Molecular Cloning and Characterization of a Novel Human G-protein-coupled Receptor, EDG7, for Lysosphatidic Acid*

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Lysosphatidic acid (LPA), together with sphingosine 1-phosphate, is a bioactive lipid mediator that acts on G-protein-coupled receptors to evoke multiple cellular responses, including Ca2+ mobilization, modulation of adenyl cyclase, and mitogen-activated protein (MAP) kinase activation. In this study, we isolated a human cDNA encoding a novel G-protein-coupled receptor, designated EDG7, and characterized it as a cellular receptor for LPA. The amino acid sequence of the EDG7 protein is 53.7 and 48.8% identical to those of the human functional LPA receptors EDG2 and EDG4, respectively, previously identified. LPA (oleoyl) but not other lysospholipids induced an increase in the [Ca2+]i of EDG7-overexpressing Sf9 cells. Other LPA receptors, EDG4 but not EDG2, transduced the Ca2+ response by LPA when expressed in Sf9 cells. LPAs with an unsaturated fatty acid but not with a saturated fatty acid induced an increase in the [Ca2+]i of EDG7-expressing Sf9 cells, whereas LPAs with both saturated and unsaturated fatty acids elicited a Ca2+ response in Sf9 cells expressing EDG4. In EDG7- or EDG4-expressing Sf9 cells, LPA stimulated forskolin-induced increase in intracellular cAMP levels, which was not observed in EDG2-expressing cells. In PC12 cells, EDG4 but not EDG2 or EDG7 mediated the activation of MAP kinase by LPA. Neither the EDG7- nor EDG4-transduced Ca2+ response or cAMP accumulation was inhibited by pertussis toxin. In conclusion, the present study demonstrates that EDG7, a new member of the EDG family of G-protein-coupled receptors, is a specific LPA receptor that shows distinct properties from known cloned LPA receptors in ligand specificities, Ca2+ response, modulation of adenyl cyclase, and MAP kinase activation.

Lyosphatidic acid (LPA) and sphingosine 1-phosphate (SIP) are lipid mediators with diverse biological properties (1–3). The cellular responses elicited by LPA vary widely. The effects of LPA on the cell cycle are either mitogenic or antimotic. LPA stimulates phospholipase C (PLC) activation and consequent Ca2+ mobilization, inhibits adenyl cyclase, activates mitogen-activated protein (MAP) kinase, and stimulates the transcription of serum response element transcriptional reporter genes, such as c-fos, in various types of cells. It also exerts an effect on the cytoskeleton that can lead to changes in cell shapes and motility, which include inducing stress fiber production and stimulating chemotaxis, cell migration, and tumor cell invasiveness. These actions of LPA are believed to be mediated by seven-transmembrane G-protein-coupled receptor(s) (GPCR) on the cell surfaces. Some functional studies have suggested that multiple subtypes of LPA receptors with distinct signaling properties mediate the diverse cellular effects of LPA (4–6). Indeed, several subtypes of LPA receptors, which are GPCRs, were identified recently.

The EDG (endothelial cell differentiation gene) family of orphan receptors comprises EDG1 (7), EDG2/Rec1.3/Vzg-1 (8, 9), EDG3 (10), EDG4 (11), AGR16/H218 (12, 13), and EDG6 (14), and their amino acid sequences show 36–58% homology with one another. Hecht et al. (9) first reported that EDG2/Rec1.3/Vzg-1 increased responsiveness to LPA in cell rounding and adenyl-cyclase inhibition assays when overexpressed in cerebral cortical cell lines, showing that EDG2/Rec1.3/Vzg-1 is a receptor for LPA. Because LPA and SIP are structurally related, this finding has enabled scientists to examine whether members of EDG family function as receptors for LPA and SIP. At present, according to their amino acid sequence homologies, ligand specificities, and genomic structures (15), these GPCRs of the EDG family fall into two major groups that interact either with LPA (EDG2 (9) and EDG4 (11)) or with SIP (EDG1 (16–18), EDG3 (19), and AGR16/H218 (20)). The ligand of EDG6, which is expressed predominantly in lymphoid tissue (14), has not been elucidated yet. A novelGPCR, named PSP24, which does not show significant sequence similarity with any member of the EDG family, has also been isolated from Xenopus oocytes as a functional receptor for LPA (21). Because some LPA-responsive cells do not express known LPA receptors (EDG2, EDG4, and PSP24), it was expected that unidentified subtypes of LPA receptors were suggested to be present in mammals (11, 22). To understand the biological functions of LPA fully, we attempted to identify novel subtypes of LPA.

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receptors. In this study, we identified and characterized a novel GPCR, EDG7, the third functional LPA receptor belonging to the EDG family.

**MATERIALS AND METHODS**

**Lipids**—1-Oleoyl-LPA, 1-palmitoyl-LPA, 1-stearoyl-LPA, 1-oleoyl-lyso phosphatidylcholine (LPC), 1-oleoyl-lyso phosphatidylethanolamine (LPE), 1-oleoyl-lyso phosphatidylserine (LPS), porcine liver lyso phosphatidylino nitol (LPSI), SI1, egg yolk phosphatidic acid (PA), dioleoyl phosphatidylserine, and platelet-activating factor (PAF C16) were purchased from Avanti polar lipids (Alabaster, AL), 1-oleoyl-2-linoleoyl phosphatic acid (2-acyl-LPA) was prepared from egg yolk PA (5 nmol) as follows. PA was incubated with Rhizopus delenner lipase (20 mg/ml; Seikagaku-kogyo, Tokyo, Japan) in a 50 mM Tris-malate buffer, pH 5.7, at 37°C for 2 h in the presence of one-quarter volume of diethyl ether. After the free fatty acids had been extracted with diethyl ether/petroleum ether (1:1 v/v) four times, the remaining lysophospholipids were extracted by the method of Bligh and Dyer (23). 2-acyl-LPA contains mostly oleic acid and linoleic acid, because egg yolk PA is prepared from egg yolk phosphatidylcholine, which contains those fatty-acid chains at the sn-2 position. Because the 2-acyl-1-lyso phospholipids were not stable, they were stored at −80°C in chloroform/methanol (2:1 v/v) and used within 24 h after mixing with a buffer solution. Cyclic PA (cPA) and its analogs were prepared as described (24).

**Amplification of the Novel G-protein-coupled Receptor with Degenerate PCR**—A PCR was performed on about 10⁷ human Jurkat T cells, and 5.0 μg was reverse-transcribed into DNA using the cDNA Cycle Kit (Invitrogen, Carlsbad, CA) with an oligo(dT) primer. The degenerate PCR primers were designed based on the amino acid sequences of the second and sixth of the seven transmembrane regions of the G-protein-coupled receptors EDG2 and EDG4. The oligonucleotides used were: GCCGCGCGCAACATGACTAGATGGACTACAAAGACGATGACGATAAAGCTGCCAGCCCGCTCAGTCCTGTTGGTTGGGTTGAGC (for EDG4). The resulting DNA fragment was digested by EcoRI and ligated into pFASTBAC1. Recombinant baculoviruses were prepared using the Bac-to-Bac system (Invitrogen). cDNA sequences corresponding to the 5'- and 3'-noncoding regions and mRNA clones were amplified by degenerate PCR primers designed based on the amino acid sequences of cDNAs prepared by reverse transcription-PCR were confirmed by DNA sequencing.

**Co²⁺ Measurements**—S9 cells were harvested 2 days after baculovirus infection, washed gently with an HBS buffer (20 mM Hepes, pH 7.4, containing 120 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl₂, 1.25 mM CaCl₂, 1.2 mM KH₂PO₄, and 10 mM glucose), and loaded with 2 μM Fura-2 acetoxyethyl ester (Fura-2 AM; Molecular Probes Inc., Eugene, OR) for 45 min. Free Fura-2 AM was washed out, and the cells were resuspended in the HBS buffer to produce a concentration of 10⁶ cells/ml. Agonist-induced Fura-2 AM fluorescence of samples in quartz cuvettes kept at 27°C was monitored at excitation wavelengths of 340 and 380 nm and an emission wavelength of 500 nm using a CAF-110 spectrofluorimeter (Japan Spectroscop, Inc., Tokyo, Japan). Fluorescence was recorded before and after addition of LPA and other phospholipids, which were dissolved in phosphate-buffered saline with 0.01% (v/v) of fatty acid-free bovine serum albumin (Sigma).

**Pertussis Toxin and U73122 Treatment**—24 h after baculovirus infection of the S9 cells, PTX (100 ng/ml; Calbiochem, La Jolla, CA) was added to the culture medium, and incubation was continued for an additional 48 h. Then the cells were collected, and their Ca²⁺ responses were tested as described above. The phosphodiesterase inhibitor U73122 (Calbiochem, La Jolla, CA) was added to S9 cells 3 min before LPA was added.

([³H]LPA Binding)—S9 cells (5 x 10⁶) infected by each baculovirus for 48 h were washed with a binding buffer (phosphate-buffered saline containing 0.25% bovine serum albumin) and incubated for 60 min at 0°C in the same buffer containing various concentrations of [³H]LPA in a 96-well microplate filter plate (pore size, 65 nm; Millipore, Orlando, FL). The cells were resuspended three times with a fresh buffer (phosphate-buffered saline containing 1% bovine serum albumin) using a Multi-screen Filtration System (Millipore), and the radioactivity bound to the cells was quantified using a β-counter. Total and nonspecific binding was evaluated in the absence and presence of 10 μM nonradioactive LPA, respectively. To examine the specificity of LPA binding, the amounts of LPA bound (using 10 μM of [³H]LPA) in the presence of excess nonradioactive LPA (10 μM), LPS (10 μM), LPC (1 μM), LPI (10 μM), LPE (10 μM), SI1 (10 μM), and PAF (1 μM) (10 μM of PAF or PAF caused cell lysis) were determined, and the specific binding value was calculated by subtracting the nonspecific binding value (cpm) from the total binding value (cpm).

**cAMP Measurements**—S9 cells were infected with recombinant baculoviruses and harvested 2 days after infection. Cells were incubated with 5 μM forskolin in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (0.5 μM) for 10 min following a 20-min stimulation with 2.5 μM LPA with various acyl chains in an HBS buffer. cAMP levels were determined using a cAMP enzyme immunoassay system (Biotrak; Amersham Pharmacia Biotech), following the instruction of the manufacturer's protocol. To measure MAP kinase-mediated signal transduction, we employed the PathDetect ELISA Trans-Reporting System (Strategene, La Jolla, CA). This assay employs a fusion protein that contains the DNA-binding domain of GAL4 and the transactivation domain of Elk1 to induce expression of a luciferase reporter driven by an artificial promoter containing five GAL4-binding sites. Phosphorylation of the transactivation domain of
Elk1 by MAP kinase, in turn, activates the transcription of the luciferase gene from the reporter plasmid. cDNAs encoding FLAG-tagged EDG7, EDG2, and EDG4 were inserted into EcoRI/NcoI sites of the mammalian expression plasmid pcDNA3 (Invitrogen, Carlsbad, CA) (FLAG-EDG7-pcDNA3, FLAG-EDG2-pcDNA3, and FLAG-EDG4-pcDNA3). Transient transfections were performed using SuperFect transfection reagent (Qiagen, Hilden, Germany). Briefly, 100,000 PC12 cells were seeded in 24-well plates 24 h before the transfection. Each point was transfected with 200 ng of FLAG-EDG7-pcDNA3 (or FLAG-EDG2-pcDNA3, FLAG-EDG4-pcDNA3, or empty pcDNA3 for the control experiment), 200 ng of pFR-Luc, 25 ng of pFA-Elk1 (as described by Stratagene), and 200 ng of pRL-TK (as described by Promega). The transfected cells were rinsed twice with phosphate-buffered saline and incubated in a serum-free medium for another 12 h. The cells were then stimulated with 10 μM of LPA (oleyl) for 10 h at 37°C and lysed in 500 μl of an extraction buffer, 100 μl of which was used to measure luciferase activity, following the instructions of the manufacturer. Expression of each protein was confirmed by immunofluorescence using anti-FLAG monoclonal antibody M5.

Northern Blot Analysis—Human Multiple Tissue Northern blots were purchased from CLONTECH (Palo Alto, CA), DNA probes (nucleotide positions 307–730) were labeled by random priming with [α-32P]dCTP, and hybridization was carried out at 65 °C for 4 h in a rapid hybridization buffer (Amersham Pharmacia Biotech). Each blot was rinsed with 2× SSC at room temperature for 5 min, washed twice with 0.5× SSC containing 0.1% SDS at 65 °C for 40 min, and then autoradiographed using Kodak X-Omat AR film at 80 °C with an intensifying screen for 12 h. Finally, each blot was rehybridized with a gliceraldehyde-3-phosphate dehydrogenase cDNA probe (CLONTECH, Palo Alto, CA) as an internal standard.

RESULTS

PCR Amplification, Cloning, and Sequencing of EDG7 cDNA—We investigated a previously unidentified GPCR belonging to the EDG family by subjecting cDNA of human Jurkat T cells to PCR amplification. Two blocks of conserved amino acid sequences in EDG2 and EDG4, one from the second and one from the sixth transmembrane domain, were chosen for the synthesis of the degenerate oligonucleotide primers DP5 and DP6 (see “Materials and Methods”). The PCR reaction was performed, yielding a 554-bp fragment containing a GPCR-like sequence distinct from those of known members of the EDG family. Using 5′- and 3′-RACE, we isolated a DNA fragment that covered the entire open reading frame of this novel gene. Complete sequencing of the DNA revealed a 1059-bp open reading frame flanked by a 42-bp 5′-untranslated region and a 44-bp 3′-untranslated region. The translational initiation site (ATG) was assigned to the first methionine codon (nucleotide positions 43–45), because an in-frame stop codon was present upstream of this methionine residue and flanking sequences (CCACA) were present, fulfilling Kozak’s criteria for initiation (25). An in-frame translational termination codon (TAA) was present after nucleotide 1,101. Therefore, we concluded that this new GPCR contains 535 amino acids and that its molecular mass is 40,128 Da (Fig. 1A).

A comparison of the deduced amino acid sequence with those of known EDG sequences revealed that the primary structure of the predicted protein was similar to those of the GPCRs of the EDG family, with overall sequence identities to human EDG1, human EDG2 (Vzg-1), human EDG3, human EDG4, rat H218, and human EDG6 of 34.8, 53.7, 36.3, 48.8, 33.8, and 35.5%, respectively. Therefore, the protein encoded by the cloned cDNA was named EDG7 (Fig. 1B). A high degree of similarity between EDG2 and EDG4 (approximately 50%; Fig. 1B) was observed among EDG7, EDG2, and EDG4. To gain better understanding of the relationships involved in the molecular evolution of the EDG family, a phylogenetic tree was constructed using the neighbor joining method (Fig. 1C). According to this phylogenetic tree, the EDG family can be classified into two distinct groups: EDG1, EDG3, H218/AGR16, and EDG6 belong to one, and EDG2, EDG4, and EDG7 belong to the other. Because EDG1, EDG3, and H218/AGR16 have been reported to function as S1P receptors and EDG2 and EDG4 to function as LPA receptors, the EDG7-expressing recombinant baculovirus (data not shown)
increased the [Ca\(^{2+}\)]. However, no such Ca\(^{2+}\) response was observed in Sf9 cells infected with wild-type baculovirus (Fig. 2B) or in uninfected control cells (data not shown), even if the cells were treated with 10 \(\mu\)M LPA. The structurally related lipids 1-oleoyl-LPC, 1-oleoyl-LPE, 1-oleoyl-LPS, 2-oleoyl-LPS, 1-acyl-LPI, PAF, and S1P, each at a concentration of 1 \(\mu\)M, failed to elicit significant increases in the [Ca\(^{2+}\)] (Fig. 2C).

We also expressed known LPA receptors EDG2 and EDG4 in Sf9 cells and compared their Ca\(^{2+}\) response. Protein expression was confirmed by Western blotting using anti-FLAG antibody (Fig. 2A). The expression of EDG2 protein was much higher than those of EDG4 and EDG7, but it failed to transmit a detectable Ca\(^{2+}\) signal in response to LPA (oleoyl) (Fig. 2B), consistently with the observation by Zondag et al. (17). On the other hand, EDG4 mediated a Ca\(^{2+}\) signal like EDG7 in Sf9 cells (Fig. 2B). Thus, both EDG4 and EDG7 but not EDG2 mediate the Ca\(^{2+}\) response by LPA in Sf9 cells.

LPA Binding—We investigated the specific binding of \(^{3}H\)oleoyl-LPA to Sf9 cells expressing FLAG-EDG7, FLAG-EDG2, and FLAG-EDG4 using various doses of LPA. EDG7- and EDG4-expressing Sf9 cells increased the specific binding of \(^{3}H\)LPA in comparison with wild-type baculovirus and EDG2-infected cells (Fig. 3A). The apparent \(K_d\) values of EDG7 and EDG4 for \(^{3}H\)LPA are 206 and 73.6 nM, respectively (Fig. 3B). Thus, the binding affinity for LPA of EDG7 is relatively lower than that of EDG4. We also examined the competition between the binding of \(^{3}H\)ILPA and related nonradioactive lipids to FLAG-EDG7-expressing Sf9 cells. LPA (10 \(\mu\)M) reduced \(^{3}H\)ILPA binding, whereas the other related lipids examined (LPC, LPE, LPS, LPI, S1P, and PAF) did not (Fig. 3C).
FIG. 2. LPA-induced increases in the intracellular $[\text{Ca}^{2+}]$ of SF9 cells expressing EDG7. A, expression of FLAG-tagged EDG7, EDG2, and EDG4 proteins in SF9 cells. SF9 cells were infected with each baculovirus, and FLAG-tagged protein expression was examined by Western blotting with an anti-FLAG (M5) monoclonal antibody. The predicted molecular mass of FLAG-EDG7 is about 35 kDa. The size of the molecular mass marker is shown on the right. B, $\text{Ca}^{2+}$ response of FLAG-EDG7-, FLAG-EDG2-, and FLAG-EDG4-expressing SF9 cells to LPA. SF9 cells were infected with each baculovirus, loaded with the fluorescent $\text{Ca}^{2+}$ indicator Fura-2 AM, and stimulated with 1-oleoyl-LPA. A result from cells infected with the wild-type virus is also shown. C, structurally related phospholipids did not evoke the $\text{Ca}^{2+}$ response. Fura-2 AM-loaded SF9 cells expressing FLAG-EDG7 were stimulated sequentially with 1 µM each phospholipid, and the $\text{Ca}^{2+}$ response was examined as described in the legend to B.
These data clearly demonstrate that EDG7 represents a specific receptor for LPA.

Ligand Specificity of EDG7—Several molecular species of LPA with saturated (stearoyl-(18:0), palmitoyl-(16:0)) or unsaturated (oleoyl-(18:1), linoleoyl-(18:2), arachidonoyl-(20:4)) acyl chains have been detected in activated platelets (26). We then investigated which structural features of LPA are important for the EDG7-dependent activation of Ca\textsuperscript{2+} mobilization.

1-Oleoyl-LPA is a good ligand for EDG7 (Fig. 2B). As shown in Fig. 5, however, 1-acyl-2-lysophosphatidic acids with saturated acyl chains (1-stearoyl-, 1-palmitoyl-, and 1-myristoyl-LPA), at a concentration of 10 \textmu M, failed to elicit significant increases in [Ca\textsuperscript{2+}]. Furthermore, 2-acyl-LPA, which contains a mixture of oleic and linoleic acids at the sn-2 position (see “Materials and Methods”), elicited a significant increase in [Ca\textsuperscript{2+}] (Fig. 4, A–E). No such Ca\textsuperscript{2+} response of Sf9 cells infected with wild-type baculovirus was induced by 2-acyl-LPA (data not shown).

The ligand specificity of EDG4 was also examined in this system. In marked contrast with EDG7, 1-myristoyl-, 1-palmitoyl-, 1-stearoyl-, 1-oleoyl-, and 2-acyl-LPA equally elicited a significant increase in the [Ca\textsuperscript{2+}], in Sf9 cells expressing EDG4 (Fig. 4, F–J).

We also examined the ability of cPA, which was first isolated from the slime mold Physarum polycephalum (27), to increase the [Ca\textsuperscript{2+}]. As shown in Fig. 5, cPA with oleoyl acid at the sn-1 position of the lipid (18:1 cPA, PHYLPA-8) increased the [Ca\textsuperscript{2+}], whereas 10 \textmu M cPAs with palmitic acid (16:0 cPA, PHYLPA-5) and cyclopropane-containing hexadecanoic acid (PHYLPA-1) were inactive. These data demonstrated that EDG7 prefers LPA with unsaturated fatty-acyl chains at the sn-1 or sn-2 position.

Characterization of the EDG7-transduced Ca\textsuperscript{2+} Response—We next examined the effects of PTX and the PLC inhibitor U73122 on EDG7-transduced Ca\textsuperscript{2+} responses. As shown in Fig. 6, pretreatment of FLAG-EDG7-expressing Sf9 cells with 100 ng/ml PTX for 24 h did not affect the Ca\textsuperscript{2+} response evoked by 1 \textmu M LPA, whereas U73122 inhibited effectively the Ca\textsuperscript{2+} response transduced by EDG7, demonstrating that EDG7 is coupled to PTX-insensitive G-protein(s). The Ca\textsuperscript{2+} response transduced by EDG4 was also inhibited by PLC inhibitor U73122 but unaffected by PTX pretreatment (Fig. 6).

Effect on cAMP Level—It has been repeatedly demonstrated that LPA inhibits adenylyl cyclase via a G\texttextsubscript{i}-mediated signaling event in mammalian cells. To examine whether EDG7 participates in the inhibition of adenylyl cyclase in response to LPA, Sf9 cells expressing each LPA receptor were pretreated with forskolin to raise the intracellular cAMP level and then treated with LPA. Unexpectedly, LPA did not suppress forskolin-induced cAMP accumulation but rather increased intracellular cAMP level in EDG7-expressing Sf9 cells (Fig. 7A). The in-
crease in cAMP level by LPA in EDG7-expressing Sf9 cells was insensitive to PTX (Fig. 7B) and observed only when the cells were pretreated with forskolin (data not shown). The similar response was observed in EDG4-expressing Sf9 cells (Fig. 7B).

cAMP level in EDG2-expressing Sf9 cells was unaffected upon stimulation with LPA (Fig. 7A), as described previously (17).

Effect on MAP Kinase—MAP kinase in insect Sf9 cells has not been characterized yet. In addition we could not detect MAP kinase activity using anti-human MAP kinase antibodies or substrates for MAP kinase such as oligopeptide from human EGF-receptor (data not shown). Thus we used the mammalian expression system for this experiment. To determine whether EDG7 mediates MAP kinase activation, we used the PathDetect™ Elk1 trans-Reporting System. Elk1 is a transcription factor that is phosphorylated and activated by MAP kinase (28). PC12 cells were transfected with FLAG-tagged EDG2, EDG4, or EDG7 and subjected to an assay of MAP kinase activation. Expressions of each receptor were confirmed by immunofluorescence in PC12 cells (data not shown). LPA activated the transcription of the luciferase gene through activation of the Elk1 in EDG4-expressing PC12 cells but not in

**Fig. 4. Substrate specificity of EDG7 and EDG4.** The activities of LPA and its acyl chain analogs to induce rapid, transient increases in [Ca^{2+}]_{i} in Sf9 cells expressing EDG7 (A–E) or EDG4 (F–J) were measured. Cells, loaded with Fura-2 AM, were stimulated with various concentrations of LPAs, and changes in [Ca^{2+}]_{i} were analyzed using CAF-110 spectrofluorimeter. The means were calculated from the results of three separate experiments. A and F, 1-oleoyl-LPA; B and G, 2-acyl-LPA; C and H, 1-stearoyl-LPA; D and I, 1-palmitoyl-LPA; E and J, 1-myristoyl-LPA.

**Fig. 5. Cyclic PA with oleic acid elicits Ca^{2+} response in Sf9 cells expressing EDG7.** FLAG-EDG7-expressing Sf9 cells were loaded with the fluorescent Ca^{2+} indicator Fura-2 AM and stimulated sequentially with PHYLPA-1 (10 μM), PHYLPA-3 (palmitoyl (16:0)-cPA, 10 μM), PHYLPA-8 (oleoyl (18:1)-cPA, 10 μM), and 1-oleoyl-LPA (1 μM).

**Fig. 6. Effects of PTX and PLC inhibitor (U73122) on the LPA-induced Ca^{2+} response of Sf9 cells expressing EDG7 (A and B) and EDG4 (C and D).** A and C, Sf9 cells expressing FLAG-EDG7 (A) or FLAG-EDG4 (C) were pretreated with (open circle) or without (closed circle) PTX (100 ng/ml) for 24 h before the Ca^{2+} response was examined using various doses of oleoyl-LPA. B and D, Sf9 cells expressing FLAG-EDG7 (B) or FLAG-EDG4 (D) were pretreated with PLC inhibitor, U73122 (3 μM), for 3 min before the Ca^{2+} response was examined. Oleoyl-LPA (1 μM) was used.
EDG2- or EDG7-expressing cells (Fig. 8), suggesting that only EDG4 is coupled to MAP kinase activation among the cloned LPA receptors.

**Tissue and Cellular Distributions of EDG7—**The tissue distribution of EDG7 was examined by subjecting several human tissues and cancer cell lines to Northern blot analysis. EDG7 transcripts of about 4.3 kilobases were detected in the heart, pancreas, prostate, and testis and, to a lesser extent, in the lung and ovary (Fig. 9). EDG7 transcripts were also found weakly in the human cancer cell lines HeLa, K562, SW480, A549, and G361, the last of which, a melanoma cell line, contained the highest level (data not shown).

**DISCUSSION**

In this study, we isolated a new member of the EDG family of G-protein-coupled receptors, EDG7, and showed that it functions as a cellular receptor for LPA. Interestingly, EDG7 transduce increases in both [Ca\(^{2+}\)](i) and the cAMP level upon stimulating only with LPA containing an unsaturated fatty-acyl chain at the sn-1 or sn-2 position but not with LPA containing any of saturated fatty acids. EDG7 also reacted with cPA with unsaturated fatty acid (Fig. 5). In contrast, EDG4 can be stimulated by LPAs with both saturated and unsaturated fatty acids (30). In addition, Tokumura et al. (31) reported that degree of unsaturation in the acyl moiety of LPA affects proliferation of cultured vascular smooth muscle cells from rat aorta by LPA. A newly identified LPA receptor, EDG7, may account for these responses.

Several molecular species of LPA with saturated (stearoyl-
induced Ca\textsuperscript{2+} mobilization can be transduced through both PTX-sensitive (32) and PTX-insensitive (33–35) G-protein followed by activation of PLC. An et al. (1998) J. Biol. Chem. 265, 9308–9313


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