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PIP3 but not PIP2 increases GLUT4 surface expression and glucose metabolism mediated by AKT/PKC ζ / λ phosphorylation in 3T3L1 adipocytes

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Abstract

PIP3 (phosphatidylinositol-3,4,5-triphosphate) and PIP2 (phosphatidylinositol-4,5-bisphosphate) are two well-known membrane bound polyphosphoinositides. Diabetes is associated with impaired glucose metabolism. Using a 3T3L1 adipocyte cell model, this study investigated the roles of PIP3 and PIP2 on insulin stimulated glucose metabolism in high glucose (HG) treated cells. Exogenous PIP3 supplementation (1, 5, or 10 nM) increased the phosphorylation of AKT and PKC ζ / λ , which in turn upregulated GLUT4 total protein expression as well as its surface expression, glucose uptake, and glucose utilization in cells exposed to HG (25 mM); however, PIP2 had no effect. Comparative signal silencing studies with antisense AKT2 and antisense PKC ζ revealed that phosphorylation of PKC ζ / λ is more effective in PIP3 mediated GLUT4 activation and glucose utilization than in AKT phosphorylation. Supplementation with PIP3 in combination with insulin enhanced glucose uptake and glucose utilization compared to PIP2 with insulin, or insulin alone, in HG-treated adipocytes. This suggests that a decrease in cellular PIP3 levels may cause impaired insulin sensitivity in diabetes. PIP3 supplementation also prevented HG-induced MCP-1 and resistin secretion and lowered adiponectin levels. This study for the first time demonstrates that PIP3 but not PIP2 plays an important role in GLUT4 upregulation and glucose metabolism mediated by AKT/PKC ζ / λ phosphorylation. Whether PIP3 levels in blood can be used as a biomarker of insulin resistance in diabetes needs further investigation.

Keywords

PIP3; PIP2; AKT; PKC ζ / λ ; GLUT4; Glucose metabolism; Diabetes

Introduction

Phosphatidylinositol and its phosphorylated derivatives (referred to as phosphoinositides, PI) has long been known to have an important regulatory role in cell physiology [1].

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Author Contributions

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Disclosure Statement

The authors have declared that no conflict of interest exists.

Phosphatidylinositol-3,4,5-triphosphate (PIP3) and phosphatidylinositol-4,5-bisphosphate (PIP2) are two well known lipid second messengers [2]. The regulatory role of PIP3 is of fundamental importance in higher eukaryotes, as it mediates a large variety of effects including cell proliferation, apoptosis, metabolism, motility, and immune responses [3]. PIP3 target proteins are diverse in nature and include protein kinases, adaptor molecules, and GAP and GEF for small GTPases [4, 5]. This diversity in PIP3 signaling makes this molecule an important lipid second messenger downstream from growth factor and oncogene signaling cascades. PIP2 on the other hand regulates a variety of diverse cellular activities, including modulation of the actin cytoskeleton, endocytosis, exocytosis, and ion channel activity [6].

Insulin stimulated glucose uptake and metabolism is one of the fundamental mechanisms responsible for the maintenance of glucose homeostasis in the body. Impaired insulin action or insulin resistance leads to diabetic pathophysiology. PI3K (phosphoinositide-3-kinase) and PTEN (phosphatase and tensin homologue deleted on chromosome 10) play central roles in the insulin signaling cascade and glucose metabolism [7]. In response to an extracellular signal the majority of intracellular PIP3 is synthesized via the activation of a Class I PI3K enzyme, which phosphorylates PIP2 into PIP3 [7]. The PIP3 signaling event is terminated either by the phosphatase PTEN, which dephosphorylates the D3 position of PIP3, or by the phosphatase SHIP (SH2 domain-containing inositol 5- phosphate SHIP), which dephosphorylates the D4 position of PIP3 [7]. Thus the intracellular PIP3 concentration is regulated by the PI3K/PTEN or SHIP equilibrium. Studies in the literature have shown that inhibition of PTEN expression using PTEN antisense oligonucleotides normalized blood glucose levels in ob/ob (obese) mice [8] and that overexpression of PTEN resulted in inhibition of PIP3 production and glucose uptake in 3T3L1 adipocytes [9]. Similarly, various studies using knockout mice and cell models of PTEN, GLUT4, and PI3K have documented that inhibition of PTEN, and activation of PI3K and AKT signaling molecules, are crucial in insulin signaling pathways and to the maintenance of whole body glucose metabolism [10]. Cells that lack PTEN showed elevated PIP3 levels and activated AKT-dependent signaling [7]. These findings suggest that PIP3 can play a role in the insulin signaling pathway [1, 3]. However, at present there is no report in the literature concerning whether PIP3 or PIP2 has a direct effect on glucose uptake and glucose utilization at the cellular level. Using an adipocyte cell model, this study demonstrates that PIP3 but not PIP2 increases both glucose uptake and glucose utilization and that the effect is mediated by the activation of the AKT/PKC ζ /GLUT4 signaling pathway. Comparative signal silencing studies using antisense AKT2 and antisense PKC ζ demonstrated that PKC ζ is significantly more active in PIP3 mediated GLUT4 activation and glucose uptake as well as in glucose utilization. Using a 3T3L1 adipocyte cell model, this study for the first time provides evidence for a novel mechanism by which PIP3 but not PIP2 increases GLUT4 surface expression and glucose metabolism mediated by AKT/PKC ζ /GLUT4 phosphorylation.

Materials and Methods

Materials

GLUT4 antibody was purchased from Abcam, Inc. (Cambridge, MA). GLUT2 antibody was purchased from Millipore (Billerica, MA). AKT2, phospho AKT (serine 473), phospho PKC ζ/λ (threonine 410/403), and PKC ζ were purchased from Cell Signaling Technology (Beverly, MA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise mentioned.

3T3L1 adipocyte cell culture and differentiation

The murine 3T3L1 fibroblast cell line was obtained from American Type Culture Collection (ATCC, Manassas, VA). These cells were cultured in high glucose DMEM containing 10% (v/v) FCS, 100 U/mL penicillin, 100 μ g/mL streptomycin, and maintained at 37°C in a humidified atmosphere containing 5% (v/v) CO₂. Three days after achieving confluence, to allow for differentiation into adipocytes, cells were incubated in high glucose DMEM containing 10% (v/v) FBS, 100 U/mL penicillin, and 100 μ g/mL streptomycin supplemented with 100 mU/mL insulin, 0.5 mM IBMX (3-isobutyl-1-methylxanthine) and 250 nM dexamethasone for 2 days. The cells were then placed in the same medium containing insulin but lacking any other supplements for an additional 2 days. The media were replaced every 2 days thereafter until more than 85% of the cells contained lipid droplets. Seven to 10 days after the induction of differentiation, 3T3L1 adipocytes were ready to be used in experiments [11]. The cells were incubated with serum-free low-glucose DMEM during the experimental incubation period.

Treatment of adipocytes with high glucose (HG), PIP3 and PIP2

Cells were treated with normal glucose (7 mM) and HG (25 mM) with and without PIP3 and PIP2 separately. In this study, cells were exposed to 25 mM HG. Many previous studies have reported that glucose concentrations as high as 50 mM have been found in the blood of patients with uncontrolled diabetes [12]. It is true that blood glucose levels in patients are not likely to stay as high as 25 mM for 24 h. However, tissue damage in diabetic patients occurs over many years of countless hyperglycemic episodes. Thus, the glucose concentration of 25 mM used in this cell-culture study does not seem unreasonable. Anionic phosphatidylinositol phosphate derivatives were delivered across the cell membrane by complexing them with a positive lysine-rich histone carrier following the procedure described by the manufacturer (Echelon Biosciences, Inc., Salt Lake City, UT). Unlabeled Histone H1 (Echelon Biosciences) and PIP3 or PIP2 (Echelon Biosciences) were mixed at a molar ratio of 1:1, vortexed, subjected to sonication, and incubated for 10 minutes at room temperature. The mixture was then added to the medium of adipocytes to a final concentration of 1, 5, or 10 nM for 2 or 4 h respectively, followed by HG (25 mM) for the next 20 h. After treatment, cells were lysed in RIPA supplemented with protease and phosphatase inhibitors. Lysates were cleared by centrifugation and total protein concentrations were determined using a BCA assay kit (Pierce/Thermo Scientific, Rockford, IL).

siRNA transient transfection studies

AKT2 siRNA and control siRNA were purchased from Cell Signaling Technology (Beverly, MA). PKC ζ siRNA was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). 3T3L1 adipocytes grown in 12-well plates were transiently transfected with 100 nM siRNA complex using LipofectamineTM2000 transfection reagent (Invitrogen, Carlsbad, CA) following the method as described earlier [11]. Control siRNA, a fluorescein conjugated scrambled nonspecific 25 nucleotide RNA duplex with no sequence homology with any of the genes, was used as a negative control as well as to monitor the transfection efficiency.

Glucose utilization, glucose uptake, cell viability, and cytokine secretion

Glucose utilization assays were done at 0 h and at other specified times. The glucose utilization level was determined by subtracting glucose values at specified times (leftover glucose) from the 0 h glucose level. All assays were done in duplicate at each time point [11]. An Advantage Accu- Chek glucometer (Boehringer Mannheim Corporation, Indianapolis, IN) was used for the glucose utilization assay. The glucose uptake assay was performed using 6-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (6-NBDG; Invitrogen), a fluorescent analogue of 2-deoxyglucose, following the method of Jung *et al.* [13]. Results were expressed as relative fluorescence units (RFU). Cell viability was determined using the Alamar Blue reduction bioassay (Alamar Biosciences, Sacramento, CA). This method is based upon Alamar Blue dye reduction by live cells. The MCP-1 (monocyte chemoattractant protein-1) and resistin levels in the supernatants of treated cells were measured by the sandwich ELISA method using commercially available kits from R&D Systems, Inc. (Minneapolis, MN) with an intra-assay coefficient of variation (CV) of less than 5% and interassay CV of less than 4%. Adiponectin levels were determined using a kit and reagents from ALPCO Diagnostics (Salem, NH) with intra-assay and interassay CVs of 4.6 and 7.5%, respectively. All appropriate controls and standards as specified by each manufacturer's kit were used. In the cytokine assay, control samples were analyzed each time to check the variation from plate to plate on different days of analyses.

Immunoblotting

All samples contained approximately the same amount of protein (~20–40 μ g) and were run on 8%–10% SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were blocked at room temperature for 2 h in blocking buffer containing 1% BSA to prevent non-specific binding and then incubated with: anti-AKT (AKT2) (1:1000 dilution), anti- PKC ζ (1:1000), anti-GLUT4 (1:1000), anti-phosphorylated AKT (serine 473) (1:500), or anti-phosphorylated PKC ζ / λ (1:500) (threonine 410/403) primary antibodies at 4°C overnight. The membranes were washed in TBS-T (50 mmol/L Tris-HCl, pH 7.6, 150 mmol/L NaCl, 0.1% Tween 20) for 30 min and incubated with the appropriate HRP conjugated secondary antibody (1:5000 dilution) for 2 h at room temperature and developed using the ultrasensitive ECL substrate (Millipore, MA). The intensity of each immunoblotting band was measured using the histogram tool of Adobe Photoshop CS5.

Detection of GLUT4 surface expression

GLUT4 surface expressions were determined using flow cytometry as described earlier [14] with some modifications. After treatment cells were washed in FACS buffer (PBS without Mg^{2+} and Ca^{2+} , with the addition of 2% FBS and 0.002% sodium azide), centrifuged, suspended in the FACS buffer and incubated for 2 h at 4°C with appropriate anti-GLUT4 antibody (Santa Cruz Biotechnology) at a 1:50 dilution. Instead of antibody, polyclonal rabbit serum was added to the control sample. The cells were then washed in the buffer for FACS and incubated with a FITC conjugated appropriate secondary antibody (Abcam) at a 1:40 dilution on ice for 30 min in the dark. After the incubation, 1 mL washing buffer for FACS was added to each sample. The samples were then vortexed, centrifuged, the supernatant removed, and 0.5 mL washing buffer for FACS and 1% formaldehyde was added. In each experiment, a minimum of 15,000 cells was analyzed (per treatment condition) by flow cytometer. Gates were set to exclude nonviable cells, cell debris, and cells of abnormal size and shape. Results were expressed as mean fluorescence intensity (MFI) per 15,000 cells.

Statistical analysis

Data were analyzed statistically using ANOVA with Sigma Stat statistical software (Jandel Scientific, San Rafael, CA). When data passed a normality test, all groups were compared using the Student–Newman–Keuls method. A difference was considered significant at the $p < 0.05$ level.

Results

Effect of PIP3 and PIP2 on glucose uptake and glucose utilization in high glucose exposed adipocytes

Figure 1A–B illustrates the effect of PIP3 and PIP2 on glucose uptake and glucose utilization levels in adipocytes exposed to HG (25 mM, 20 h). It shows that treatment with different concentrations (1, 5, or 10 nM) of PIP3 for 4 h increased the glucose uptake levels. The glucose utilization study also revealed that at doses of 5 and 10 nM for 4 h, PIP3 treatment showed optimum glucose utilization levels. However, under the identical conditions, compared to the HG exposed cells, PIP2 supplementation (1, 5, or 10 nM) did not cause any significant changes in either glucose uptake or glucose utilization levels.

Role of PIP3 and PIP2 on activation of the AKT/PKC ζ /GLUT4 signaling pathway

Figure 2A–D shows that treatment with PIP3 increased the phosphorylation of both AKT (2A) and PKC ζ / λ (2B) and the GLUT4 (2C) total protein expression in 3T3L1 adipocytes exposed to HG compared to those seen in cells treated with HG alone. Different concentrations of PIP2 supplementation had no significant effect on the phosphorylation of AKT and PKC ζ / λ , nor on GLUT4 total protein expression in HG-treated cells. The surface expression of GLUT4 was also investigated using flow cytometric analysis (2D). We observed that PIP3 supplementation increased the GLUT4 surface expression to a level ~75% higher than that observed in HG- treated cells. However, no significant differences were observed on the GLUT4 surface expression in cells treated with PIP2 and HG.

Signal silencing studies with siAKT2 and siPKC ζ

Comparative signal silencing studies with either AKT2 siRNA or PKC ζ siRNA are shown in Figures 3–4. Figure 3 shows that transfection with either AKT2 siRNA or PKC ζ siRNA significantly downregulated the respective protein expression (~ 75%) compared to that seen in cells transfected with control siRNA. We observed that transient transfection with either AKT2 siRNA or PKC ζ siRNA decreased glucose metabolism (4A, 4B) compared to that seen in normal cells. The effect of PKC ζ silencing on glucose utilization was found to be more pronounced than that of AKT2. Exogenous addition of PIP3 improved both glucose uptake (4A) and glucose utilization (4B) in the AKT2 silencing cells, but no such effect was observed in the PKC ζ silencing cells. The same effect was also observed in GLUT4 expression (4C). PIP3 supplementation improved GLUT4 expression (4C) against HG exposure in the adipocytes transfected with AKT2 siRNA. However, compared with AKT2 silencing, PIP3 treatment had no effect on GLUT4 expression in the adipocytes transfected with PKC ζ siRNA. Treatment with PIP2 had no significant effect on the GLUT4 expression and glucose utilization in either the AKT2 siRNA or the PKC ζ siRNA transfected cells compared to those in the HG-treated group.

Effect of insulin with and without PIP3 or PIP2 on glucose uptake and glucose utilization in adipocytes exposed to HG

Figure 5 shows the effect of insulin alone or in combination with PIP3 or PIP2 supplementation (5 nM) on glucose uptake and glucose utilization levels in cells exposed to HG. It has been observed that PIP3 supplementation (5 nM) along with insulin (10, 25, 50, or 100 nM) significantly boosted the glucose uptake (~2 fold) and glucose utilization (~4 fold) levels compared to those of the cells supplemented with insulin alone exposed to HG. Interestingly, equimolar PIP2 supplementation along with insulin had no significant effect on either glucose uptake or glucose utilization levels compared to those of cells treated with insulin alone. The outcome of this study suggests that the decrease in cellular PIP3 levels plays a role in the impaired insulin sensitivity in T2D.

Effect of PIP3 and PIP2 on the secretion of MCP-1, adiponectin, and resistin in adipocytes

The data in Figure 6A show that HG treatment increased the MCP-1 secretion in adipocytes. However, pretreatment with PIP3 attenuated this phenomenon. Similarly, PIP3 supplementation increased the adiponectin secretion in adipocytes exposed to HG (6B). HG exposure also increased resistin secretion from adipocytes, but supplementation with PIP3 prevented HG induced resistin secretion (6C). PIP2 treatment had no effect on the secretion of MCP-1, adiponectin, or resistin compared to that in HG-treated cells. This suggests that PIP3 but not PIP2 can mediate inhibition of MCP-1 and resistin secretion and an increase in adiponectin levels in adipocytes exposed to HG.

Discussion

PIP3 and PIP2 regulate a complex intracellular signaling network. Cellular concentration of these two phospholipids is regulated by the PI3K/PTEN equilibrium. Dysregulation of this pathway has been implicated in many human diseases including diabetes [15]. Adipose tissue is crucial in regulating glucose metabolism. Using a 3T3L1 adipocyte cell model, this

study demonstrates for the first time that treatment with PIP3 but not PIP2 per se increases GLUT4 surface expression and glucose uptake as well as glucose utilization at the cellular level mediated by AKT/PKC ζ / λ phosphorylation.

PIP3 recruits a number of proteins having a pleckstrin homology (PH) domain such as PDK1, PDK2, AKT/PKB and PKC ζ / λ [4, 5]. A direct and vital target of PIP3 is AKT, a serine-threonine protein kinase essential for both cell survival and metabolism. PIP3 binds with the PH domain of AKT/ PKB and promotes its activation, which stimulates GLUT4 translocation and glucose uptake [16]. In line with AKT/GLUT4 signaling, several lines of recent evidence suggest that PKC ζ / λ (atypical protein kinase C zeta/lambda) also plays an important role in insulin stimulated GLUT4 translocation/glucose uptake in muscles [17, 18] and adipose tissues [19, 20]. The binding of acidic lipids such as PIP3 with the regulatory domain of PKC ζ / λ leads to molecular unfolding and an increase in enzymatic activity [21]. Therefore, binding with PIP3 activates PKC ζ / λ , which enhances more rapid GLUT4 translocation. Although most cell types also constitutively express the GLUT1 isoform at the cell surface, we focused our study on GLUT4 because the insulin-stimulated increase in plasma membrane-associated GLUT4 protein accounts for the majority of post-prandial glucose disposal in both muscle and adipose tissue [22]. In this study exogenous PIP3 supplementation increased the phosphorylation of AKT and PKC ζ / λ and GLUT4 total protein expression, as well as the levels of glucose uptake and glucose utilization in adipocytes treated with HG. Different concentrations of PIP3 treatment also increased the GLUT4 surface expression in a dose dependent manner. However, under the same conditions equimolar treatment with PIP2 had no significant effect on the AKT/PKC ζ / λ / GLUT4 signaling pathway as well as glucose metabolism in adipocytes treated with HG compared to those observed in cells treated with HG alone.

Comparative signal silencing studies with either antisense AKT2 or antisense PKC ζ revealed that PKC ζ plays a greater role in GLUT4 activation and glucose utilization than does AKT2. The same effect was also observed in GLUT4 surface expression. Treatment with PIP3 increased the glucose utilization and GLUT4 activation against HG exposure in the siAKT2 transfected cells but the effect was significantly attenuated in the siPKC ζ silencing study. In our earlier study we observed that HG exposure decreases the intracellular PIP3 levels in adipocytes and that this could be upregulated the exogenous PIP3 supplementation [11]. Thus it seems likely that in adipocytes, defects in insulin stimulated glucose metabolism are due to the uncoupling of AKT/PKC ζ / λ /GLUT4 signaling pathways, which may in turn be linked with the decreased production of PIP3 in the hyperglycemic environment. Interestingly, it has been observed that PIP3 supplementation in combination with insulin significantly enhanced glucose uptake and glucose utilization compared to treatment with either PIP2 with insulin or insulin alone in HG-treated adipocytes. This suggests that a decrease in cellular PIP3 levels may be linked to impaired insulin sensitivity and glucose metabolism in T2D.

In an earlier study Jiang *et al.* [23] reported that insulin stimulated glucose transport in adipocytes was inhibited by the PI3K inhibitor, wortmannin, but that supplementation with exogenous diC8-PIP3/AM (dioctanoyl-PIP3-acetoxymethyl ester) was capable of overcoming the inhibitory effect of wortmannin in insulin stimulated glucose transport.

Using 3T3L1 adipocytes, Funaki *et al.* [24] reported that in the presence of a cell permeable phosphoinositide-binding peptide (PBP10), PIP2 functions as a second messenger in GLUT4 activation and glucose uptake, possibly through regulation of F-actin remodeling. However, at present there is no report in the literature concerning whether PIP3 or PIP2 has a direct effect on glucose uptake and glucose utilization at the cellular level. This study demonstrates for the first time that PIP3 but not PIP2 increases GLUT4 total protein expression as well as its surface expression and glucose metabolism mediated by AKT/PKC ζ/λ phosphorylation.

Adiponectin, in addition, has also been shown to upregulate the glucose uptake and fatty acid oxidation in skeletal muscle cells or a muscle cell line [25]. Resistin, an adipocyte secreted factor, is well known for its potential links to obesity and development of insulin resistance [26]. MCP-1 plays an important role in vascular inflammation [27]. This study shows that PIP3 supplementation increased adiponectin secretion and reduced MCP-1 and resistin secretion in adipocytes exposed to HG. This suggests that PIP3 can also positively influence the metabolic actions of insulin via inhibition of MCP-1 and resistin secretion and increased adiponectin levels.

Studies in the literature reported that atypical protein kinase C (aPKC, including ζ and λ/ϵ) activation is defective in type 2 diabetic patients [28, 29]. The role of aPKC in glucose metabolism is well established [28, 29] and it has been reported that it can be activated by different lipid components, such as phosphatidylinositols, phosphatidic acid, arachidonic acid, and ceramide [30, 31]. PIP3 plays an important role for the complete and stable activation of PKC ζ [30, 31]. Metformin is an insulin sensitizing drug widely used in the treatment of type 2 diabetic patients. Farese and colleagues reported that the responsiveness of PIP3 to activate immunoprecipitated PKC ζ is impaired in the muscles of type 2 diabetic or obese humans [28, 29, 32, 33]. Interestingly, PIP3 supplementation (10 μ mol/L) causes a significant increase in PKC ζ activity in the immunoprecipitates prepared from the muscles of long term metformin-treated type 2 diabetic subjects compared to the muscles of diabetic subjects who were on or off long-term metformin treatment [34]. This improved responsiveness of PKC ζ to PIP3 suggests that PIP3 may play an important role in improving insulin-stimulated aPKC activation during long term metformin treatment. However, the effect of metformin on intracellular PIP3 levels in different tissues of diabetic subjects is still lacking and needs to be investigated.

In conclusion, this study demonstrates that PIP3 increases the glucose uptake and glucose utilization mediated via the activation of AKT/PKC ζ/λ /GLUT4 signaling pathways in murine 3T3L1 adipocytes. Figure 7 outlines a schematic representation of a proposed mechanism by which PIP3 can positively modulate insulin stimulated glucose metabolism. Diabetes is associated with hyperglycemia and increased oxidative stress, which can upregulate PTEN and downregulate PI3K, leading to a decrease in cellular PIP3. Using an adipocyte cell model, this study provides evidence for a molecular mechanism by which PIP3, but not PIP2, can modulate the AKT/PKC ζ/λ /GLUT4 signaling cascade to improve glucose metabolism and that a decrease in cellular PIP3 may be linked with insulin resistance and impaired glucose metabolism in diabetes. Future studies on the status of PIP3 in various tissues are needed to validate the role of PIP3 as a biomarker for insulin resistance

and impaired glucose homeostasis in diabetes. If this role is so, validated approaches for the discovery of new therapeutics should also target improvement of PIP3 status in diabetes.

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Figure 1.

Effect of PIP3 and PIP2 on glucose uptake and glucose utilization in adipocytes (3T3L1) exposed to HG. A: glucose uptake and B: glucose utilization. Cells were pretreated with either PIP3 (1, 5, or 10 nM) or PIP2 (1, 5, or 10 nM) for 4 h followed by HG (25 mM) exposure for the next 20 h. Values are expressed as mean $\pm$ SE (n=3). Difference between * vs @ is considered significant at the $p < 0.05$ level.

Figure 2.

Effect of PIP3 and PIP2 on the expression of phospho AKT (serine 473)/ AKT (AKT2), phospho PKCζ/λ (threonine 410/403)/PKCζ, GLUT4 and levels of GLUT4 surface expression in adipocytes (3T3L1) exposed to HG. A: phospho AKT/ AKT, B: phospho PKCζ/λ /PKCζ, C: GLUT4, and D: GLUT4 surface expression. Cells were pretreated with either PIP3 (1, 5, or 10 nM) or PIP2 (1, 5, or 10 nM) for 4 h followed by HG (25 mM) exposure for the next 20 h. Values are expressed as mean ± SE (n=3). Difference between * vs @ is considered significant at the $p < 0.05$ level.

Figure 3.

Effect of PIP3 and PIP2 on AKT2 or PKC ζ in adipocytes (3T3L1) exposed to HG after transfection with either AKT2 siRNA or PKC ζ siRNA. Cells were transfected with either AKT2 siRNA (100 nM) or PKC ζ siRNA (100 nM) followed by treatment with PIP3 or PIP2 with or without HG. Values are expressed as mean $\pm$ SE (n=3).

Figure 4.

Effect of PIP3 and PIP2 on glucose uptake, glucose utilization, and GLUT4 expression in adipocytes (3T3L1) exposed to HG after transfection with either siAKT2 or siPKC ζ . A: glucose uptake, B: glucose utilization, and C: GLUT4 expression. The left panel shows the result with siAKT2 and the right panel with siPKC ζ . Cells were transfected with either siAKT2 (100 nM) or siPKC ζ (100 nM) followed by treatment with PIP3 or PIP2 with or without HG as described in the Methods section. Values are expressed as mean $\pm$ SE (n=3). Difference between * vs @ is considered significant at the $p < 0.05$ level.

Figure 5.

Effect of PIP3 and PIP2 in combination with insulin on glucose metabolism (both glucose uptake and glucose utilization) in adipocytes (3T3L1) exposed to HG. A: glucose uptake and B: glucose utilization. Cells were pretreated with either insulin (10, 25, 50, or 100 nM) alone or in combination with PIP3 (5 nM) or PIP2 (5 nM) for 4 h followed by HG (25 mM) exposure for the next 20 h. Values are expressed as mean $\pm$ SE (n=3). Difference between * vs @ is considered significant at the $p < 0.05$ level.

Figure 6.

Effect of PIP3 and PIP2 supplementation on secretion of MCP-1, adiponectin, and resistin in adipocytes (3T3L1) exposed to HG. A: MCP-1, B: adiponectin, and C: resistin. Cells were pretreated with either PIP3 (1, 5, or 10 nM) or PIP2 (1, 5, or 10 nM) for 4 h followed by HG (25 mM) exposure for the next 20 h. Values are expressed as mean $\pm$ SE (n=3). Difference between * vs @ is considered significant at the $p < 0.05$ level.

Figure 7. Schematic diagram of proposed mechanism of the effect of PIP3, but not PIP2, on glucose metabolism mediated via the activation of AKT/PKC ζ/λ /GLUT4 signaling cascade in adipocytes.