Lysophosphatidic acid-mediated augmentation of cardiomyocyte lipoprotein lipase involves actin cytoskeleton reorganization

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Submitted 17 November 2004; accepted in final form 24 January 2005

Heart disease is a leading cause of mortality in diabetic patients, with coronary vessel disease being the primary reason for the increased incidence of cardiovascular dysfunction (27, 52). More recent evidence suggests that heart failure during diabetes can also occur secondary to altered cardiac energy metabolism where impaired glucose transport and utilization switches ATP production exclusively to oxidation of fatty acids (FA) (48). This adaptive mechanism could eventually become counterproductive leading to “lipotoxicity” where FA accumulate and can, either by themselves or via production of second messengers such as ceramides, provoke cell death (28).

Heart tissue acquires energy from metabolism of two major substrates, glucose and FA, the latter being the preferred substrate consumed (44, 48). FA delivery to the heart involves 1) release from adipose tissue and transport to the heart after complexing with albumin (26), 2) provision through the breakdown of endogenous cardiac triglyceride (TG) stores (37), 3) internalization of whole lipoproteins (55), and 4) hydrolysis of circulating TG-rich lipoproteins to FA by coronary lumen lipoprotein lipase (LPL) (8). The molar concentration of FA bound to albumin is ~10-fold less than that of FA in lipoprotein-TG (30), and, recently, LPL-mediated hydrolysis of lipoproteins was suggested to be the principal source of FA for cardiac utilization (5).

Because coronary endothelial cells do not synthesize LPL, this enzyme is primarily synthesized in cardiomyocytes (11). LPL, secreted as an active dimer, binds to myocyte cell surface heparan sulfate proteoglycans (HSPG) before it is translocated onto comparable HSPG binding sites on the luminal side of the vessel wall (15). At this location, LPL is heparin releasable (HR-LPL), exhibiting TG hydrolytic activity (11, 15, 36). Given the dependence of the diabetic heart on FA, luminal LPL is expected to increase to supply additional FA to meet the metabolic demand (43, 47). Indeed, after hypoinsulinemia induced by diazoxide (DZ) (42) or streptozotocin (40), we reported augmentation of LPL at the coronary luminal surface. Additionally, we established that this increased LPL in the hyperglycemic heart is likely an outcome of empty luminal HSPG binding sites being occupied by enzyme that is recruited from the cardiac myocyte (40).

Mediators responsible for LPL transfer from the underlying parenchymal cells to the vascular lumen have not been extensively characterized. In coculture experiments, lysophosphatidylcholine (lysoPC) has been suggested to be one such mediator, which, by secreting heparanase-like compounds from endothelial cells, cleaves and transports adipocyte surface LPL to the endothelial apical surface (38). We have demonstrated that exposure of isolated control hearts to lysoPC also enhances luminal LPL (41, 42). This LPL-augmenting property of lysoPC likely required obligatory formation of lysophosphatidic acid (LPA), leading to mobilization of enzyme from the myocyte to the coronary lumen (41). Indeed, when cardiomyocytes were incubated with LPA, myocyte surface LPL activity increased (41).
LPA is a pluripotent lipid mediator with multiple biological actions, including promotion of cell proliferation and migration (32, 56). LPA binds to specific cell surface G protein-coupled receptors, initiating a variety of signal transduction pathways involving RhoA/Rho kinase (ROCK), tyrosine kinase, and MAPKs (2, 32, 59). The precise mechanism by which LPA induces LPL secretion from the cardiomyocyte is not clearly understood. Given that the actin cytoskeleton has been implicated in regulating cardiomyocyte LPL (16) and that actin cytoskeleton rearrangement during neurite retraction is one of the best-characterized effects of LPA (51), we hypothesized that the increase in LPL secretion after LPA involves actin cytoskeleton reassembly. We demonstrate that after exposure to LPA, the increase in cardiomyocyte LPL activity is likely secondary to activation of ROCK, which in turn modulates actin cytoskeleton reorganization.

MATERIALS AND METHODS

Experimental animals. The investigation conforms to the guide for the care and use of laboratory animals published by the US National Institutes of Health and the University of British Columbia. Adult male Wistar rats (220–240 g) were obtained from The University of British Columbia Animal Care Unit and supplied with a standard laboratory diet (PMI Feeds; Richmond, VA) and water ad libitum.

Isolated heart perfusion. Hearts were isolated and perfused as described previously (40–42). Briefly, rats were anesthetized with 65 mg/kg pentobarbital sodium, and the hearts were carefully excised. After cannulation of the aorta, hearts were secured by tying below the coronary flow (7–8 ml/min) was controlled by a peristaltic pump.

Isolated cardiac myocytes. Ventricular calcium-tolerant myocytes were prepared by a previously described procedure (40–42). Briefly, rats were anesthetized with 65 mg/kg pentobarbital sodium, and the hearts were carefully excised. After cannulation of the aorta, hearts were secured by tying below the coronary flow (7–8 ml/min) was controlled by a peristaltic pump.

Isolated cardiomyocytes. In addition to laminar LPL, a considerable amount of enzyme is also located on the surface of and within myocytes. Ventricular calcium-tolerant myocytes were prepared by a previously described procedure (40–42). Cardiac myocytes were suspended at a final cell density of 0.4 × 10⁶ cells/ml and incubated at 37°C, and basal LPL activity in the medium was measured. To release surface-bound LPL activity, heparin (5 U/ml) was added to the myocyte suspension, aliquots of the cell suspension were removed at different time points, and the medium was separated by centrifugation and assayed for LPL activity.

Measurement of LPL activity. LPL catalytic activity in coronary perfusates and myocyte medium was determined by measuring the in vitro hydrolysis of a sonicated [1H]triolein substrate emulsion (49). One hundred microliters of either myocyte medium or coronary perfusate were used to measure LPL activity. Results are expressed as nanomoles of oleate released per hour per milliliter (coronary perfusate) or per 10⁶ cells (myocyte medium).

Western blot analysis for RhoA and ROCK I and II. To determine RhoA and ROCK activation, migration of these proteins from the cytosol to the membrane was determined using immunoblot analysis (25, 39). Briefly, ventricles (200 mg) or myocytes (2 × 10⁶) that were subjected to various agents were homogenized in ice-cold buffer A (containing 20 mM Tris·HCl, 2 mM EDTA, 5 mM EGTA, 50 mM 2-mercaptoethanol, 1 mM DTT, 25 μg leupeptin, and 4 μg aprotinin; pH 7.5) and centrifuged for 1 h at 35,000 rpm; the supernatant was used as the cytosolic fraction. The pellet was resuspended in buffer B (containing 1% NP-40, 0.1% SDS, 0.5% deoxycholic acid, and 5 mM EGTA; pH 7.5), sonicated for 30 s, and centrifuged at 35,000 rpm for 1 h; the supernatant was used as the membrane fraction. The protein contents of both cytosolic and membrane fractions were determined using a Bradford protein assay kit (Bio-Rad). Cytosolic and membrane proteins (50 μg) were fractionated using 11% SDS-PAGE and transferred onto nitrocellulose membranes (polyvinylidene difluoride membranes were used when RhoA was estimated). The membranes were blocked with Tris-buffered saline containing 5% skim milk for 2 h at room temperature. To identify RhoA and ROCK I and II, primary antibodies were used at dilutions of 1:250 and 1:180, respectively. This was followed by incubation with goat anti-mouse (RhoA) or goat anti-rabbit (ROCK) horseradish peroxidase-conjugated secondary antibodies at dilutions of 1:3,000 and 1:5,000 respectively. Membranes were washed, and the reaction products were visualized using chemiluminescence. Blots were quantified by densitometry.

Cardiac LPL gene expression. LPL gene expression was measured in the indicated groups using RT-PCR (40, 43). Briefly, total RNA from myocytes (100 mg) was extracted using TRIzol (Invitrogen). After spectrophotometric quantification and resolving of RNA integrity using a formaldehyde agarose gel, RT was carried out using an oligo-(dT) primer and superscript II reverse transcriptase (Invitrogen). cDNA was amplified using the following LPL-specific primers: 5'-ATCCAGCTGGGCTAAGTTT-3' (left) and 5'-AATGGCTCTTC-CATGTTGCG-3' (right). The β-actin gene was amplified as an internal control using 5'-TTGTCGGATGCTGCTAAGG-3' (left) and 5'-ATCCGTACTCAGTGGCTTGG-3' (right). The linear range was found to be between 15 and 30 cycles. The amplification parameters were set at 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min, for a total of 30 cycles. The PCR products were electrophoresed on a 1.7% agarose gel containing ethidium bromide. Expression levels were represented as the ratio of signal intensity for LPL mRNA relative to β-actin mRNA.

Immunolocalization of filamentous and globular cardiomyocyte actin. Briefly, myocytes were plated on laminin-coated coverglass slides and rinsed with PBS. Myocytes were fixed for 10 min with 4% paraformaldehyde in PBS, permeabilized with 0.1% Triton X-100 in PBS for 3 min, treated with PBS containing 1% BSA for 20 min, and finally rinsed with PBS. Cells were double stained with DNAaseI Alexa fluor 594 and rhodamine 488-phalloidin to colocalize monomeric globular actin (G-actin; red) and polymerized filamentous actin (F-actin; green) (3, 19). The unbound fluorescent probe was rinsed with PBS buffer, and slides were visualized using a Bio-Rad 600 confocal microscope at ×1,260 magnification. For quantitative estimation of F- and G-actin, cells were plated on a laminin-coated 24-well plate at a density of 75,000 myocytes/well. After incubation, cells were rinsed, fixed, and permeabilized as described above. The increase in green and red fluorescence was monitored in a spectrofluorometer (green: excitation 485 nm and emission 530 nm; red: excitation 530 nm and emission 590 nm). Background was identified in wells without cells. Data are expressed as fluorescence units (FU) after the background was subtracted. An increase in the F-actin-to-G-actin ratio was assumed to represent polymerization of actin filaments (6).

Adipose tissue LPL activity. Epididymal adipose tissue was isolated from fed rats and weighed (58). After incubation of adipose tissue with or without LPA, basal and HR-LPL activity was measured in the incubation medium. Results are expressed as milliliters of LPL per gram of tissue.

Treatments. To investigate the influence of LPA on LPL, isolated hearts and myocytes were treated with LPA for 1 h, and LPL activity was determined. LPA was solubilized in chloroform-methanol and subsequently in Krebs-Henseleit-HEPES buffer. In the indicated experiment, control rat hearts were perfused with LPA (1 nM) for 60 min, and HR-LPL activity was subsequently determined. Control myocytes were also incubated with increasing concentrations of LPA (1–100 nM) for 60 min, and LPL activity was measured. The effects of LPA in myocytes were also determined in the presence or absence of 1 μM cytochalasin D (CTD; an actin polymerization inhibitor).
appreciably enhanced basal and HR-LPL activity in the medium (Fig. 1) without influencing intracellular LPL in the myocyte pellet (unpublished observations). Interestingly, changes in LPL activity were independent of shifts in mRNA, suggesting a posttranscriptional increase in myocyte LPL (Fig. 1, inset).

**Augmentation of HR-LPL in LPA-perfused isolated hearts.** LPL at the coronary lumen is an outcome of translocation of the enzyme from the myocyte cell surface (40, 47). To determine whether the influence of LPA on myocyte LPL is subsequently reflected in an increase in the enzyme at the vascular lumen, isolated control hearts were perfused retrogradely with LPA for 1 h, and, subsequently, heparin was added to release luminal LPL into the coronary perfusate (Fig. 2). LPL discharge was rapid, and peak activity, likely representing LPL located at or near the endothelial surface, was observed within 1 min. Compared with control rat hearts, there was a substantial increase in coronary LPL activity (~200%) at the vascular lumen after 1 h of LPA perfusion (Fig. 2).

**Inhibition of LPA-mediated augmentation of myocyte HR-LPL.** Functional effects of LPA require activation of an endothelial gene-differentiating family of receptors (2, 32). We attempted to block these receptors using Sur (12). The ability of LPA to augment myocyte HR-LPL was inhibited by this putative LPA receptor antagonist (Fig. 3), suggesting that the effect of this lysophospholipid is receptor mediated. When cells were incubated for 30 min, Sur per se did not influence myocyte LPL (control: 195 ± 12 nmol·h⁻¹·10⁻⁶·cells and Sur: 221 ± 42 nmol·h⁻¹·10⁻⁶·cells). To investigate the involvement of the actin cytoskeleton in LPA-mediated augmentation of myocyte LPL, myocytes were pretreated with an actin polymerization inhibitor, CTD (34), before incubation with LPA. CTD abolished the effect of LPA to increase myocyte HR-LPL (Fig. 3) without any effect on basal activity (control: 195 ± 12 nmol·h⁻¹·10⁻⁶·cells and CTD: 127 ± 16 nmol·h⁻¹·10⁻⁶·cells).

**RESULTS**

**LPA augments myocyte LPL activity.** Myocardial distribution of LPL protein in the mouse heart demonstrates that 78% of total LPL is present in myocytes, 3–6% in the interstitial space, and 18% in the capillary endothelium (7, 8). We evaluated whether LPA can augment LPL in myocytes. Incubation of myocytes with graded concentrations of LPA (1–100 nM)
LPA induces cardiomyocyte actin polymerization. The actin cytoskeleton has been implicated in managing myocyte LPL activity (16). To determine whether LPA-mediated augmentation of myocyte LPL involves F-actin polymerization, cells were double stained with DNAse I Alexa fluor 594 and rhodamine 488-phalloidin to colocalize monomeric G-actin (red) and polymerized F-actin (green). In the resting cardiomyocyte, the proportion of G-actin is consistently higher than polymerized F-actin and is predominantly localized along the cell periphery. LPA induced formation of a dense and organized network of thick and parallel F-actin stress fibers with a complete absence of G-actin (Fig. 4). Pretreatment with CTD markedly reduced F-actin, whereas G-actin increased, consistent with F-actin depolymerization. The ratio of fluorescence of rhodamine and DNAse I (F-to-G-actin ratio) was used to quantify actin cytoskeleton organization; an increase in the F-to-G-actin ratio indicates actin polymerization. CTD pretreatment abolished LPA’s ability to increase this ratio (>60%; Fig. 4).

LPA-induced augmentation of HR-LPL requires RhoA and ROCK activation. Actin cytoskeleton organization is regulated by small Rho GTPases (18, 50), a subgroup of the Ras superfamily, with RhoA being its most extensively characterized member. After stimulation, cytosolic RhoA translocates to the cell membrane (50). Effectors of RhoA include serine/threonine kinases like ROCK I and ROCK II (46), which phosphorylate downstream substrates (46). Similar to RhoA, ROCKs also migrate to the cell membrane upon activation (46). Growth factors and LPA are characterized as upstream regulators of RhoA (32, 50, 56). Indeed, incubation of myocytes with LPA for 1 h caused significant membrane translocation of RhoA (Fig. 5, top) and ROCK I (Fig. 5, bottom) compared with control myocytes. ROCK II remained unaf-

Fig. 3. Blockade of the LPA effect on myocyte HR-LPL activity. Myocytes were pretreated with suramin (Sur) or cytochalasin D (CTD) for 30 min before incubation with 1 nM LPA for 1 h. Hep was then added to the cell suspension, and, after another 60 min, a 1-ml aliquot was aspirated and centrifuged, and the supernatant separated. The released LPL activity in the medium was determined. Results are means ± SE of 4 rats/group. *Significantly different from all other groups (P < 0.05).

Fig. 4. Myocyte actin rearrangement after LPA treatment. Myocytes were fixed, permeabilized, and double stained with DNAse I Alexa fluor 594 and rhodamine 488-phalloidin to colocalize G-actin (red) and F-actin (green). Slides were visualized using a confocal microscope at ×1,260 magnification. For the quantitative estimation of actin polymerization, cells were plated, fixed, and permeabilized as described above at a density of 75,000 myocytes/well. The increase in red and green fluorescence was monitored in a spectrofluorometer. Data are expressed as fluorescence units (FU) after the background was subtracted. *Significantly different from other groups (P < 0.05). An increase in the F-to-G-actin ratio was assumed to represent polymerization of actin filaments.
fected by LPA (unpublished observations). Incubation of myocytes with Y-27632 not only blocked membrane translocation of ROCK [as measured by densitometry; control: 1.3 FU; LPA: 2.2 FU; and LPA + Y-27632: 1.6 FU, \( P < 0.05 \)] but also appreciably reduced basal and HR-LPL activity obtained with LPA (Fig. 6). When incubated for 30 min, Y-27632 per se did not influence myocyte basal LPL activity (control: 195 ± 12 nmol·h\(^{-1}\)·10\(^{-6}\) cells and Y-27632: 143 ± 12 nmol·h\(^{-1}\)·10\(^{-6}\) cells).

**LPA augments adipose tissue LPL activity.** Given the difficulty in differentiating sarcomeric from nonsarcomeric actin in determining the LPL secretory function of LPA, we evaluated whether this lysophospholipid can augment LPL in adipose tissue. Incubation of adipose tissue with 1 nM LPA significantly enhanced basal and HR-LPL activity in the medium (Fig. 7).

RhoA and ROCK are activated during acute hyperglycemia.

We have previously reported that DZ, a selective ATP-sensitive K\(^+\) channel opener, decreases insulin secretion and causes hyperglycemia within 1 h (42). In addition, there was a substantial increase in coronary LPL activity and immunofluorescence in DZ-treated hearts. To evaluate whether this change in LPL during hyperglycemia is related to RhoA and ROCK activation, we measured membrane translocation of these mediators in DZ-treated hearts. DZ-treated hearts demonstrated significant membrane translocation of RhoA (Fig. 8, top) and ROCK 1 (Fig. 8, bottom) compared with control. To verify whether DZ-mediated RhoA and ROCK I activation was secondary to hyperglycemia and not DZ per se, insulin was

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**Fig. 5.** Effect of LPA on RhoA and Rho kinase (ROCK) 1 in isolated cardiomyocytes. Myocytes were incubated with LPA for 60 min, and homogenates were prepared for Western blot analysis. Homogenates were subjected to cytosolic (C) and membrane (M) separation, and respective protein contents were determined using a Bradford protein assay kit. Identification of RhoA and ROCK I protein was carried out using mouse monoclonal anti-RhoA or rabbit polyclonal anti-ROCK I primary antibodies at dilutions of 1:250 and 1:180, respectively, followed by incubation with goat anti-mouse (RhoA) or goat anti-rabbit (ROCK) horse-radish peroxidase-conjugated secondary antibodies at dilutions of 1:3,000 and 1:5,000, respectively. Results are means ± SE of 5 rats/group. *Significantly different from Con membrane fraction (\( P < 0.05 \)).

**Fig. 6.** ROCK inhibition and myocyte HR-LPL activity. Myocytes were pretreated with 10 \( \mu \)M Y-27632, an inhibitor of ROCK, for 30 min before incubation with 1 nM LPA for 1 h. Hep was then added to the cell suspension, and, after 1 h, a 1-ml aliquot was aspirated and centrifuged, and the supernatant was separated for measurement of LPL activity. Data are means ± SE of 4 different hearts/group. *Significantly different from its corresponding basal value; #significantly different from Con HR-LPL; *significantly different from control basal value (\( P < 0.05 \)). Insert: single representative Western blot of cardiac ROCK I in cytosolic and membrane fractions of myocytes incubated with or without 1 nM LPA either in the presence or absence of 10 \( \mu \)M Y-27632.
administered to DZ-treated animals. Insulin not only normalized blood glucose and cardiac LPL (42) but also abrogated the DZ-mediated membrane translocation of ROCK I [as measured by densitometry; control: 0.36 FU; DZ: 2.33 FU; and DZ + insulin: 0.39 FU, \( P < 0.05 \)] and RhoA [as measured by densitometry; control: 0.58 FU; DZ: 3.97 FU; DZ + insulin: 1.12 FU, \( P < 0.05 \)].

DISCUSSION

Hydrolysis of TG-rich lipoproteins by LPL positioned at the endothelial surface of the coronary lumen is suggested to be the principal source of FA for myocardial energy utilization (5). Endothelial cells do not produce LPL, and hence the enzyme is synthesized in myocytes (11). After enzyme activation and transport by mechanisms that are largely unknown, LPL is transported onto myocyte cell surface HSPG before it is translocated onto comparable HSPG binding sites on the luminal side of the coronary vessel wall (15). Heparan sulfate oligosaccharides, fragments of myocyte HSPG, have then been shown to act as extracellular chaperones for LPL, transporting the enzyme in its active form toward the apical surface of endothelial cells (36, 38). Mediators responsible for the cleavage and transfer of LPL from the parenchymal cell surface to the lumen include the lipolytic byproduct lysoPC (41). At least in the whole heart, the LPL-augmenting property of lysoPC likely required endothelial PKC activation and formation of LPA (41). One of the best-characterized effects of LPA is actin cytoskeleton reassembly (2, 32, 56, 59). Given that incubation of cultured cardiomyocytes with insulin and dexamethasone stimulated basal and HR-LPL activities, a process involving actin cytoskeleton reorganization (16), we hypothesized that LPA augments cardiomyocyte LPL secretion through its effects on actin cytoskeleton. In this study, for the first time, we demonstrate that the LPA-mediated augmentation of cardiomyocyte LPL involves the actin cytoskeleton.

ATP-dependent reversible polymerization of G- to F-actin provides the cell structural framework, in addition to mechanical force that allows for changes in contraction (13), locomotion, chemotaxis, and, more importantly vesicular transport (53). This polymerized F-actin network beneath the cell membrane has been proposed to be a negative modulator of vesicular secretion, acting as a physical barrier as it depolymerizes during exocytosis (33, 57). Other studies have suggested an

![Fig. 7. Changes in adipose LPL activity after incubation with LPA. Epididymal adipose tissue fat pads (0.5 g) were incubated either in the absence or presence of 1 nM LPA for 1 h. Hep (5 U/ml) was then added to the cell suspension, and, after another 60 min, a 1-ml aliquot was aspirated and centrifuged, and the supernatant was separated for measurement of LPL activity. LPL activity is expressed as milliunits of activity per gram of tissue. Results are means ± SE of 3 rats/group. *Significantly different from its corresponding basal value; #significantly different from control HR-LPL; + significantly different from control basal value (\( P < 0.05 \)).](http://ajpheart.physiology.org/)

![Fig. 8. RhoA and ROCK I in hearts isolated from acutely hyperglycemic rats. Diazoxide (DZ; 100 mg/kg) was administered to Con animals, and hearts were isolated after 4 h. Whole heart homogenates were prepared for Western blot analysis. Homogenates were subjected to cytosolic and membrane separation, and respective protein contents were determined using a Bradford protein assay kit. Identification of RhoA and ROCK I protein was carried out as described above. Results are means ± SE of 5 rats/group. *Significantly different from control membrane fraction (\( P < 0.05 \)).](http://ajpheart.physiology.org/)

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The obligatory role for F-actin formation in facilitating ligand-induced protein secretion (35). Thus, in adrenal chromaffin cells, mobility of secretory granules is mediated by actin filament formation, a process that is blocked by lantrunculins, which disassemble cortical actin (54). In addition, insulin-mediated GLUT-4 translocation in skeletal muscle (9) and adipocytes require intact F-actin (21). LPA is an established upstream mediator of actin polymerization, and, in adrenal tumor PC12 cells, LPA-stimulated release of acetylcholine was inhibited when actin reorganization was disrupted using butulinum toxin (20). In the present study, incubation of myocytes with LPA induced formation of a dense and organized network of thick and parallel F-actin fibers, an effect that was abrogated by pretreatment with CTD. Because this effect of LPA on F-actin formation occurred at a time when HR-LPL activity was augmented, our data suggest that actin polymerization likely facilitates secretion of this enzyme in myocytes.

LPA-induced polymerization of actin requires activation of an endothelial gene-differentiating family of G protein-coupled receptors (2, 32, 56, 59). In this study, we confirmed that the effects of LPA on the actin cytoskeleton and myocyte LPL are receptor mediated because pretreatment with Sur, a putative LPA receptor antagonist, blocked these effects. G proteins that participate in LPA receptor-mediated effects include Goq, Gq, and Gi2/13 (14), with activation of the Gi2/13 pathway being most closely linked to actin polymerization (22). Stimulation of Gi2/13 activates small Rho GTPases (10, 14), specifically RhoA, a subgroup of the Ras superfamily that is identified as the essential upstream regulator of actin polymerization. After receptor activation, cytosolic RhoA translocates to the cell membrane (50). Effectors of RhoA include serine/threonine kinases like ROCK I and ROCK II (46), which also migrate to the cell membrane upon RhoA-induced activation, with ensuing phosphorylation of downstream substrates (46). Exposure of myocytes to LPA for 60 min facilitated significant membrane translocation of RhoA and ROCK I. Interestingly, Y-27632 pretreatment not only blocked membrane translocation of ROCK I but also appreciably reduced basal and HR-LPL activity obtained with LPA. Overall, these results indicate that LPA likely facilitates secretion of preformed LPL, a process that is dependent on polymerized actin.

In adipose tissue, FA for TG storage are largely obtained through LPL-catalyzed hydrolysis of circulating lipoproteins (61). Like in the heart, dietary and hormonal factors also influence the enzyme in this tissue. For example, with fasting, LPL activity decreases (58), whereas insulin is known to augment adipose tissue LPL (17). To evaluate whether the effects of LPA on myocyte LPL can be duplicated in adipose tissue, we measured HR-LPL after treatment with LPA. Because incubation of adipose tissue with LPA significantly enhanced basal and HR-LPL activity, our data suggest that sarcomeric actin likely has a limited role in influencing the LPL secretory function of LPA in the myocyte. Interestingly, peroxisome proliferator-activated receptor (PPAR)-γ was recently characterized as an intracellular receptor for LPA (29), and PPAR-γ activation is known to enhance adipose tissue LPL activity (23).

During diabetes, LPL increases to guarantee FA supply to the diabetic heart to compensate for the diminished contribution of glucose as an energy source, allowing the heart to maintain its function (43, 47, 48). Thus, after acute hypoinsulinemia induced with DZ, HR-LPL activity and protein increase at the coronary lumen (42). Because these effects of DZ were inhibited after suppression of circulating TG hydrolysis, lipolytic byproducts like lysoPC were implicated in explaining this enzyme increase (42). LPA is a known metabolite of lysoPC (4). We hypothesized that the increase in HR-LPL activity after DZ could be related to an LPA-induced activation of Rho GTPases. Given that the effects of LPA on myocyte LPL were observed at 1 nM and the technical difficulty associated with measuring trivial concentrations of this lysophospholipid, we measured the activation of downstream mediators facilitating actin polymerization in hearts from DZ-treated animals. Similar to the results obtained when myocytes were incubated with LPA, hyperglycemia also caused significant membrane translocation of RhoA and ROCK I in whole hearts. Interestingly, injection of insulin normalized blood glucose and luminal LPL (42) in addition to preventing DZ-induced membrane translocation of RhoA and ROCK I. Hence, a mechanism for the acute effect of hyperglycemia in augmenting cardiac HR-LPL could involve RhoGTPase activation and actin reorganization.

In summary, LPA-induced increases in myocyte and adipose tissue LPL occurred via posttranscriptional mechanisms and processes that likely required RhoA activation and actin polymerization (Fig. 9). In congenital generalized lipodystrophy, where LPA conversion to phosphatidic acid is impaired, levels of LPA increase with augmented TG deposition in skeletal muscle (1) and associated insulin resistance (31). Whether this process occurs via increases in LPL merits further investigation. In the heart, LPA, by increasing LPL, may initiate lipid oversupply with resulting muscle fiber degeneration (24), extensive lipid deposition (24), proliferation of mitochondria and peroxisomes (24), and eventual impairment of contractile function (60).
REFERENCES


