Biochemical and Biophysical Research Communications **259,** 78–84 (1999)
Article ID bbrc.1999.0627, available online at http://www.idealibrary.com on IDEAL®

Activation of G_{i1} by Lysophosphatidic Acid Receptor without Ligand in the Baculovirus Expression System[1]

Akira Yoshida and Hiroshi Ueda[2]
Department of Molecular Pharmacology and Neuroscience, Nagasaki University School of Pharmaceutical Sciences, Nagasaki 852-8521, Japan

Received March 31, 1999

Lysophosphatidic acid (LPA) receptor has been attracting many neuroscientists' concerns, since it was reported to have a potential role in the neurogenesis, which occurs in the ventricular zone of the developing and adult brain. In the present experiments using baculovirus expression system, the LPA receptor encoded by ventricular zone gene 1 (Edg-2/Vzg-1) was found to be functionally coupled to G_{i1}, G_{oA}, and G_{11}, but not to G_S. The coexpression of LPA receptor markedly decreased the expression of G protein α_{i1} or α_{oA} subunit, while the basal [^{35}S]GTPγS binding significantly increased in the G_{i1}-preparation. The Scatchard Plot analysis indicates that the expression of LPA-receptor (Edg-2/Vzg-1) showed stimulation of G_{i1} without agonist. These results suggest the Edg-2/Vzg-1 has an intrinsic acctivity on G_{i1}. © 1999 Academic Press

Lysophosphatidic acid (1-acyl-*sn*-glycero-3-phosphate, LPA), a water-soluble phospholipid, can elicit diverse biological responses (1). These actions were found to be mediated through activation of a variety of second messenger pathways, including stimulation of phospholipase C and D (2–4), inhibition of adenylyl cyclase (5), elevation of intracellular Ca^{2+} (6), activation of ras and mitogen-activated protein kinases (7, 8) and activation of rho (9, 10). These observations also suggest that LPA exerts such actions through various kinds of G proteins and/or G protein-coupled receptors. Indeed some effects of LPA are pertussis toxin (PTX)-sensitive (8).

On the other hand, there are accumulating reports that LPA has a potential role in neurogenesis, based on the knowledge of biological activities of LPA and biological events during neurogenesis (9, 11–15). Chun and his colleagues (16) have succeeded in cloning an LPA receptor as a G protein-coupled receptor (Edg-2/Vzg-1) which is 96% identical to an sheep sequence termed edg-2. The Edg-2/Vzg-1 is preferentially expressed in the subventricular zone of the brain, where neurogenesis is actively occurring in the embryonic, neonatal and adult stage (17, 18). At the same time of this work, another type of metabotropic LPA receptor (PSP24) was isolated from a *Xenopus* oocytes library (19). However, little is known of details of their signaling mechanisms.

The best way to study the signaling mechanisms of cloned metabotropic receptors is to perform a reconstitution experiments. For this purpose, baculovirus/insect cell expression system has been recently utilized. This expression system has advantages that a large amounts of recombinant proteins are expressed, followed by posttranslational modifications such as isoprenylation and glycosylation (20). In the present study we demonstrate the functional coupling of Edg-2/Vzg-1 with some G protein species in this expression system. During the characterization of such couplings, we found the intrinsic activity of the receptor to activate a specific G protein.

MATERIALS AND METHODS

Materials. The anti-G protein antibodies were kindly donated by Dr. T. Haga at the University of Tokyo, Faculty of Medicine at Tokyo Japan. The anti-Edg-2/Vzg-1 antibody was kindly donated by Dr. J. Chun at University of California, San Diego, CA.

Cloning of mouse LPA receptor cDNA. A male ddY mouse was decapitated and the brain was removed. A pair of amygdala was then dissected and immediately homogenized with a Teflon-glass homogenizer in TRIzol (Gibco BRL, Grand Island, NY). cDNAs were generated by reverse transcriptase, SuperScript II (Gibco BRL) with random hexamer oligonucleotide. A coding region of mouse Edg-2/Vzg-1 homologue was amplified by Taq polymerase using primers, 5′-CCAAACTACAGCACTGTCATG-3′ and 5′-CCGGCTGGCTTCCTTCTAAA-3′. The PCR product was cloned into pGEM-T vector (pT-VZG1).

[1] Parts of this study were supported by Grants-in-Aid from the Ministry of Education, Science, Culture, and Sports of Japan, and by a research grant from Ono Medical Research Foundation.

[2] To whom correspondence and reprint requests should be addressed at Department of Molecular Pharmacology and Neuroscience, Nagasaki University School of Pharmaceutical Sciences, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan. Fax: +81-95-844-4248. E-mail: ueda@net.nagasaki-u.ac.jp.

0006-291X/99 $30.00

Generation of recombinant baculovirus for expression of Edg-2/Vzg-1 LPA receptor and human D1 dopamine receptor. The baculovirus transfer vector containing sequence for Edg-2/Vzg-1 was constructed as follows. The *Nco*I-*Sal*I fragment containing the coding region was excised from pT-VZG1, where the *Nco*I site was filled in with T4 DNA. The fragment was inserted between *Bam*HI and *Sal*I sites of pFASTBAC1 (pF-VZG1), where the *BamH*I site was filled in with T4 DNA polymerase. The pF-VZG1 was transformed into DH10BAC for transposition of the donor plasmid containing Edg-2/Vzg-1 to a bacmid. The recombinant bacmid DNA was transfected by using CELLFECTIN Reagent (Gibco BRL) into *Spodoptera frugiperda* (Sf21).

The baculovirus transfer vector containing sequence for human D1 dopamine receptor was constructed, as follows. A coding region of human D1 dopamine receptor was amplified from the cDNA, kindly given by Dr. M. Caron at Duke University Medical Center, NC, by Taq polymerase using primers, 5′-AAGGATCCAAGATGAGGACTCTGAACAC-3′ and 5′-TTGAATTCTCAGGTTGGGTGCTGACC-3′with 5′ flanking region containing *Bam*HI and *Eco*RI site respectively. The PCR product was cloned into pGEM-T vector (pThD1R). The *Bam*HI-*Eco*RI fragment containing the human D1 dopamine receptor coding region was excised from pThD1R. The fragment was inserted between *Bam*HI and *Eco*RI sites of pFASTBAC1 (pFhD1R). The pFhD1R was transformed into DH10BAC for transposition of the donor plasmid containing hD1R to a bacmid. The recombinant bacmid DNA was transfected by using CELLFECTIN Reagent into Sf21. The generated recombinant virus was amplified by infection and the amplified virus (1×10^8 pfu/ml) was stored at 4°C.

Coexpression of recombinant G proteins and LPA receptor in Sf21 cells. Sf21 cells (1.0×10^7 cells) were infected with the recombinant viruses at a multiplicity of infection (m.o.i.) of 1 for mouse LPA receptor, while at m.o.i. of 10 for $G\beta_1/\gamma_2$ and various kinds of $G\alpha$-subunits. Baculoviruses for these G protein subunits have been prepared, as previously reported (21). However, $G\beta_1$ and $G\gamma_2$ subunits were placed downstream of two polyhedrin promoters arranged back-to-back in the recombinant virus coding both subunits. Cells were harvested 2 ~ 3 days after infection at 27°C.

Membrane preparation from Sf21 cells and [^{35}S]GTPγS binding assay. The Sf21 cells were washed once and homogenized using a glass-Teflon homogenizer in 1 ml of TES (0.32 M sucrose, 0.1 mM EDTA, 25 mM Tris-HCl, pH 7.5) and centrifuged at 17,600 × g for 40 min at 4°C. The pellet was resuspended with buffer A (50 mM HEPES-KOH, 1 mM EGTA, 1 mM DTT, 100 mM NaCl and 5 mM $MgCl_2$, pH 7.4) and centrifuged at 400 × g for 5 min. The supernatant was stored on ice until use.

[^{35}S]GTPγS binding assay was performed as described (22) with some modifications. The membrane preparations (20 μl) were incubated in buffer A with 10 μM GDP, [^{35}S]GTPγS ($2–5 \times 10^5$ cpm/assay NEM, Boston, MA) and drugs in a total volume of 100 μl for 60 min at 30°C. Bound and free [^{35}S]GTPγS were separated by a rapid filtration through a nitrocellulose filter (0.45 μm, ADVANTEC Toyo, Tokyo, Japan) which was set into 96-well manifold kit. Bound radioactivity was determined using BioImaging Analyzer (FUJIX BAS 1000, Fuji Film, Tokyo, Japan). Non-specific activity was determined by the difference between the [^{35}S]GTPγS binding in the presence of 1 μM unlabeled GTPγS. In this concentration of GTPγS, the LPA (10 μM)-dependent activation of [^{35}S]GTPγS binding to the membrane expressing G_{i1} and Edg-2/Vzg-1 was 0.95 ± 0.42% (n = 3) of that in the absence of unlabeled GTPγS. The data were analyzed using Student's *t*-test.

SDS-PAGE and immunoblotting. SDS-PAGE by using 12% polyacrylamide gel and immunoblotting were performed as described (23) with some modifications. Visualization of immunoreactive bands was performed by using an enhanced chemiluminescent substrate for detection of horse radish peroxidase, SuperSignal Substrate (PIERCE, Rockford, IL). The intensities of immunoreactive bands were analyzed by NIH imaging after scanning exposed films.

RESULTS

LPA-Induced Stimulation of G_{i1}, G_{oA}, and G_{11} through Edg-2/Vzg-1 LPA Receptor

In previous studies, a pertussis toxin (PTX)-sensitive G protein has been postulated to be in part involved in various biological actions of lysophosphatidic acid (LPA). To examine whether a cloned mouse LPA receptor, Edg-2/Vzg-1 couples PTX-sensitive G proteins, G_{i1} or G_{oA}, the α subunit of either G protein was expressed simultaneously with the β_1 and γ_2 subunit of G protein with Edg-2/Vzg-1 in Sf21 cells by recombinant baculovirus infection, and [^{35}S]GTPγS binding assay was performed. The specific [^{35}S]GTPγS binding to the membranes expressing G_{i1} or G_{oA} and Edg-2/Vzg-1 significantly increased with LPA concentrations in the range from 0.1 to 10 μM (Figs. 1A and 1B). The addition of 1 μM LPA increased the specific [^{35}S]GTPγS binding to the G_{i1}-expressed membrane by 58% of control, and to the G_{oA}-one by 86% of control. The absolute value of LPA-induced increase in [^{35}S]GTPγS binding in the preparations expressing G_{i1} or G_{oA} and Edg-2/Vzg-1 was 670.7 fmole/mg protein or 886.3 fmole/mg protein respectively. No LPA-dependent activation was found in the membranes with Edg-2/Vzg-1 alone nor with either G protein alone (data not shown). These results indicate that the Edg-2/Vzg-1 couples G_{i1} or G_{oA} in the present coexpression experiments.

In a preparation expressing G_{11}, LPA stimulated the [^{35}S]GTPγS binding through Edg-2/Vzg-1 in a concentration-dependent manner in the range from 0.1 μM to 10 μM (Fig. 1C). The addition of 10 μM LPA showed a significant increase by 30% of control. On the other hand, 10 μM LPA showed a weak, but non-significant increase (16.6%) in the preparation expressing G_s (Fig. 1D). In order to confirm that G_s was properly expressed, we performed the coexpression of G_s and D1 dopamine receptor. As shown in Fig. 1E, SKF-82958, a dopamine D1 agonist, markedly stimulated the [^{35}S]GTPγS binding in a concentration-dependent manner.

LPA-Induced Kinetic Changes in the [^{35}S]GTPγS Binding to G_{i1} or G_{oA}

To further characterize the functional coupling between Edg-2/Vzg-1 and either G protein, LPA-stimulated [^{35}S]GTPγS binding was determined under the condition of various concentrations of [^{35}S]GTPγS in the reaction mixture. The Scatchard Plot analysis showed the single component of [^{35}S]GTPγS binding in either preparation expressing G_{i1} or G_{oA}, and in both cases in the absence or pres-

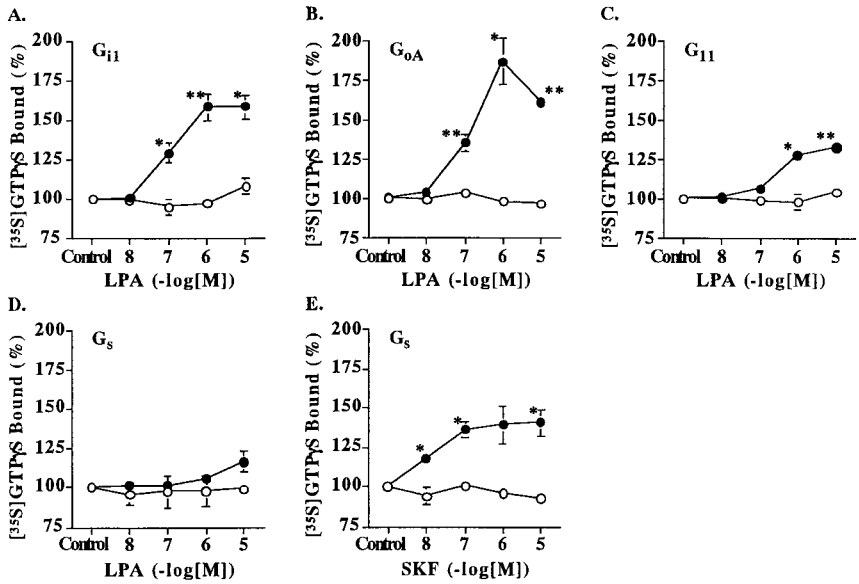

FIG. 1. Effect of LPA or SKF-82958 concentrations on the [^{35}S]GTPγS binding to membrane. [^{35}S]GTPγS binding activity in the membrane containing G protein (A, G_{i1}; B, G_{oA}; C, G_{11}; and D and E, G_s) was assayed. (A-D) The membrane contained G protein with (●) or without (○) Edg-2/Vzg-1. (E) The membrane contained G_s with (●) or without (○) D1 dopamine receptor. Each sample was incubated for 1 hr at 30°C. * < 0.05, ** < 0.01, compared with value of the membrane without Edg-2/Vzg-1. Data are expressed as a percentage of control (no LPA nor SKF-82958) and represent the mean ± S.E.M. of three independent experiments.

ence of 1 μM LPA (Figs. 2A and 2B). The K_d value of [^{35}S]GTPγS binding in the absence of LPA in the preparation expressing Edg-2/Vzg-1 and G_{i1} was 337.5 nM as shown in Fig. 2C. When 1 μM LPA was added, the K_d was decreased to one third of control (85.7 nM). Similar decrease in the K_d value was found in the membrane expressing Edg-2/Vzg-1 and G_{oA}. The K_d in the absence and presence of 1 μM LPA was 191.0 and 46.1 nM, respectively (Fig. 2C). These results indicate that LPA activates G_{i1} and G_{oA} through the Edg-2/Vzg-1.

Expression of G Proteins in Baculovirus Expression System

Although the recombinant G_s was found to be functional from the coexpression experiment using D1 receptor and G_s, the possibility cannot be excluded that the loss of coupling between Edg-2/Vzg-1 and G_s might be attributed to the poor expression of Edg-2/Vzg-1 or the selective inhibition of G_s-expression by coexpression of Edg-2/Vzg-1. The relative amounts of Vzg-1 calculated from their immunoreactivities in the G_{i1},

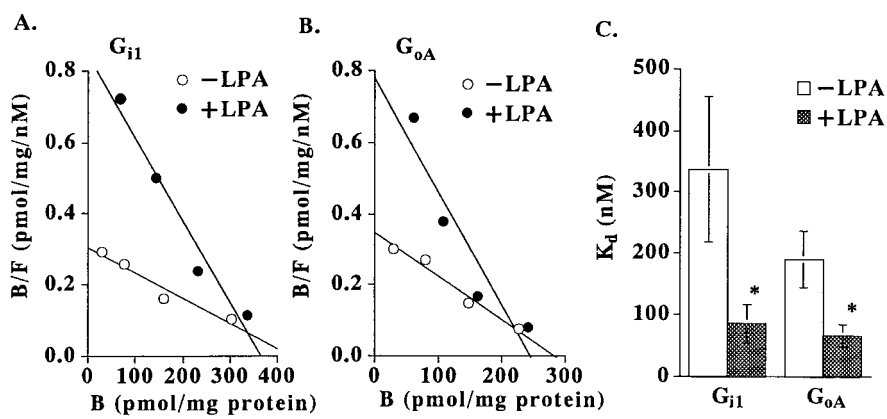

FIG. 2. Scatchard analysis of binding of [^{35}S]GTPγS to Sf21 membranes G-proteins. [^{35}S]GTPγS binding activity in the membrane containing the G protein (A, G_{i1}; B, G_{oA}) with Edg-2/Vzg-1 was assayed in the various concentration of GTPγS in the absence (○) or presence (●) of 1 μM of LPA. (C) LPA-dependent change of K_d values of [^{35}S]GTPγS binding to G_{i1} or G_{oA} with Edg-2/Vzg-1. * < 0.05, compared with the value of the membrane without LPA. Data represent the mean ± S.E.M. of four independent experiments.

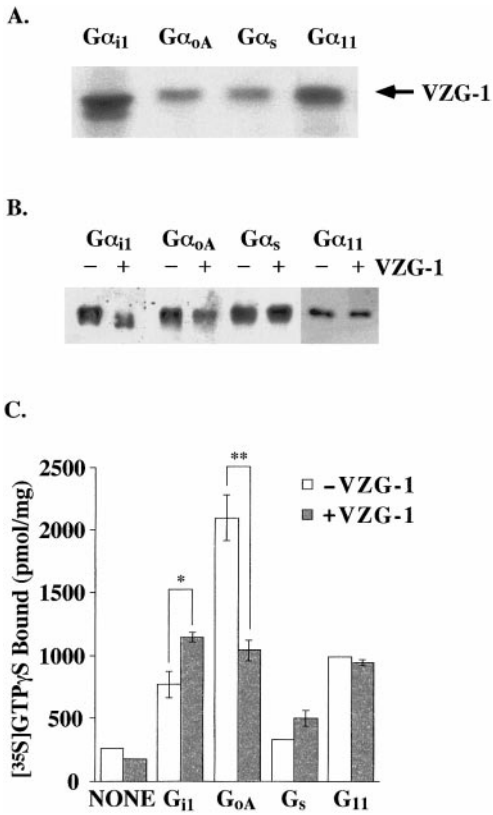

FIG. 3. Immunoblot analysis of expression of LPA receptor and G protein α subunits, and basal activity of [^{35}S]GTPγS binding. (A and B) Immunoblot analysis of expressed Edg-2/Vzg-1 (A) or G protein α subunits (B) in the infected Sf21 cells. Membrane proteins (1.3 μg) of the Sf21 cells expressing the indicated G protein with (+) or without (−) Edg-2/Vzg-1 were analyzed by immunoblotting using the specific antibody against the Edg-2/Vzg-1 or the corresponding type of α subunit respectively. (C) Basal activity of [^{35}S]GTPγS binding in the absence of LPA. [^{35}S]GTPγS binding activity in the membrane containing indicated G protein was assayed in the absence of LPA. Each sample was incubated for 1 hr at 30°C. * < 0.05, ** < 0.01, compared with the value of the membrane without Edg-2/Vzg-1. Data represent the mean ± S.E.M. of three independent experiments.

G_{oA}, G_s, and G_{11} are 1, 0.41, 0.38, and 0.84, respectively (Fig. 3A). The linearity of dilution-immunoreactivity relationship was observed in the range from the immunoreactivity in G_s preparation to that in G_{i1} one. It demonstrated that the amount of the Edg-2/Vzg-1 in the G_s-expressed membrane was similar to that in the G_{oA}-expressed membrane in which the significant increase in [^{35}S]GTPγS binding was detected. Figure 3B shows that all G protein α-subunits including $G\alpha_s$ were expressed in the reconstituted membranes with or without Edg-2/Vzg-1 by immunoblot analysis. Each antibody stained only one band corresponding to the expected molecular weight of recombinant α subunit. No significant immunoreactivity for G protein α subunits was observed by use of these antibodies in the insect cells which were uninfected or infected with wild type baculovirus (data not shown). Interestingly, the immunoreactivity of $G\alpha_{i1}$ and $G\alpha_{oA}$ was markedly decreased by co-infection with Edg-2/Vzg-1.

Intrinsic Activity of Edg-2/Vzg-1 to Stimulate G_{i1}

Figure 3C shows the basal [^{35}S]GTPγS binding in the preparation expressing each G protein with or without Edg-2/Vzg-1. As expected from the results with immunoblot analysis (Fig. 3B), the basal binding activity in the G_{oA}-preparation was markedly decreased in the presence of Edg-2/Vzg-1. There was no significant change in the basal binding in the G_s- or G_{11}-preparation between the preparations with or without Edg-2/Vzg-1 (Fig. 3C), being consistent with the results from the immunoblot analyses.

However, the basal binding in the G_{i1}-preparation was rather increased in the Edg-2/Vzg-1-coexpressed membrane (Fig. 3C), being inconsistent with the result of immunoblot analysis. In order to further characterize the increase in the basal [^{35}S]GTPγS binding in the G_{i1}-preparation, the LPA-dependent [^{35}S]GTPγS binding was normalized by dividing with the maximal [^{35}S]GTPγS binding which was obtained from the Scatchard Plot analysis by use of various concentrations of [^{35}S]GTPγS. As shown in Fig. 4A, there was no change in [^{35}S]GTPγS binding by various concentrations of LPA in the preparation expressing G_{i1} alone, and the LPA-induced changes in [^{35}S]GTPγS binding were found in the preparation expressing G_{i1} plus Edg-2/Vzg-1 as described above. Of interest is the finding that the normalized basal [^{35}S]GTPγS binding in the absence of LPA was significantly higher in the preparation expressing G_{i1} plus Edg-2/Vzg-1 than that expressing G_{i1} alone. On the other hand, there was no significant change in the basal binding between the preparations expressing G_{oA} and G_{oA} plus Edg-2/Vzg-1 (Fig. 4B).

We further characterized the kinetic changes in basal G protein activities by co-expression with Edg-2/Vzg-1 in the absence of LPA. In the Scatchard Plot analyses, the affinity for [^{35}S]GTPγS was increased by Edg-2/Vzg-1 coexpression in G_{i1}-preparation but not in G_{oA}-preparation (Figs. 5A and 5B). Average (± S.E.M.) of K_d value of [^{35}S]GTPγS binding in the G_{i1}-preparation was decreased from 695 ± 227 to 377 ± 119 nM by Edg-2/Vzg-1 expression (Fig. 5C).

DISCUSSION

The present study revealed two major findings, 1) LPA receptor, Edg-2/Vzg-1, selectively couples some species of G proteins including G_o in coexpression experiments, 2) Edg-2/Vzg-1 has an intrinsic activity to stimulate G_{i1}. Here we found that LPA strongly stimulates G_{i1} and G_{oA}, and weakly stimulates G_{11} through Edg-2/Vzg-1. The LPA-induced activation of G_{i1} is con-

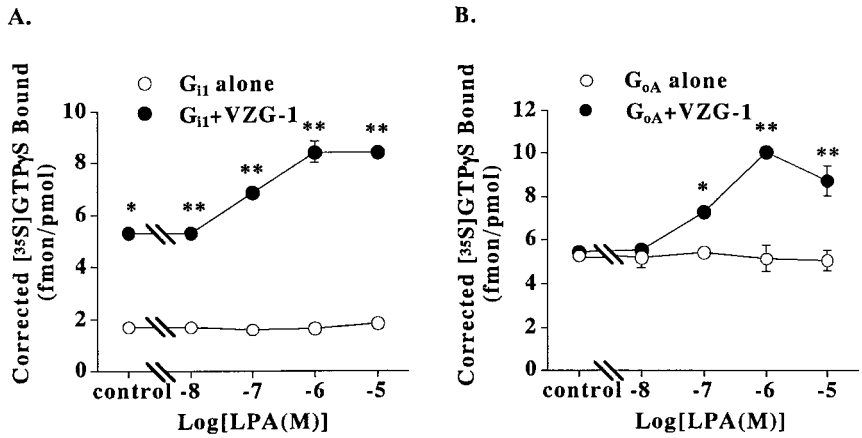

FIG. 4. Intrinsic stimulation of [^{35}S]GTPγS binding by expression of Edg-2/Vzg-1. A value of specifically bound [^{35}S]GTPγS per membrane proteins (fmol/mg protein) was divided by the value of maximal binding (pmol/mg protein) calculated in the presence of LPA. The membrane contained G protein with (●) or without (○) Edg-2/Vzg-1. * < 0.05, ** < 0.01, compared with the value of the membrane without Edg-2/Vzg-1. Data represent the mean ± S.E.M. of three independent experiments.

sistent with some previous reports which showed that the LPA stimulation inhibited an adenylate cyclase in the Edg-2/Vzg-1-expressed neuroblast cell line (16), and activated an endogenous G_{i1} in the membrane from the Edg-2/Vzg-1-expressed hepatoma cell line (24). However, the activation of G_o which is an abundant membrane bound protein in the brain (25) through Edg-2/Vzg-1 has not been reported. In this study, we demonstrate that G_{oA} is also activated through the Edg-2/Vzg-1 in the similar range of concentration of LPA to activate G_{i1}. Some parts of LPA-induced cellular events in the brain may be mediated through an activation of G_o.

We observed that LPA weakly but significantly activated G_{11} through Edg-2/Vzg-1 in the coexpression experiments. As the stoichiometry of receptor coupling to $G_{q/11}$ is generally very low, compared to G_i, G_o or G_s-coupled receptors (26), we should not neglect the physiological significance of functional coupling between Edg-2/Vzg-1 and G_{11}. LPA was reported to activate phospholipase C through a PTX-insensitive G protein (8, 27). The present result suggests that Edg-2/Vzg-1-mediated activation of G_{11} may lead to activate phospholipase C and to mobilize an increase in the concentration of intracellular calcium ion.

G_s could not be coupled to Edg-2/Vzg-1 in the expression system. It is possible that lower expression of functional receptors caused the apparent uncoupling. Because the LPA has the lipophilic detergent property, it was difficult to analyze ligand-receptor interactions (16, 28, 29). Therefore, we determined that the amount of LPA receptor in the G_s-expressed membrane was

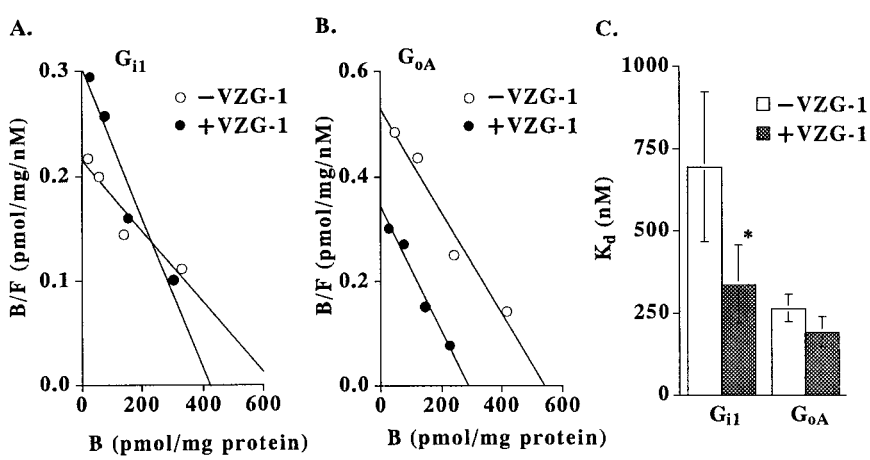

FIG. 5. Scatchard analysis of binding of [^{35}S]GTPγS in the absence of LPA. [^{35}S]GTPγS binding activity in the membrane containing the G protein (A, G_{i1}; B, G_{oA}) with (●) or without (○) Edg-2/Vzg-1 was assayed in the various concentration of [^{35}S]GTPγS in the absence of LPA. (C) Edg-2/Vzg-1-induced change of K_d values of [^{35}S]GTPγS binding to G_{i1} or G_{oA}. * < 0.05, compared with the value of the membrane without LPA. Data represent the mean ± S.E.M. of four independent experiments.

similar to that in the G_{oA}-expressed membrane by immunoblot analysis. The reason that Edg-2/Vzg-1 could not coupled to G_s might be not that the receptor expression was low.

Interestingly, the amount of G_{i1} or G_{oA} in the preparation coexpressing Edg-2/Vzg-1 was decreased compared with that of G_{i1} or G_{oA} in the preparation in the absence of Edg-2/Vzg-1. Some reports showed that the amount of G protein was decreased by the stimulation of its downstream effector (30, 31). Therefore, the decrease in both protein expressions found in this study may reflect down regulation caused by the downstream mechanisms in the infected cells.

We found that the addition of LPA increased the affinity (or decreased the K_d) of [^{35}S]GTPγS binding to either G_{i1} or G_{oA}. However, during the detailed analysis we noticed that the basal activity of [^{35}S]GTPγS binding to G_{i1} without LPA was higher in the Edg-2/Vzg-1 expressed cells than in unexpressed ones, despite the immunoreactive G_{i1} expression was markedly decreased by Edg-2/Vzg-1 expression. Further analysis revealed that the affinity of [^{35}S]GTPγS binding to G_{i1} in the absence of LPA was increased by this receptor expression, indicating that the Edg-2/Vzg-1 has an intrinsic activity to stimulate G_{i1}. Although we washed the membranes with serum-free buffer, the contamination of residual LPA or related compounds derived from serum used in the infection of Sf21 cells with baculoviruses can not excluded. However, as there was no significant increase in the affinity of [^{35}S]GTPγS binding to the reconstituted G_{oA}, it is unlikely that such a contamination exerts significant effects. These results indicate that the G_{i1} is selectively activated by Edg-2/Vzg-1 in the absence of any ligand. It is possible that no intrinsic activity observed in G_{oA}-expressed membranes resulted from lower expression of Edg-2/Vzg-1 indicated by immunoblot analysis. However, because of higher activity of LPA-induced [^{35}S]GTPγS binding in G_{oA}-expressed membranes than in G_{i1}-expressed membranes, the amount of the functional receptor may be enough to intrinsically activate G_{oA} if it was possible. Therefore the intrinsic activity of the receptor to G_{oA} may be relatively weak. Several reports suggested that some receptors were found to show an intrinsic activity in the absence of their ligands (32, 33). These receptors affected the basal level of second messengers, such as cAMP (34), but the kinetic change in a specific G protein through the intrinsic activity of receptors has not been reported.

It is interesting to discuss the physiological roles of intrinsic activity of the Edg-2/Vzg-1. cAMP is one of major second messenger to regulate neuronal differentiation such as neurite extension and gene expression (35, 36). As it is expected that the Edg-2/Vzg-1 itself activates G_i and inhibited cAMP production, the developmental change of Edg-2/Vzg-1 during neurogenesis (37) may have an influence on the cAMP signaling pathway and the neuronal differentiation. On the other hand, there are reports that some compounds possess the inverse agonist activity which represents the inhibition of G protein activity or second messenger production through metabotropic receptors (38–41). The observation of the intrinsic activity of Edg-2/Vzg-1 may lead to a discovery of an unknown inverse agonist to Edg-2/Vzg-1 LPA receptor.

In conclusion, we demonstrated here that a single type of LPA receptor, Edg-2/Vzg-1 is functionally coupled with some G proteins, G_{i1}, G_{oA} and G_{11}, and the receptor has an intrinsic activity to stimulate G_{i1}.

ACKNOWLEDGMENTS

We thank Dr. T. Haga at the University of Tokyo for providing us with the anti-G protein antibodies, and Dr. J. Chun at University of California for the anti-Edg-2/Vzg-1 antibody. We thank N. Nomura and K. Kakizawa (Nagasaki Univ.) for technical assistance.

REFERENCES

1. Moolenaar, W. H., Kranenburg, O., Postma, F. R., and Zondag, G. C. (1997) *Curr. Opin. Cell Biol.* **9,** 168–173.
2. van der Bend, R. L., de Widt, J., van Corven, E. J., Moolenaar, W. H., and van Blitterswijk, W. J. (1992) *Biochem. J.* **285,** 235–240.
3. Malcolm, K. C., Sable, C. L., Elliott, C. M., and Exton, J. H. (1996) *Biochem. Biophys. Res. Commun.* **225,** 514–519.
4. Schmidt, M., Lohmann, B., Hammer, K., Haupenthal, S., Nehls, M. V. C., and Jakobs, K. H. (1998) *Mol. Pharmacol.* **53,** 1139–1148.
5. Tigyi, G., Dyer, D. L., and Miledi, R. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1908–1912.
6. Jalink, K., van Corven, E. J., and Moolenaar, W. H. (1990) *J. Biol. Chem.* **265,** 12232–12239.
7. Howe, L., and Marshall, C. J. (1993) *J. Biol. Chem.* **268,** 20717–20720.
8. van Corven, E. J., Hordijk, P. L., Medema, R. H., Bos, J. L., and Moolenaar, W. H. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1257–1261.
9. Jalink, K., van Corven, E. J., Hengeveld, T., Morii, N., Narumiya, S., and Moolenaar, W. H. (1994) *J. Cell Biol.* **126,** 801–810.
10. Buhl, A. M., Johnson, N. L., Dhanasekaran, N., and Johnson, G. L. (1995) *J. Biol. Chem.* **270,** 24631–24634.
11. Stewart, G. R., and Pearlman, A. L. (1987) *J. Neurosci.* **7,** 3325–3333.
12. Lo Turco, J. J., and Kriegstein, A. R. (1991) *Science* **252,** 563–566.
13. Checovich, W. J., and Mosher, D. F. (1993) *Arterioscler. Thromb.* **13,** 1662–1667.
14. Zhang, Q., Checovich, W. J., Peters, D. M., Albrecht, R. M., and Mosher, D. F. (1994) *J. Cell Biol.* **127,** 1447–1459.
15. Chenn, A., and McConnell, S. K. (1995) *Cell* **82,** 631–641.
16. Hecht, J. H., Weiner, J. A., Post, S. R., and Chun, J. (1996) *J. Cell Biol.* **135,** 1071–1083.
17. Takahashi, T., Nowakowski, R. S., and Caviness, V. S. Jr. (1993) *J. Neurosci.* **13,** 820–833.

18. Doetsch, F., Garcia-Verdugo, J. M., and Alvarez-Buylla, A. (1997) *J. Neurosci.* **17,** 5046–5061.
19. Guo, Z., Liliom, K., Fischer, D. J., Bathurst, I. C., Tomei, L. D., Kiefer, M. C., and Tigyi, G. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 14367–14372.
20. Cha, K., Bruel, C., Inglese, J., and Khorana, H. G. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 10577–10582.
21. Fukushima, N., Kohno, M., Kato, T., Kawamoto, S., Okuda, K., Misu, Y., and Ueda, H. (1998) *Peptides* **19,** 811–819.
22. Tokuyama, S., Hirata, K., Ide, A., and Ueda, H. (1997) *Neurosci. Lett.* **233,** 141–144.
23. Yoshida, A., Ogura, A., Imagawa, T., Shigekawa, M., and Takahashi, M. (1992) *J. Neurosci.* **12,** 1094–1100.
24. Fukushima, N., Kimura, Y., and Chun, J. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 6151–6156.
25. Neer, E. J., Lok, J. M., and Wolf, L. G. (1984) *J. Biol. Chem.* **259,** 14222–14229.
26. Pang, I. H., and Sternweis, P. C. (1990) *J. Biol. Chem.* **265,** 18707–18712.
27. Hildebrandt, J. (1995) *Membr. Biol.* **144,** 49–58.
28. van der Bend, R. L., Brunner, J., Jalink, K., van Corven, E. J., Moolenaar, W. H., and van Blitterswijk, W. J. (1992) *EMBO J.* **11,** 2495–2501.
29. Thomson, F. J., Perkins, L., Ahern, D., and Clark, M. (1994) *Mol. Pharmacol.* **45,** 718–723.
30. Gupta, S. K., and Mishra, R. K. (1992) *J. Mol. Neurosci.* **3,** 197–201.
31. Sands, S. A., Dickerson, D. S., Morris, S. J., and Chronwall, B. M. (1997) *Endocrine* **6,** 325–333.
32. Costa, T., and Herz, A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7321–7325.
33. Freissmuth, M., Selzer, E., and Schutz, W. (1991) *Biochem. J.* **275,** 651–656.
34. Kozell, L. B., and Neve, K. A. (1997) *Mol. Pharmacol.* **52,** 1137–1149.
35. Tigyi, G., Fischer, D. J., Sebok, A., Marshall, F., Dyer, D. L., and Miledi, R. (1996) *J. Neurochem.* **66,** 549–558.
36. Gan, L., Perney, T. M., and Kaczmarek, L. K. (1996) *J. Biol. Chem.* **271,** 5859–5865.
37. Chun, J., and Jaenisch, R. (1996) *Mol. Cell. Neurosci.* **7,** 304–321.
38. Jin, J., Mao, G. F., and Ashby, B. (1997) *Br. J. Pharmacol.* **121,** 317–323.
39. Merkouris, M., Mullaney, I., Georgoussi, Z., and Milligan, G. (1997) *J. Neurochem.* **69,** 2115–2122.
40. Newman-Tancredi, A., Conte, C., Chaput, C., Spedding, M., and Millan, M. J. (1997) *Br. J. Pharmacol.* **120,** 737–739.
41. Ueda, H., Misawa, H., Fukushima, N., Katada, T., Ui, M., and Satoh, M. (1996) *J. Neurochem.* **66,** 845–851.