Lysophosphatidic acid stimulates glucose transport in *Xenopus* oocytes via a phosphatidylinositol 3'-kinase with distinct properties

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Lysophosphatidic acid (LPA) stimulated the transport of deoxyglucose into oocytes isolated from *Xenopus laevis*. This stimulation was accounted for entirely by an increase in the $V_{\max}$ for transport. Various LPAs with different acyl groups in the *sn*-1 position and phosphatidic acid stimulated deoxyglucose (deGlc) transport in these cells with a rank order potency of 1-oleoyl-LPA > 1-palmitoyl-LPA > phosphatidic acid = 1-stearoyl-LPA > 1-myristoyl-LPA. The phosphatidylinositol 3'-kinase inhibitor LY294002 completely blocked LPA-stimulated deoxyglucose uptake ($IC_{50} \sim 2 \mu\text{M}$). In marked contrast, wortmannin, which can completely block both insulin-like growth factor-I (IGF-I)-stimulated deGlc uptake in oocytes and phosphatidylinositol 3'-kinase activation at concentrations as low as 20 nM [Gould, Jess, Andrews, Herbst, Plevin and Gibbs (1994) J. Biol. Chem. **269**, 26622–26625], was a relatively poor inhibitor of LPA-stimulated deGlc transport, even at concentrations as high as 100 nM. We further show that LPA stimulates phosphatidylinositol 3'-kinase activity(s) that can phosphorylate both phosphatidylinositol and

phosphatidylinositol 4,5-bisphosphate, and that this stimulation is inhibited by LY294002 but is relatively insensitive to wortmannin, again in marked contrast to IGF-I-stimulated phosphatidylinositol 3'-kinase activity. Antibodies against the p85 regulatory subunit of phosphatidylinositol 3'-kinase or anti-phosphotyrosine antibodies immunoprecipitated IGF-I-stimulated but not LPA-stimulated phosphatidylinositol 3'-kinase activity. We conclude that LPA stimulates glucose uptake in *Xenopus* oocytes by a mechanism that may involve activation of a form of phosphatidylinositol 3'-kinase that is distinguished from other isoforms by its resistance to wortmannin and by its substrate specificity. Since the LPA-activated form of phosphatidylinositol 3'-kinase is pharmacologically and immunologically distinct from that which is involved in IGF-I-stimulated glucose transport in these cells, we suggest that distinct isoforms of this enzyme are able to function with the same biological effect, at least in the regulation of sugar transport.

INTRODUCTION

Exposure of resting cells to mitogens causes activation of numerous signal transduction pathways leading to cell growth and division. Since growing cells have an increased energy requirement, stimulation of glucose transport is an effect that is common to all mitogens. The time course of mitogen-induced stimulation of glucose transport is biphasic; an acute response occurs within minutes that is followed by a chronic effect after ~4–12 h [1–7]. The acute stimulation of glucose uptake by mitogens is thought to result from the translocation of intracellular glucose transporters to the cell surface [5,8], whereas the chronic effect is associated with increased glucose-transporter-gene transcription [1–6,9] and protein synthesis [3,5].

Lysophosphatidic acid (1-acyl-2-hydroxy-*sn*-glycero-3-phosphate; LPA) is a naturally occurring lipid that is capable of inducing a multiplicity of biological effects, including DNA synthesis and cell division in fibroblasts and epithelial cell lines [10,11], by interacting with specific G-protein-coupled cell-surface receptors [12,13]. Although the mitogenic action of LPA is well documented, the effect of this lipid on sugar transport and the molecular mechanisms that are potentially involved in this response have yet to be defined.

Recently, phosphatidylinositol 3'-kinase (PI 3-kinase) activation has been implicated as an important component of the intracellular signalling pathways that are activated following growth factor stimulation of cells [14–18]. Moreover, synthetic phosphotyrosine-containing peptides that stimulate PI 3-kinase activity by interacting with the SH2 domain of the regulatory subunit stimulate glucose transport when microinjected into *Xenopus* oocytes [18], indicating that PI 3-kinase may have a role in the control of glucose transport by mitogens.

Here we show that LPA stimulates deoxy-D-glucose (deGlc) uptake in oocytes by increasing the $V_{\max}$ for transport. The biological characteristics of this effect are consistent with LPA binding to a cell-surface receptor that is pharmacologically distinct from that previously reported for other cells. Furthermore, LPA-stimulated deGlc transport is blocked by the PI 3-kinase inhibitor LY294002. However, a distinct PI 3-kinase inhibitor, wortmannin, that was previously shown to block completely insulin-like growth factor-I (IGF-I)-stimulated deGlc uptake in oocytes [18], had only a weak inhibitory effect on LPA-stimulated deGlc uptake. We extend this analysis to show that LPA stimulates the activity of a PI 3-kinase in oocytes that can phosphorylate both phosphatidylinositol (PtdIns) and phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5) P_2]. This activity is

Abbreviations used: LPA, lysophosphatidic acid; IGF-I, insulin-like growth factor-I; deGlc, deoxyglucose; PI 3-kinase, phosphatidylinositol 3'-kinase; PtdIns, phosphatidylinositol; PtdIns(4,5) P_2 , phosphatidylinositol 4,5-bisphosphate; oleoyl-LPA, 1-oleoyl-2-hydroxy-*sn*-glycero-3-phosphate; stearoyl-LPA, 1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphate; myristoyl-LPA, 1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphate; palmitoyl-LPA, 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphate; PA, phosphatidic acid; PtdIns3P, phosphatidylinositol 3-phosphate.

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LY294002 sensitive but relatively wortmannin resistant when compared with other PI 3-kinases [18,19]. We further show that the PI 3-kinase activity(s) activated by LPA is immunologically distinct from that activated by IGF-I and is not immunoprecipitated with anti-phosphotyrosine antibodies. These results indicate an important role for a wortmannin-resistant PI 3-kinase in the mechanism of LPA-stimulated glucose transport. Since the LPA-activated form of PI 3-kinase is pharmacologically and immunologically distinct from that which is involved in IGF-I-stimulated glucose transport in these cells, we suggest that distinct isoforms of this enzyme are able to function with the same biological effect, at least in the regulation of sugar transport.

MATERIALS AND METHODS

Materials

1-Oleoyl-2-hydroxy-*sn*-glycero-3-phosphate (oleoyl-LPA), 1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphate (stearoyl-LPA), 1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphate (myristoyl-LPA), 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphate (palmitoyl-LPA), PtdIns and 1,2-dipalmitoyl phosphatidic acid were purchased from Avanti Polar Lipids (Alabaster, AL, U.S.A.). PtdIns(4,5) P_2 was purchased from Boehringer Mannheim (Lewes, East Sussex, U.K.). Cytochalasin B and wortmannin were obtained from Sigma (Poole, Dorset, U.K.). [2,6- ^3H]deGlc (8.1 Ci/mmol) was purchased from DuPont-NEN (Stevenage, Herts., U.K.) and [γ - ^{32}P]ATP from Amersham (Little Chalfont, Bucks., U.K.). The monoclonal antibody against the p85 subunit of PI 3-kinase was bought from Upstate Biotechnology (Lake Placid, NY, U.S.A.) and anti-phosphotyrosine antibody was purchased from Santa Cruz Biotech (Santa Cruz, CA, U.S.A.). 2-Morpholino-8-phenyl-4*H*-1-benzopran-4-one (LY294002) was kindly given by Professor Philip Cohen (MRC Protein Phosphorylation Unit, University of Dundee, Dundee, U.K.). All other reagents were as described previously [18,20].

Isolation of oocytes from *Xenopus laevis*

Female wild caught *X. laevis* were purchased from the South African Xenopus Facility (Noordhoek, R.S.A.). Stage V and VI oocytes were isolated using watchmaker's forceps and incubated in Barth's buffer [5 mM Hepes/NaOH, pH 7.4, 88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO₃, 0.82 mM MgSO₄, 0.41 mM CaCl₂ and 0.33 mM Ca(NO₃)₂] as described previously [20]. Before experimentation, damaged oocytes were removed. Where required, healthy oocytes were microinjected as described previously [20].

Sugar transport assays in oocytes

The transport of deGlc was assayed as described previously [18,20]. Briefly, groups of 10 oocytes were incubated in 0.2 ml of Barth's buffer in 13.5 ml centrifuge tubes. Transport measurements were initiated by the addition of an aliquot of [2,6- ^3H]deGlc to the required concentration. After incubation at room temperature for the indicated times, the reaction was stopped by quickly aspirating the media and washing the oocytes with 5 ml of ice-cold PBS (10 mM sodium phosphate, pH 7.4, and 150 mM NaCl). Oocytes were washed in this fashion a further two times and dispensed into scintillation vials (one oocyte per vial) containing 1.0 ml of 1% SDS. The vials were incubated with agitation before scintillant was added and radioactivity was measured. For kinetic analysis of deGlc uptake, a range of substrate concentrations was used. The amount of sugar non-specifically associated with the oocytes was accounted for by

performing duplicate uptake measurements in the presence of 10 μM cytochalasin B, a potent inhibitor of facilitative glucose transporters. We have previously shown that under these conditions transport of deGlc and not its subsequent phosphorylation is rate limiting [21].

Measurement of oocyte PI 3-kinase activity

Oocytes were incubated in Barth's buffer at room temperature, with or without stearoyl-LPA (100 μM). At the indicated times, the media was aspirated and the oocytes washed six times in 4 ml of 20 mM Tris/HCl (pH 7.2)/140 mM NaCl. Oocytes were resuspended in 20 mM Tris/HCl (pH 7.2)/140 mM NaCl/1 mM sodium orthovanadate/10% (v/v) glycerol/0.5 mM dithiothreitol/0.1 μM pepstatin A/2 mM di-isopropyl fluorophosphate/200 μM aprotinin/0.1 mM *L-trans*-epoxy-succinyl-L-leucylamido-(4-guanidino) butane (30 oocytes/600 μl) and homogenized by 20 strokes of a Dounce homogenizer. The homogenate was centrifuged at 100000 *g* and 4 °C for 60 min, and the supernatant was snap-frozen in liquid N₂ and stored at -80 °C before assaying. Where indicated, oocyte PI 3-kinase was immunoprecipitated with either antibodies against the 85 kDa subunit of PI 3-kinase or monoclonal anti-phosphotyrosine antibodies and protein A.

PI 3-kinase activity in oocyte homogenates, immunoprecipitates or post-precipitation supernatant was assayed by the phosphorylation of PtdIns or PtdIns(4,5) P_2 with [^{32}P]ATP as previously described [22–24]. Reactions (final vol. = 50 μl) were terminated by the addition of 80 μl of 1 M HCl, and the lipids were extracted with 180 μl of CHCl₃/methanol (1:1; v/v). Extracted lipids (50 μl) were spotted on a 5 cm $\times$ 20 cm Silica Gel 60 plate (Whatman, Maidstone, Kent, U.K.), and phosphatidyl-inositol phosphates were separated on the ability of phosphatidylinositol 4-phosphate, but not phosphatidylinositol 3-phosphate (PtdIns3*P*), to form a borate complex with the *cis*-diol present in the inositol ring as previously described [25]. The position of the PtdIns3*P* spot was determined using a ^{32}P -labelled PtdIns3*P* standard followed by autoradiography. The corresponding section of the plate was excised, and the ^{32}P incorporated was measured by scintillation counting.

Statistical analysis

Statistical analysis of results was performed using StatView Software (Abacus, CA, U.S.A.) running on a Macintosh computer.

RESULTS

Effect of LPAs with different acyl groups in the *sn*-1 position on deGlc transport into oocytes

Exogenously added LPA can induce DNA synthesis and cell division in fibroblasts [10,11]. Since a common action of mitogens is to increase glucose transport, we examined the effect of LPA on glucose transport in *Xenopus* oocytes. We incubated oocytes with stearoyl-LPA, a mitogen in fibroblasts [11], and measured deGlc uptake as described in the Materials and methods section. After a 30 min incubation with LPA, deGlc uptake into oocytes was significantly increased above that measured in the absence of lipid (0.29 ± 0.02 versus 0.11 ± 0.02 pmol/min per oocyte; $n = 10$; $P < 0.05$), and the increased rate of glucose transport was still evident after 2 h incubation with LPA (0.31 ± 0.01 versus 0.12 ± 0.02 pmol/min per oocyte; $n = 10$; $P < 0.05$). In contrast, when LPA was microinjected into the oocytes, there was no significant increase in deGlc transport, even after 2 h incubation

Figure 1 Effect of LPAs with different acyl chain lengths on deGlc uptake into *Xenopus* oocytes

Oocytes were incubated with various concentrations of LPA or PA for 1 h. [^3H]deGlc (50 μM , 0.5 $\mu\text{Ci}/\text{tube}$) was added and the oocytes were incubated for a further 1 h before uptake was stopped. DeGlc uptake values were corrected for non-specific association of deGlc with the oocytes by performing parallel assays in the presence of 10 μM cytochalasin B, a potent inhibitor of facilitative glucose transporters (this value typically amounted to $\sim 10\%$ of the total uptake in unstimulated oocytes). The lipids used were stearoyl-LPA (■); oleoyl-LPA (◆); myristoyl-LPA (▲); palmitoyl-LPA (●); and PA (▼). Each point on the graph represents the mean value for 10 oocytes. S.E.M. were typically less than 10% in each case but have been omitted for clarity.

$[0.12 \pm 0.005 \text{ pmol}/\text{min per oocyte} (n = 8)]$ in stearoyl-LPA-injected oocytes compared with $0.125 \pm 0.01 \text{ pmol}/\text{min per oocyte}$ in vehicle-injected oocytes, not statistically different] (results not shown). These data strongly suggest that LPA stimulates an increase in deGlc transport in oocytes by acting at an external site of action, probably a membrane-associated receptor [13].

Previously, LPAs with different *sn*-1 position fatty acid side chains have been shown to exhibit a distinct rank order of potency in binding to the LPA receptor [13] and in inducing DNA synthesis in fibroblasts [11]. To determine the potencies of a variety of lipids in stimulating sugar transport, oocytes were incubated with LPAs with different *sn*-1 position acyl groups or with phosphatidic acid (PA) (Figure 1). Oleoyl-LPA was the most potent lipid tested ($\text{EC}_{50} = 30 \text{ nM}$). Palmitoyl-LPA, stearoyl-LPA and 1,2-dipalmitoyl PA were less potent than oleoyl-LPA. Interestingly, myristoyl-LPA, the least potent LPA, stimulated a substantially smaller maximal increase in deGlc uptake than the other LPA species did. The rank order potency of the LPAs for deGlc transport in oocytes was oleoyl-LPA > palmitoyl-LPA > stearoyl-LPA = PA > myristoyl-LPA. In contrast, other lipids, such as lysophosphocholine and monoacylglycerol (both at 100 μM), did not stimulate deGlc transport in oocytes (results not shown), suggesting that the effect of LPA on deGlc transport is specific to this and other structurally related lipids.

Figure 2 Effect of PI 3-kinase inhibitors on LPA-stimulated deGlc uptake in oocytes

Top, effect of LY294002 on LPA-stimulated deGlc uptake. Oocytes were incubated with stearoyl-LPA (100 μM) (●) or vehicle (○) in the presence of LY294002 for 1 h. [^3H]deGlc (50 μM , 0.5 $\mu\text{Ci}/\text{tube}$) was then added, and the oocytes were incubated for an additional 1 h before uptake was stopped. The uptake values presented here have been corrected for non-specific association of deGlc with the oocytes by performing parallel incubations in the presence of cytochalasin B (10 μM), and are expressed as percentages of the deGlc uptake measured in the absence of any agonist or inhibitor. * indicates a statistically significant inhibition compared with control values ($P < 0.05$). Bottom, effect of wortmannin on LPA-stimulated deGlc uptake. Assays were performed exactly as in the top panel, except that wortmannin was used instead of LY294002. DeGlc uptake values are expressed as percentages of basal uptake measured in the absence of agonist or inhibitor and have been corrected for non-specific association of isotope with the oocytes as described above. * indicates a statistically significant inhibition compared with control values ($P < 0.05$).

Analysis of the kinetics of basal and LPA-stimulated deGlc transport in oocytes indicated that the LPA effect may be accounted for entirely by an increased V_{max} for transport (results

Figure 3 Effect of LPA on oocyte PI 3-kinase activity

Left, time course of LPA-stimulated PtdIns3P formation. Oocytes were incubated with stearoyl-LPA (100 μ M) (●) or with vehicle (□) for the times shown. Oocytes were then washed and cell homogenates were prepared as described. PI 3-kinase activity in homogenates was determined by measurement of the phosphorylation of PtdIns in the presence of [γ - 32 P]ATP as described in the Materials and methods section. Values represent the means $\pm$ S.E.M. of four experiments performed on a minimum of two separate oocyte preparations. * indicates a statistically significant increase compared with vehicle-treated oocytes ($P < 0.05$). Middle, effect of LY294002 on LPA-stimulated PtdIns3P production. Oocytes were incubated with stearoyl-LPA (100 μ M) (●) or with vehicle (○) for 10 min, after which cell homogenates were prepared as described in the Materials and methods section. Homogenates were pre-incubated with LY294002 for 10 min before measuring PtdIns3P formation by the phosphorylation of PtdIns in the presence of [γ - 32 P]ATP. Values are expressed as percentages of basal activity and represent the means $\pm$ S.E.M. for four separate experiments performed on a minimum of two independent oocyte preparations. * indicates a statistically significant inhibition compared with control ($P < 0.05$). Right, effect of wortmannin on LPA-stimulated PtdIns3P production. As in the middle panel, except that homogenates were incubated with the indicated concentration of wortmannin for 10 min before initiation of the PI 3-kinase assay. Values are from three independent experiments expressed as percentages of basal activity. * indicates a statistically significant inhibition compared with control ($P < 0.05$).

not shown). This may result from increased activity of pre-existing transporters in the plasma membrane, the recruitment of additional glucose transporters to the cell surface, or both (for reviews, see [26,27]). At present, methodologies are not available to distinguish between these possibilities, since oocytes contain relatively low levels of immunoreactive GLUT1-like protein [28].

Evidence for a role for PI 3-kinase activation in LPA-stimulated deGlc transport

Recent evidence has implicated PI 3-kinase activation as an important consequence of mitogen stimulation of many cells [14–17], and previous studies from this laboratory have shown that PI 3-kinase activation plays a role in IGF-I-stimulated deGlc transport in oocytes [18]. To determine if PI 3-kinase activation has a role in the mechanism of LPA-induced glucose transport in oocytes, we investigated the effect of the PI 3-kinase inhibitor LY294002 [29] on this response (Figure 2, top). LPA-stimulated deGlc uptake was completely inhibited by LY294002 at concentrations as low as 10 μ M. This is within the concentration range used to inhibit purified bovine brain PI 3-kinase [29]. At higher concentrations of LY294002 ($> 3 \mu$ M) there was a small ($< 20\%$) inhibition of basal transport, but this effect was not statistically significant.

In marked contrast, a distinct PI 3-kinase inhibitor, wortmannin, that has been previously shown to block completely IGF-I-stimulated deGlc uptake in oocytes at concentrations as low as 20 nM and PI 3-kinase activation in these cells [18] was a relatively poor inhibitor of LPA-stimulated deGlc transport, even at concentrations as high as 100 nM (Figure 2, bottom). Although LPA-induced deGlc uptake was significantly reduced

by wortmannin, $\sim 40\%$ of the LPA-stimulated response remained at a concentration of 100 nM wortmannin. [Note that we have tested concentrations of wortmannin up to 250 nM and observed no further inhibition of LPA-stimulated deGlc transport.] These data contrast with those that were observed previously for IGF-I [18] and were confirmed in this study using the same oocyte preparations in parallel experiments (results not shown). IGF-I-stimulated deGlc uptake was completely inhibited by 20 nM wortmannin [18] but was only partially blocked ($< 40\%$) by LY294002 even at concentrations of 100 μ M, 50-fold higher than the concentration that completely inhibits LPA-stimulated transport and PI 3-kinase activation. These data suggested to us that LPA-stimulated deGlc transport in oocytes may involve the activation of a PI 3-kinase isoform that has distinct pharmacological properties from to that which is activated in response to IGF-I, but that both of these isoforms may serve to regulate glucose transport.

LPA activates a PI 3-kinase with distinct pharmacological properties

In an effort to address the above point, we set out to examine LPA-stimulated PI 3-kinase activity in oocyte extracts. Oocytes were incubated with stearoyl-LPA (100 μ M), and lysates were prepared and assayed for PI 3-kinase activity using PtdIns as a substrate (Figure 3, left panel). LPA stimulated a 2-fold increase in PtdIns3P levels after 10 min, compared with control (vehicle-treated) oocytes, consistent with an increase in PI 3-kinase activity. LPA-stimulated increases in PtdIns3P levels were still evident after a 30 min incubation with LPA but had declined almost to basal levels by 1 h (Figure 3, left panel).

The LPA-stimulated increase in oocyte PI 3-kinase activity was completely inhibited by LY294002 (Figure 3, middle panel). However, LPA-stimulated PI 3-kinase activity is resistant to wortmannin, a result that is in marked contrast to that reported for IGF-I-stimulated PI 3-kinase activity in oocytes [18] (Figure 3, right panel). Note that we have obtained identical pharmacological profiles of the effects of these inhibitors when added either directly to homogenates for PI 3-kinase assay (Figure 3) or when oocytes were pre-incubated with inhibitors before stimulation with LPA and preparation of homogenates. Neither wortmannin nor LY294002 had any significant inhibitory effect on basal production of PtdIns3P. The reason for this observation is unclear but may reflect the involvement of either a PI 3-kinase that is insensitive to both inhibitors or a PI 3-kinase-independent route of PtdIns3P production.

A wortmannin-resistant, PtdIns-specific PI 3-kinase that is distinct from the p85/p110 family of enzymes and devoid of detectable PtdIns(4,5) P_2 3-kinase activity has been identified in bovine adrenoreticula and yeast cells [30,31]. To determine whether the LPA-stimulated enzyme in oocytes displays this distinct substrate specificity, we performed assays of oocyte PI 3-kinase activity using PtdIns(4,5) P_2 as a substrate. Extracts from oocytes treated with LPA (10 min) were found to exhibit a 3-fold increase in PtdIns(4,5) P_2 3-kinase activity (8.3 ± 1.0 fmol/ μ g in controls versus 26.0 ± 0.9 fmol/ μ g in LPA-treated oocyte homogenates). On the basis of this different substrate specificity, it would seem likely that the LPA-stimulated, wortmannin-resistant PI 3-kinase is distinct from certain wortmannin-resistant forms that have been previously identified [30,31].

In an effort to characterize further the LPA-activated PI 3-kinase, we immunoprecipitated the p85/p110 form of this enzyme with antibodies that recognize the p85 subunit and assayed PI 3-kinase activity in the immunoprecipitates. There was no significant increase in PI 3-kinase activity in anti-p85 immunoprecipitates from LPA-stimulated oocytes when compared with controls (results not shown). However, there was a significant increase in PI 3-kinase activity in post-precipitation supernatant from LPA-stimulated oocytes (59.9 ± 1.0 fmol/ μ g versus 35.6 ± 7.9 fmol/ μ g in controls; $n = 4$). Anti-phosphotyrosine antibodies did not immunoprecipitate LPA-stimulated PI 3-kinase activity, whereas PI 3-kinase activity was stimulated 9-fold in immunoprecipitates from IGF-I-treated oocytes. In conclusion, it would appear that LPA stimulates the activity of a PI 3-kinase in oocytes that is immunologically distinct from the p85/p110 family and is activated by a mechanism other than tyrosine phosphorylation.

DISCUSSION

Significant quantities of LPA are produced following cell activation [32–34] and, when released into the extracellular milieu, may interact with receptors and have a variety of biological actions. Indeed, exogenously added LPA can promote platelet aggregation [35,36], alter neuronal cell morphology [37], induce smooth muscle contraction [38,39], elicit a chemotactic response in *Dictyostelium* [40], induce an increase in a Ca^{2+} -dependent Cl^- conductance in *Xenopus* oocytes [37,41], and trigger DNA synthesis and cell division in fibroblasts and epithelial cells [10,11]. In this study, we show, for the first time, that LPA can also stimulate an acute increase in deGlc uptake into cells (*Xenopus* oocytes). Stimulation of deGlc transport by LPA was dependent upon the lipid being present in the bathing medium (microinjected LPA was ineffective) and was apparently selective for LPA and structurally related lipids (such as PA), suggesting that LPA may induce sugar transport by acting at the LPA receptor [13].

It is interesting to note that the rank order of potencies for LPA-stimulated deGlc transport differed from that observed in studies of LPA-induced DNA synthesis in Rat 1 fibroblasts [10,11,42] and in their affinity for the rat brain and fibroblast LPA receptor [13]. This difference in potency may be accounted for by the existence of a different LPA receptor subtype(s) in *Xenopus* oocytes and fibroblasts.

Of the many different receptor species that are endogenous to *Xenopus* oocytes, only IGF-I receptor activation has previously been shown to elicit an increase in glucose transport [18,43]. The LPA and IGF-I receptors are structurally dissimilar and signal through distinct intracellular pathways: the LPA receptor being G-protein coupled [10,44–46] whereas the IGF-I receptor belongs to the tyrosine kinase receptor family [47]. Nevertheless, activation of both receptor types has the same biological effect, i.e. activation of sugar transport, suggesting that the signal transduction pathways activated by these distinct receptors converge.

The evidence provided here and in our previous studies [18] suggests that within the context of the regulation of glucose transport the common link between LPA and IGF-I receptor signalling may involve the activation of PI 3-kinase(s). However, since LPA- and IGF-I-stimulated sugar transport and PI 3-kinase activation are differentially affected by wortmannin and LY294002, it appears likely that distinct PI 3-kinase forms participate in the control of sugar transport by these two growth factors. This situation may reflect differences in the coupling of different receptors to distinct PI 3-kinase isoforms, as IGF-I activates PI 3-kinase via the intermediacy of SH2 domain interactions with phosphotyrosine residues, whereas LPA may activate a distinct PI 3-kinase isoform, perhaps via $\beta\gamma$ complexes released from heterotrimeric G-proteins [48].

Previous studies have shown that LPA can activate p85/p110 PI 3-kinases in Swiss 3T3 cells [49,50]. However, biochemical characterization of the LPA-stimulated PI 3-kinase activity in oocytes suggests that the oocyte enzyme is distinct from the p85/p110 family. We were unable to immunoprecipitate LPA-stimulated PI-3 kinase activity using antibodies against the p85 subunit. Furthermore, the LPA-stimulated PI 3-kinase activity in oocytes was relatively resistant to wortmannin, in contrast with the p85/p110-PI 3-kinases [18,19]. Wortmannin-resistant PI 3-kinases that are distinct from the p85/p110 family have been identified in mammalian and yeast cells [30,31]. The LPA-stimulated PI 3-kinase activity in oocytes phosphorylates both PtdIns and PtdIns(4,5) P_2 , suggesting that this enzyme differs from the PtdIns-specific enzyme(s) [30,31]. It would seem therefore that LPA-stimulated PI 3-kinase in oocytes has greatest biochemical similarity with the PI 3-kinase in myeloid-derived cells that is activated by G-protein $\beta\gamma$ subunits, is relatively wortmannin resistant and can utilize both PtdIns and PtdIns(4,5) P_2 as substrates [48]. However, to our knowledge, this is the first demonstration of a physiological role for this enzyme in non-myeloid cells.

The precise role of PI 3-kinase in the regulation of glucose transport remains to be identified. p70 ribosomal S6 kinase is a known down-stream effector of PI 3-kinase in certain cells [51]. However, preliminary results indicate that rapamycin, a potent inhibitor of p70 S6 kinase activation, is without effect on glucose transport in oocytes (F. J. Thomson, T. J. Jess and G. W. Gould, unpublished work), suggesting that the activation of this kinase is not required for growth-factor-stimulated glucose transport. In other cell types, PI 3-kinase(s) may have a role in vesicle trafficking, membrane ruffling and pinocytosis [31,52,53]. In insulin-stimulated adipocytes, PI 3-kinase activation appears to be required for GLUT4 glucose transporter translocation to the plasma membrane [19,54,55]. By analogy, PI 3-kinase activation

following stimulation of LPA and IGF-I receptors may be important for the recruitment of transporters to the cell surface, suggesting that a common role for distinct PI 3-kinase isoforms may lie in the regulation of transporter trafficking. Further studies are required to determine the molecular basis of the role of PI 3-kinase in such events.

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