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Schwann cell survival mediated by the signaling phospholipid lysophosphatidic acid

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ABSTRACT **Lysophosphatidic acid (LPA) is a bioactive phospholipid with properties of an extracellular growth factor for many cell lines, including those derived from neuroblastomas. However, the relevance of LPA signaling to the normal, developing nervous system is unknown, in part reflecting the previous unavailability of cloned receptor genes. Recent studies of the first such gene, encoding the G protein-coupled receptor LP_{A1}/VZG-1 (lysophospholipid receptor A1/ventricular zone gene-1), revealed a major locus of expression in oligodendrocytes and Schwann cells (SCs) during development, suggesting an influence of LPA on these myelinating cells. Here we report that LPA (≥10 nM) is a potent survival factor for cultured neonatal SCs, with survival activity equaling the maximal effect of neuregulin, the major peptide SC survival factor. LPA activates a pharmacologically defined signaling pathway in SCs, involving G_i and phosphoinositide 3-kinase. Moreover, LPA's effect depends on Akt, a downstream kinase that can mediate phosphoinositide 3-kinase-dependent survival, as demonstrated by both Western blot and transfection analyses. Overexpression of functional epitope-tagged LP_{A1}/VZG-1 protein decreases SC apoptosis in response to serum withdrawal. These data demonstrate a role for extracellular LPA and its receptor LP_{A1}/VZG-1 in SC survival and, more broadly, implicate G protein-coupled receptor-mediated lysophospholipid signaling as a significant mechanism in neural development.**

The simple phospholipid lysophosphatidic acid (LPA; 1-acylglycerol-3-phosphate) can serve as an extracellular signaling molecule with effects on the morphology, ionic conductance, and growth of numerous cell lines (reviewed in ref. 1). Although LPA long has been known to act through a putative G protein-coupled receptor (GPCR), the lack of cloned receptors has made it difficult to assess the biological significance of LPA signaling. The recent cloning of mammalian receptors for both LPA (2–5, 9) and the related signaling lysophospholipid sphingosine-1-phosphate (S1P) (3, 6–8, 10) has allowed the identification of potential target tissues based on receptor distribution.

The first cloned LPA receptor, LP_{A1}/VZG-1 (lysophospholipid receptor A1/ventricular zone gene-1), has been demonstrated to couple to at least two distinct G protein pathways: a pertussis toxin (PTX)-sensitive $G_{i/o}$ pathway leading to adenylate cyclase inhibition, serum response element activation, and cell cycle progression, and a PTX-insensitive pathway acting through Rho to influence the actin cytoskeleton (2, 9). Detailed expression studies of this receptor have suggested roles for LPA in the regulation of multiple cell types during nervous system development. The gene encoding LP_{A1}/VZG-1 was isolated by virtue of its expression in ventricular zone neuroblasts of the embryonic cerebral cortex (2). It is expressed throughout the neurogenetic period by these cells, which can respond to LPA application with multiple ionic conductance changes (11). In contrast, postnatal brain expression of lp_{A1}/*vzg-1* is confined to a class of glial cells, oligodendrocytes, during the period of myelination (12), suggesting a physiological role for LPA signaling in myelinating cells.

Myelin, formed by oligodendrocytes in the central nervous system and Schwann cells (SCs) in the peripheral nervous system, is a fatty glial membrane extension that ensheaths neuronal axons, insulating them and facilitating saltatory nerve conduction (13). Because a variety of neurological disorders, such as multiple sclerosis and Charcot-Marie-Tooth disease, involve disruptions of myelinating cell function (14), identification of novel signaling mechanisms influencing these cells could provide new therapeutic targets. Previous reports that serum can markedly affect the differentiation and survival of both oligodendrocytes (15–17) and SCs (18) *in vitro* predict a prominent role for LPA, which is present in serum at micromolar concentrations (19). Here we demonstrate LP_{A1}/VZG-1 gene expression in SCs both *in vivo* and *in vitro* and show that LPA can potently and specifically promote the survival of SCs through a defined G protein-mediated signaling pathway. These results demonstrate a role for LP_{A1}/VZG-1-mediated LPA signaling in myelinating cells and implicate lysophospholipids as a class of molecules influencing multiple stages of nervous system development.

EXPERIMENTAL PROCEDURES

Reagents and Pharmacological Treatments. Lyophilized LPA (1-oleoyl-2-hydroxy-sn-glycero-3-phosphate; Avanti Polar Lipids) was resuspended in 1% fatty acid-free (FAF) BSA (Sigma). S1P (Biomol, Plymouth Meeting, PA) was dissolved in methanol, lyophilized, and resuspended in 0.01% FAF BSA. S1P activity was confirmed in an independent assay by using the B103 neuroblastoma cell line (9). PI3K inhibitors wortmannin, and LY294002, and the mitogen-activated protein kinase pathway inhibitor PD98059 (Calbiochem) were dissolved in DMSO at 10 mM, 50 mM, and 100 mM, respectively, and diluted in PBS. Pharmacological inhibitors were added at the time of LPA treatment for *in situ* end labeling + (ISEL+) experiments or 2 h before LPA treatment for Akt experiments. PTX (Calbiochem) was added to cultures 18 h before serum withdrawal, at the time of serum withdrawal and LPA addition, and again 24 h later. Efficacy of PTX was confirmed in an *in vitro* ADP ribosylation assay by using SC membranes (data not shown). Truncated GST-NRGβ1 (encompassing the EGF-like motif; see ref. 20) was the generous gift of Cary Lai, Scripps Research Institute, La Jolla, CA.

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Abbreviations: LPA, lysophosphatidic acid; LP_{A1}/VZG-1, lysophospholipid receptor A1/ventricular zone gene-1; SC, Schwann cell; PI3K, phosphoinositide 3-kinase; GPCR, G protein-coupled receptor; PTX, pertussis toxin; S1P, sphingosine-1-phosphate; NRG, neuregulin; ISEL+, *in situ* end labeling +.

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Primary SC Culture. Sciatic nerves were excised from postnatal day 3 rat pups, and SCs were purified essentially as described (21). Cells were grown on poly-L-lysine (0.1 mg/ml)-coated dishes or on poly-L-lysine- and laminin (10 μg/ml)-coated eight-well plastic Chamber Slides (Nunc) and glass coverslips. Growth medium was DMEM (GIBCO) supplemented with 10% FCS, 20 μg/ml of pituitary extract (Sigma), 2 μM forskolin, and penicillin/streptomycin. The serum-free medium used was DMEM alone, with only penicillin/streptomycin added. SC cultures were >98% pure as assessed by anti-P_0 and anti-S100 (Dako) immunofluorescence. For proliferation experiments, cultures at 24 h postserum withdrawal were pulsed for an additional 24 h with BrdUrd, and cells were fixed and processed for BrdUrd immunofluorescence (BrdUrd Labeling and Detection Kit; Boehringer Mannheim).

Northern Blot Analysis and *in Situ* Hybridization. Total RNA was isolated from mouse sciatic nerves at various ages and from cultured rat SCs by using TRIzol (GIBCO). Northern blots of 10–15 μg RNA were made by using standard protocols and probed with ^{32}P-labeled ORF fragments of murine lysophospholipid (LP) family receptor cDNAs (3, 10) at 5×10^6 cpm/ml. For lp_{A1}/*vzg-1 in situ* hybridization analyses, SCs were grown on two-well glass Chamber Slides and fixed and hybridized with sense or antisense riboprobes as described (12).

ISEL+ Identification of Apoptotic Cells. SCs were grown to near confluence on eight-well Chamber Slides. Medium was changed to serum-free DMEM alone with or without LPA and/or other treatments. Cells were fixed in 4% paraformaldehyde 48 h later, and ISEL+ analyses were performed as described (22, 23). All experiments were performed at least three times in duplicate, and more than 1,000 cells were counted per well.

Akt Western Blot Analysis. SCs were grown to confluence in six-well dishes and switched to DMEM/1% FCS for 24 h to reduce basal Akt phosphorylation. Media were changed to serum-free DMEM with or without pharmacological inhibitors, and 2 h later LPA was added. Cell samples were analyzed by Western blotting by using the PhosphoPlus Ser473 Akt Antibody kit (New England Biolabs).

Transfection Studies. SCs were grown on glass coverslips to ≈80% confluency and were transfected with various expression constructs for 3 h by using Lipofectamine Plus (GIBCO) in DMEM/10% FCS. pHA-Akt(K179M) encodes an Akt protein rendered kinase-inactive by a point mutation in the catalytic domain (24, 25) and was the kind gift of David Kaplan (McGill University) and Michael Greenberg (Harvard Medical School). pHA-mΔ4–129Akt encodes a constitutively active form of Akt (26, 27) and was the kind gift of Richard Roth (Stanford University) and Michael Greenberg. pFLAG/VZG-1 (9) contains the complete ORF of murine lp_{A1}/*vzg-1* fused to an N-terminal FLAG epitope sequence in the plasmid pFLAG/CMV1 (Kodak/IBI). pFLAG/BAP control plasmid [which has a CMV vector backbone similar to that of the HA-Akt(K179M) construct] was obtained from Kodak/IBI. After transfection, cells were switched to growth medium for 12 h to allow for protein expression and then switched to serum-free DMEM with or without LPA for 24 h. Cells were processed sequentially for fluorescent ISEL (Fluorescein In Situ Cell Death Kit; Boehringer Mannheim) and anti-FLAG immunofluorescence (1:6,000, for pFLAG/BAP and pFLAG/VZG-1, anti-FLAG M2 mAb; Kodak/IBI) or antihemagglutinin (HA) immunofluorescence (1:500, for Akt constructs, anti-HA 12CA5 mAb; Boehringer Mannheim). Three experiments were performed in triplicate, and all transfected cells were counted in each coverslip. In experiments shown in Fig. 4, baseline apoptosis from transfection alone was assessed in parallel coverslips maintained in medium containing 10% FCS (in which untransfected cells exhibited <1% apoptosis), and this value was subtracted from each experimental value.

RESULTS

The LP_{A1}/VZG-1 Gene Is Expressed by Schwann Cells *in Vivo* and *in Vitro*. To understand the effects of LPA signaling on myelinating cells, primary cultures of neonatal rat sciatic nerve SCs (21) were used as a model system. During peripheral nerve myelination in the early postnatal period, the matching of SC and axon numbers is regulated by SC apoptosis (18, 28), resulting in the mature 1:1 relationship between axons and myelinating SCs (13). A similar apoptotic process can be initiated in primary cultures of SCs by withdrawal of serum (18), which includes SC survival factors. LPA is present in serum in the micromolar range (19), making it a candidate molecule for mediating its survival-promoting effects. The high-affinity LPA receptor LP_{A1}/VZG-1 (2, 3, 9) is well placed to mediate such effects because its transcript is expressed by

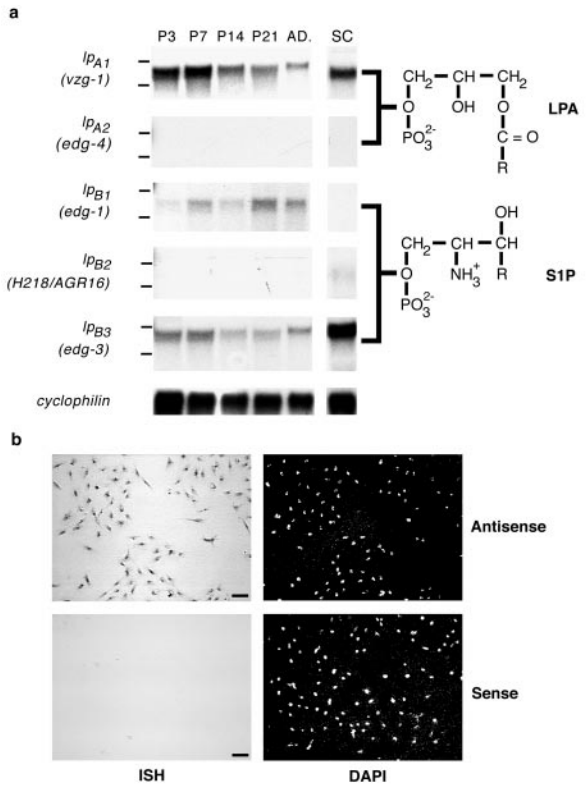

FIG. 1. Members of the lysophospholipid (*lp*) receptor gene family are expressed by SCs *in vivo* and *in vitro*. (*a*) Northern blots of total RNA from sciatic nerve at various postnatal (P) days and adult (AD) or from cultured neonatal sciatic nerve SCs (SC) were probed for transcripts encoding the five known members of the LP GPCR family (3). Lp_A genes encode LPA receptors, and lp_B genes encode S1P receptors (see refs. 3 and 10 for nomenclature; alternate gene names are given in parentheses). The structures of the two ligands are shown at right (R represents the acyl chain, which can vary in length). The LPA receptor lp_{A1}/*vzg-1* is expressed at high levels by SCs both *in vivo* and *in vitro*. Expression of a related LPA receptor gene (lp_{A2}/*edg-4*) (5) is undetectable. Of the three genes encoding S1P receptors (3, 6–8, 10), only lp_{B3} is expressed prominently by SCs *in vitro*, although both lp_{B1} and lp_{B3} are expressed *in vivo*. A cyclophilin probing is shown as a loading and transfer control. Markers represent 4.4 (upper bands) and 2.4 (lower bands) kb. (*b*) *In situ* hybridization (ISH) by using an antisense lp_{A1}/*vzg-1* riboprobe demonstrates that all SCs *in vitro* express this receptor gene. A sense probe produced no hybridization signal. Nuclear 4′,6-diamidino-2-phenylindole (DAPI) staining of the same microscope field is shown to the right of each ISH panel. (Bars = 80 μm.)

sciatic nerve SCs *in vivo* throughout the postnatal period, with highest expression observed in the first week (Fig. 1*a*). Similarly, $lp_{A1}/vzg\text{-}1$ was expressed at comparable levels by neonatal SCs *in vitro* (Fig. 1*a*). *In situ* hybridization experiments on SCs *in vitro* by using an $lp_{A1}/vzg\text{-}1$ riboprobe confirmed that all cells in the cultures expressed this gene (Fig. 1*b*). Other members of the recently identified LP GPCR family (3), including receptors for the structurally and functionally related LP S1P (6–8, 10), also were expressed by SCs (Fig. 1*a*). These data indicate the potential action of receptor-mediated LP-signaling pathways during SC maturation.

LPA Is a Potent, Specific Survival Factor for Neonatal Schwann Cells. SCs exhibit little cell death when cultured in medium containing FCS (data not shown; ref. 18). By 48 h after serum withdrawal, approximately 60–75% of SCs undergo cell death, and by 72 h, most (80–90%) SCs die (18). To examine possible survival-promoting effects of LPA, apoptosis was induced in SCs by culturing in serum-free medium (DMEM alone). In control cultures, significant cell loss comparable to that observed in prior reports (18, 29) was observed by 48 h after serum withdrawal, with many cells exhibiting apoptosis (Fig. 2*a*). Treatment of SCs with 1 μM LPA over 48 h led to a reduction (mean of $\approx$55%; see Fig. 2*b*) in the number of apoptotic cells, as identified both morphologically and by ISEL+ (22, 23), a technique that labels the fragmented DNA ends that are an apoptotic hallmark (30) (Fig. 2*a*). LPA significantly reduced SC apoptosis, assessed by using ISEL+, at low nanomolar concentrations (Fig. 2*b*), implicating the activation of the high-affinity receptor LP_{A1}/VZG-1, which continues to be expressed after serum withdrawal (Fig. 2*b Inset*). LPA treatment did not increase the number of SCs incorporating BrdUrd (Fig. 2 *a* and *c*), demonstrating that the dose-dependent maintenance of cell number in LPA-treated cultures (Fig. 2*d*) was the result of increased cell survival rather than compensatory cell proliferation. By combining the ISEL+ (Fig. 2*b*) and cell number (Fig. 2*d*) data, the percentage of SCs undergoing cell death by 48 h after serum withdrawal is estimated to be approximately 66%. This estimate is consistent with a prior report (18) that used the independent MTT assay, confirming that ISEL+ provides an accurate picture of SC apoptosis in these cultures.

Despite the prominent expression of at least one of its receptors (3, 6, 10) (Fig. 1*a*), treatment with S1P did not reduce SC apoptosis (Fig. 2*e*). These data indicate that the survival effect is LPA-specific and suggest different functions for related lipid mediators and their cognate GPCRs in SCs. The efficacy of LPA in promoting survival was compared further with that of a family of proven peptide survival factors that signal through receptor tyrosine kinases, the neuregulins [NRGs (18, 28); isoforms also termed ARIA (31), NDF (32), or GGF (33)]. LPA (1 μM) was as effective in promoting SC survival as a maximal dose (100 ng/ml) of a highly active (18, 20) NRG-β peptide encompassing the EGF-like domain (Fig. 2 *d* and *f*). LPA and NRG-β did not have a synergistic effect on survival when added together at maximal doses (Fig. 2*f*); when combined at submaximal doses, the resulting reduction in apoptosis approached that of the maximal dose of either ligand (data not shown). These data suggest that the distinct receptor-mediated signaling pathways activated by LPA and NRG-β converge on at least some common downstream effectors.

LPA Promotes Schwann Cell Survival via a Signal Transduction Pathway Involving G_i, PI3K, and Akt. Recent reports (24–26, 34) have identified a signal transduction pathway involving sequential activation of PI3K and the serine-threonine kinase Akt that can lead to the prevention of apoptosis in various cell types through phosphorylation of BAD, a procell death member of the Bcl-2 family. Because LPA can activate PI3K via the $\beta\gamma$ subunits of G_i (e.g., in COS cells; ref. 35), we asked whether this pathway is activated by

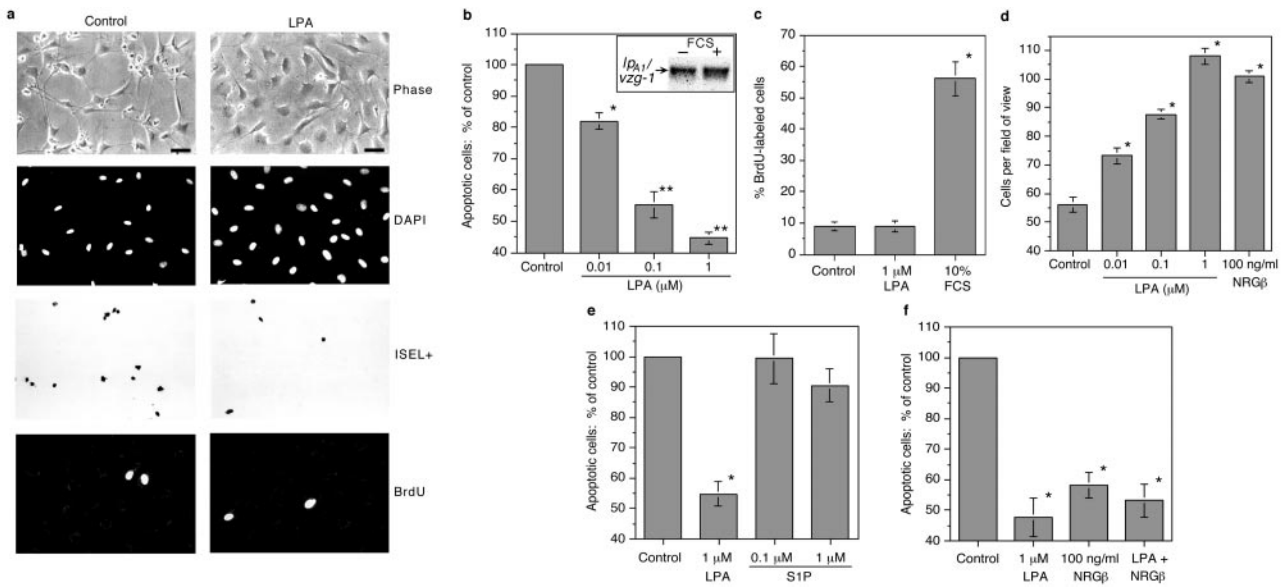

FIG. 2. LPA is a specific, potent survival factor for Schwann cells. (*a*) Control and LPA (1 μM)-treated SC cultures 48 h after serum withdrawal (DAPI and ISEL+ panels show the same fields). Total cell number is always greater in LPA-treated cultures, whereas the number of cells with apoptotic morphology or ISEL+ labeling is decreased. In contrast, LPA treatment does not affect BrdUrd incorporation. (Bars = 30 μM.) (*b*) LPA significantly decreases apoptosis as detected by ISEL+ at doses as low as 10 nM. Number of ISEL+-labeled cells is expressed as percentage of control. *, $P < 0.05$; **, $P < 0.0001$ (vs. control). (*Inset*) Northern blot analysis shows that $lp_{A1}/vzg\text{-}1$ expression is maintained after serum withdrawal. (*c*) BrdUrd incorporation is not increased by LPA treatment, as it is after addition of 10% FCS. *, $P < 0.0001$ (vs. control). (*d*) The survival-promoting effect of LPA also is evidenced by increased maintenance of cell number after 48-h serum withdrawal. *, $P < 0.0001$ (vs. control). (*e*) The LP S1P does not promote SC survival (S1P vs. control, $P > 0.3$). *, $P < 0.0001$ (vs. control). (*f*) LPA (1 μM) is as effective in promoting SC survival as a maximal dose (100 ng/ml, determined in pilot experiments) of NRG-β (ref. 18; see also *d*), a proven SC survival factor (18, 28). LPA and NRG-β do not synergize at these maximal doses. *, $P < 0.01$ (vs. control). In all experiments control is serum-free DMEM alone. (Bars in *b–f* represent means ± SEM of 3–5 experiments performed in duplicate.) *P* values are from ANOVA with Fisher's protected least significant difference post-hoc analysis.

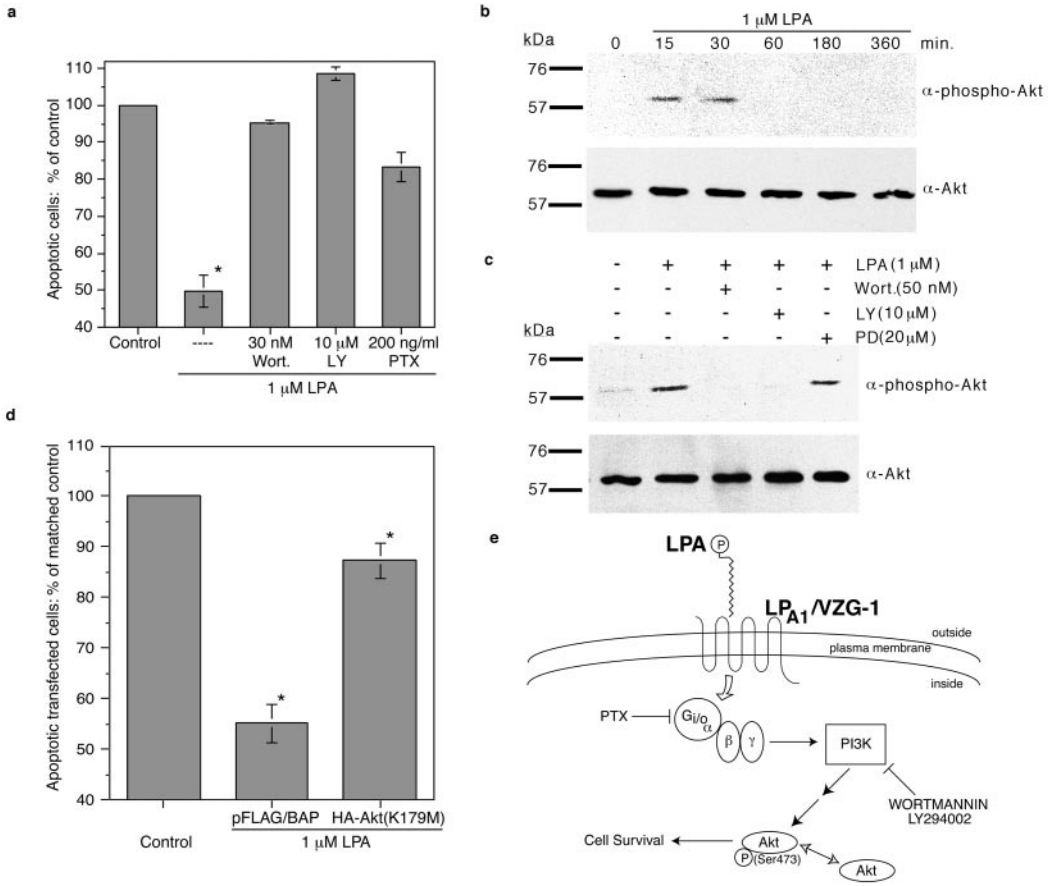

FIG. 3. Promotion of SC survival by LPA involves G_i and the PI3K/Akt pathway. (*a*) LPA-dependent survival ("—" lane) is reduced significantly by PTX (200 ng/ml), indicating involvement of G_i, and is blocked completely by wortmannin (Wort.; 30 nM) and LY294002 (LY; 10 μM), indicating dependence on PI3K. Bars represent means ± SEM of 3–4 experiments performed in duplicate. *, $P < 0.0001$ by *t* test with matched control. Other means were not significantly different from matched controls (i.e., pharmacological inhibitor alone). (*b* and *c*) Western blots probed with antibodies specific for Ser-473-phosphorylated Akt (α-phospho-Akt), with parallel control blots probed with antibodies detecting all Akt (α-Akt). Treatment of SCs with LPA induces a transient increase in Akt phosphorylation at a site required for its activation (34) (*b*) This LPA-induced phosphorylation is blocked by Wort. or LY, but not by the mitogen-activated protein kinase pathway inhibitor PD98059 (PD, *c*), consistent with data showing that PI3K activation is upstream of Akt activation (34). (*d*) The ability of 1 μM LPA to inhibit apoptosis is reduced significantly in cells transfected with an expression construct encoding a kinase-inactive form of Akt [HA-Akt(K179M)] compared with control (pFLAG/BAP)-transfected cells. *, Significant difference ($P < .01$, ANOVA with Fisher's protected least significant difference post-hoc analysis) between these two conditions; data are expressed as percentage of control values (serum-free medium alone) within each transfection condition. Bars represent means ± SEM of three experiments performed in triplicate. (*e*) A schematic illustrating the proposed signal-transduction pathway important for LPA-mediated survival in SCs. LPA binds to its receptor, LP_{A1}/VZG-1 (see Fig. 4), which activates a PTX-sensitive $G_{i/o}$ family G protein (9); G protein activation leads to the activation of PI3K (which is blocked by Wort. and LY), probably via the $\beta\gamma$ subunits (35); PI3K activation leads to the activation of Akt (34), including its phosphorylation at Ser-473; and activated Akt promotes cell survival via mechanisms likely including the phosphorylation of BAD (26).

LPA treatment in SCs. Prevention of apoptosis by LPA was inhibited by pretreatment of SCs with PTX (Fig. 3*a*), indicating the involvement of G_i (or G_o) to which LP_{A1}/VZG-1 has been demonstrated to couple directly (9). LPA-dependent survival effects also were blocked completely by two mechanistically distinct PI3K inhibitors, wortmannin and LY294002 (Fig. 3*a*). Because NRG also can activate a PI3K pathway (36), these data could explain the inability of LPA and NRG-β to synergize (see above, Fig. 2*f*). Addition of 1 μM LPA to SCs in serum-free medium induced a rapid and transient increase in the phosphorylation of Akt at a site (Ser-473) required for its activation (34), as detected by Western blot analysis (Fig. 3*b*). This accumulation of phospho-Akt in response to LPA depended on PI3K, because it was blocked by either wortmannin or LY294002, but not by the mitogen-activated protein kinase pathway inhibitor PD98059 (Fig. 3*c*). In addition, transfection of SCs with an expression construct encoding a kinase-inactive form of Akt [HA-Akt(K179M)] significantly reduced the ability of LPA to inhibit apoptosis induced by serum withdrawal (Fig. 3*d*; see Fig. 4 for examples of the transfection/apoptosis assay), confirming that Akt activation is a necessary step in LPA-mediated survival. As expected, transfection with HA-Akt(K179M) greatly increased baseline apoptosis (by ≈170%), whereas transfection with a constitutively active form of Akt (HA-mΔ4–129Akt) reduced baseline apoptosis (by ≈50%; data not shown). A schematic illustrating a signaling pathway for LPA-mediated SC survival consistent with these data and with prior reports is shown in Fig. 3*e*. Together, these data identify LPA as an activator of the PI3K/Akt pathway and demonstrate the activity of this pathway in SC survival.

The High-Affinity Receptor LP_{A1}/VZG-1 Is Involved in LPA-Dependent Schwann Cell Survival. The prominent expression of *lp_{A1}/vzg-1* in the absence of another reported LPA receptor gene (3, 5) (Fig. 1*a*), along with the effectiveness of LPA at low nanomolar doses (Fig. 2*b*), supported the hypothesis that this high-affinity receptor can mediate LPA-dependent SC survival. To address this issue, cells were transfected with an expression construct encoding a functional FLAG epitope-tagged LP_{A1}/VZG-1 (9). After transfection,

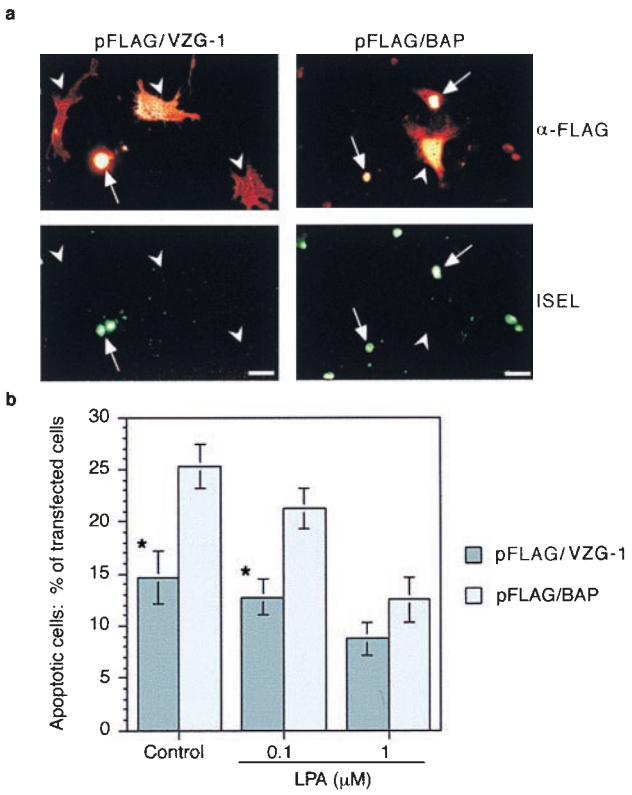

FIG. 4. Overexpression of the LPA receptor LP_{A1}/VZG-1 reduces SC apoptosis. (*a*) SCs transfected with pFLAG/VZG-1 (encoding FLAG epitope-tagged LP_{A1}/VZG-1) or with pFLAG/BAP (encoding FLAG-tagged bacterial alkaline phosphatase control protein), double-labeled for α-FLAG immunofluorescence (red) and fluorescent ISEL (green). Double-labeled, apoptotic transfected cells (arrows), as well as healthy transfected cells (arrowheads), are observed. (Bars = 30 μM.) (*b*) Overexpression of LP_{A1}/VZG-1 significantly reduces apoptosis resulting from serum withdrawal both with and without a submaximal (0.1 μM) dose of LPA. LP_{A1}/VZG-1 overexpression also modestly, but not significantly, potentiated the effect of a maximal dose (1 μM) of LPA. Bars represent means ± SEM of three experiments performed in triplicate. *, $P < 0.005$ (vs. pFLAG/BAP transfection control within each LPA treatment condition) by ANOVA and Fisher's protected least significant difference post-hoc analysis.

serum was withdrawn, and apoptotic transfected cells were identified 24 h later by double-labeling for anti-FLAG immunofluorescence and fluorescent ISEL (Fig. 4*a*). Overexpression of epitope-tagged LP_{A1}/VZG-1 significantly reduced SC apoptosis associated with serum withdrawal, both with and without a submaximal (0.1-μM) dose of LPA (Fig. 4*b*). LP_{A1}/VZG-1 overexpression only marginally (not significantly) potentiated the effect of a maximal LPA concentration (1 μM; Fig. 4*b*); this likely reflects near-maximal activation of endogenous receptors and/or downstream effectors at this dose. The decrease in apoptotic cells overexpressing LP_{A1}/VZG-1 even in the absence of LPA likely reflects residual effects of receptor activation by serum-derived LPA present in the medium prior to serum withdrawal (see *Experimental Procedures*) and/or basal receptor coupling activity, which has been observed with overexpression of GPCRs (9, 37). It is further notable that the low, effective LPA concentrations observed for SC survival (Fig. 2*b*) are consistent with those activating other cellular responses mediated by LP_{A1}/VZG-1 (2, 9). These results, together with the receptor gene expression data noted above, suggest that prevention of SC apoptosis by LPA is mediated by the high-affinity LPA receptor LP_{A1}/VZG-1.

DISCUSSION

The recent cloning of mammalian GPCRs for LPA (2–5, 9) as well as homologous receptors for S1P (3, 6–8, 10) have provided essential tools with which to ascertain the biological significance of extracellular LP signaling in complex tissues such as the nervous system. Our observation that the LPA receptor gene lp_{A1}/*vzg-1* is expressed by oligodendrocytes and SCs (ref. 12; Fig. 1) suggested a role for LPA signaling in myelinating cell biology. The data presented here identify LPA as a potent survival factor for neonatal SCs, with an efficacy equaling that of NRG.

SCs appear to receive survival and differentiation signals from growing axons during peripheral nerve development and regeneration (13, 28). Although the lipid nature of LPA makes it experimentally difficult to assess whether it is produced by peripheral nerve axons, as are NRGs (32, 38), it should be noted that, as with the NRGs, LPA can exist in both membrane-bound and soluble forms. LPA also could be released by SCs themselves as a specific, autocrine product or through lipid metabolism during the elaboration of the myelin sheath, which is about 80% lipid, including complex phospholipids (13). Moreover, because LPA is released by activated platelets (19), it is likely to be present after peripheral nerve injury, where it could influence nerve regeneration by promoting SC survival. It will be important for future studies to address the possible cellular sources of signaling LPA acting on SCs *in vivo*.

The nature of the LPA molecule and the signal transduction mechanisms involved in its cellular action lend particular novelty to these results. As a lipid-signaling molecule, LPA differs from the previously identified peptide growth and survival factors for SCs, such as the NRGs, platelet-derived growth factor (PDGF), and fibroblast growth factor (13, 18, 28). In addition, the GPCR mechanisms underlying LPA's action are distinct from those of the peptide factors, which signal through receptor tyrosine kinases (RTKs). The operation of G-protein-signaling pathways increases the repertoire of SC responses to the intracellular milieu, although it is certain that GPCR- and RTK-signaling pathways share some common components. An example is provided by our data showing that LPA treatment of SCs leads to the rapid accumulation of phosphorylated (activated) Akt in a PI3K-dependent manner and that Akt activity is necessary for LPA-mediated survival (Fig. 3 *b–d*). Peptide growth factors such as PDGF and insulin-like growth factor I also inhibit apoptosis by activating sequentially PI3K and Akt (24, 25), although the events upstream of PI3K obviously differ compared with those activated by LPA. In this regard, it should be noted that the rapidity of the Akt response observed here (Fig. 3*b*) demonstrates that the survival effects of LPA are unlikely to be mediated by release of an autocrine secondary factor. In light of these results in SCs, it is perhaps not surprising that PI3K signaling is a requirement for oligodendrocyte survival (39); expression of lp_{A1}/*vzg-1* in oligodendrocytes (12) predicts that LPA also should mediate survival of these myelinating cells.

The related LP S1P has been reported to promote cell survival through an intracellular mechanism, independent of GPCR activation (40), and hypothetically could act through one of its cognate GPCRs such as lp_{B3}/*edg-3* (Fig. 1*a*) to promote SC survival. However, S1P had no effect on SC survival, demonstrating the specificity of LPA action and also suggesting the existence of multiple, nonredundant roles for lipid signaling in myelinating cells. Functions of S1P on SCs, as well as additional effects of LPA (e.g., cytoskeletal rearrangements), need to be determined to understand fully the consequences of LP signaling for myelinating cells. As a class of molecules influencing these cells, LPs and their cognate receptors may represent pharmaceutical targets for the design of new therapeutic approaches to myelinating disorders. The data presented here identify a signaling system with potential importance for nervous system development and regeneration.

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1. Moolenaar, W. H., Kranenburg, O., Postma, F. R. & Zondag, G. C. M. (1997) *Curr. Opin. Cell Biol.* **9,** 168–173.
2. Hecht, J. H., Weiner, J. A., Post, S. R. & Chun, J. (1996) *J. Cell Biol.* **135,** 1071–1083.
3. Chun, J., Contos, J. J. A. & Munroe, D. (1999) *Cell Biochem. Biophys.*, in press.
4. An, S., Dickens, M. A., Bleu, T., Hallmark, O. G. & Goetzl, E. J. (1997) *Biochem. Biophys. Res. Commun.* **231,** 619–622.
5. An, S., Bleu, T., Hallmark, O. G. & Goetzl, E. J. (1998) *J. Biol. Chem.* **273,** 7906–7910.
6. An, S., Bleu, T., Huang, W., Hallmark, O. G., Coughlin, S. R. & Goetzl, E. J. (1998) *FEBS Lett.* **417,** 279–282.
7. Lee, M.-J., Van Brocklyn, J. R., Thangada, S., Liu, C. H., Hand, A. R., Menzeleev, R., Spiegel, S. & Hla, T. (1998) *Science* **279,** 1552–1555.
8. Zondag, G. C. M., Postma, F. R., van Etten, I., Verlaan, I. & Moolenaar, W. H. (1998) *Biochem. J.* **330,** 605–609.
9. Fukushima, N., Kimura, Y. & Chun, J. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 6151–6156.
10. Zhang, G., Contos, J. J. A., Weiner, J. A., Fukushima, N. & Chun, J. (1999) *Gene* **227,** 89–99.
11. Dubin, A. E., Bahnson, T., Weiner, J. A., Fukushima, N. & Chun, J. (1999) *J. Neurosci.* **19,** 1371–1381.
12. Weiner, J. A., Hecht, J. H. & Chun, J. (1998) *J. Comp. Neurol.* **398,** 587–598.
13. Gould, R. M., Jessen, K. R., Mirsky, R. & Tennekoon, G. (1992) in *The Cell of Schwann: An Update*, ed. Martenson, R. E. (CRC, Boca Raton, FL), pp. 123–171.
14. Scherer, S. S. (1997) *Curr. Opin. Neurol.* **10,** 386–397.
15. Raff, M. C., Miller, R. H. & Noble, M. (1983) *Nature (London)* **303,** 390–396.
16. Bologa, L., Cole, R., Chiappelli, F., Saneto, R. P. & de Vellis, J. (1988) *J. Neurosci. Res.* **20,** 182–188.
17. Louis, J. C., Magal, E., Muir, D., Manthorpe, M. & Varon, S. (1992) *J. Neurosci. Res.* **31,** 193–204.
18. Syroid, D. E., Maycox, P. R., Burrola, P. G., Liu, N., Wen, D., Lee, K. F., Lemke, G. & Kilpatrick, T. J. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 9229–9234.
19. Eichholtz, T., Jalink, K., Fahrenfort, I. & Moolenaar, W. H. (1993) *Biochem. J.* **291,** 677–680.
20. Carraway, K. L., Weber, J. L., Unger, M. J., Ledesma, J., Yu, N., Gassmann, M. & Lai, C. (1997) *Nature (London)* **387,** 512–516.
21. Brockes, J. P., Fields, K. L. & Raff, M. C. (1979) *Brain Res.* **165,** 105–118.
22. Blaschke, A. J., Staley, K. & Chun, J. (1996) *Development* (Cambridge, U.K.) **122,** 1165–1174.
23. Blaschke, A. J., Weiner, J. A. & Chun, J. (1998) *J. Comp. Neurol.* **396,** 39–50.
24. Dudek, H., Datta, S. R., Franke, T. F., Birnbaum, M. J., Yao, R., Cooper, G. M., Segal, R. A., Kaplan, D. R. & Greenberg, M. E. (1997) *Science* **275,** 661–665.
25. Franke, T. F., Yang, S.-I., Chan, T. O., Datta, K., Kazlauskas, A., Morrison, D. K., Kaplan, D. R. & Tsichlis, P. N. (1995) *Cell* **81,** 727–736.
26. Datta, S. R., Dudek, H., Tao, X., Masters, S., Fu, H., Gotoh, Y. & Greenberg, M. E. (1997) *Cell* **91,** 231–241.
27. Kohn, A. D., Summers, S. A., Mirnbaum, M. J. & Roth, R. A. (1996) *J. Biol. Chem.* **271,** 31372–31378.
28. Grinspan, J. B., Marchionni, M. A., Reeves, M., Coulaloglou, M. & Scherer, S. S. (1996) *J. Neurosci.* **16,** 6107–6118.
29. Nakao, J., Shinoda, J., Nakai, Y., Murase, S.-I. & Uyemura, K. (1997) *J. Neurochem.* **68,** 1853–1862.
30. Wyllie, A. H., Morris, R. G., Smith, A. L. & Dunlop, D. (1984) *J. Pathol.* **142,** 67–77.
31. Vartanian, T., Corfas, G., Li, Y., Fischbach, G. D. & Stefansson, K. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11626–11630.
32. Dong, Z., Brennan, A., Liu, N., Yarden, Y., Lefkowitz, G., Mirsky, R. & Jessen, K. R. (1995) *Neuron* **15,** 585–596.
33. Lemke, G. E. & Brockes, J. P. (1984) *J. Neurosci.* **4,** 75–83.
34. Franke, T. F., Kaplan, D. R. & Cantley, L. C. (1997) *Cell* **88,** 435–437.
35. Hawes, B. E., Luttrell, L. M., van Biesen, T. & Lefkowitz, R. J. (1996) *J. Biol. Chem.* **271,** 12133–12136.
36. Carraway, K. L., Soltoff, S. P., Diamonti, A. J. & Cantley, L. C. (1995) *J. Biol. Chem.* **270,** 7111–7116.
37. Milano, C. A., Allen, L. F., Rockman, H. A., Dolber, P. C., McMinn, T. R., Chien, K. R., Johnson, T. D., Bond, R. A. & Lefkowitz, R. J. (1994) *Science* **264,** 582–586.
38. Topilko, P., Murphy, P. & Charnay, P. (1996) *Mol. Cell. Neurosci.* **8,** 71–75.
39. Vemuri, G. S. & McMorris, F. A. (1996) *Development* (Cambridge, U.K.) **122,** 2529–2537.
40. Edsall, L. C., Pirianov, G. G. & Speigel, S. (1997) *J. Neurosci.* **17,** 6952–6960.