values determined at +120 mV and −120 mV. Cells were exposed to 10 μM Ki16425 for 3 min prior to addition of LPA in the presence of Ki16425. The number of cells tested in each condition is indicated above the bars. There was no effect of LPA in the presence of Ki16425 on cells infected with vector only (data not shown).
GPCRs (33). Using a DNA sequence-unbiased approach that focused on LPA-dependent cellular morphological changes produced by expression of putative orphan GPCRs, GPR92 was found to have attributes of previously described LPA GPCRs by conferring LPA responsivity following heterologous expression in B103 cells: 1) concentration-dependent neurite retraction mediated by G12/13 and Rho, 2) receptor internalization, 3) increased LPA binding, 4) increased cAMP accumulation, 5) intracellular calcium mobilization, which is mediated by Goq, and 6) evoked electrophysiological currents. In addition, GPR92 expression produces LPA-dependent actin stress fibers in RH7777 cells that, while expressing mRNA for GPR92, do not respond to LPA in the absence of G12/13-coupled LPA receptors (23). Discordant mRNA and protein expression may explain the lack of LPA responsivity in RH7777 cells that has been well documented in other systems (34–37). Taken together, the data presented here are entirely consistent with GPR92 encoding a new LPA receptor, and we therefore propose its designation as LPA5 (Fig. 8).

The approaches used to make this identity have been validated over the last decade in multiple laboratories (2–4, 23, 38). With the exception of the most recently identified receptor LPA4, the initial receptor identities were subsequently confirmed by independent assays including genetic deletion of one or more receptors that abolishes expected LPA signaling (12, 13, 16, 39). Heterologous expression of all five LPA receptors produces increased specific binding of [3H]LPA to membrane fractions, although quantitative assessment via classical receptor-ligand binding approaches has been met with varied success that may be receptor-dependent (1, 4, 23). In the present study, saturable LPA-receptor binding could not be achieved in biologically relevant ranges, similar to previous studies, and therefore a precise Kd was not established. By contrast, the EC50 for neurite retraction (Fig. 3) approximates the single-digit nanomolar concentrations observed previously in this assay for LPA1, indicating that LPA5 is likely to be a high affinity LPA receptor, comparable with those previously identified. No attempt was made in this initial study to examine other forms of LPA beyond the 1-oleyl species used. What can be said is that the LPA1- and LPA3-specific antagonist Ki16425 (41) had no effect on LPA5 at concentrations that block these two other receptors, indicating the probability of ligand-receptor selectivity, as expected from the distinct amino acid sequence of LPA5.

LPA5 complements the four previously identified GPCRs that mediate the signaling effects of LPA. While still clearly within the superfamily of GPCRs, GPR92 is notable compared with the four previously identified LPA receptors in sharing at most 35% sequence identity with LPA4 and lower identities compared with LPA1–3. Its expression pattern by Northern blot analysis is also distinct, albeit overlapping with one or more other LPA receptor genes, which suggests that LPA5 will have both distinguishable functions in vivo and within single tissues,
at least based on its ability to increase cAMP levels, which contrasts with Gt coupling observed for LPA₁₋₃ and complements the Gq signaling of LPA₄ (4). In view of the role of LPA₁ in the initiation of neuropathic pain in an animal model (17), LPA₅ expression was examined in the peripheral nervous system and was found to be expressed in dorsal root ganglia, suggesting that it too might contribute to this disease process. Similarly, its expression in other adult tissues supports LPA₅-mediated signaling as relevant to normal function, most likely in concert with previously identified receptors, although receptor expression at the level of single cell types remains to be determined. The gene expression of LPA₅ in embryonic stem cells extends its possible roles into development and stem cell functions.

With confirmation, LPA₅ would bring the number of LPA receptors to five, complementing the five S1P receptors previously identified, all of which are GPCRs (42–44). A range of biological and medicinal roles for these receptors is emerging (45, 46), and the existence of receptors with diverse amino acid composition that recognize a common ligand portends an even richer biological and chemical landscape toward understanding and manipulating this signaling system. As lysophospholipid receptors expand into evolutionarily distinct genes, it would not be surprising to encounter additional receptors for LPA and other lysophospholipids.

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