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Endothelin-1 inhibits adiponectin secretion through a phosphatidylinositol 4,5-bisphosphate/actin-dependent mechanism

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Abstract

Adiponectin is an adipokine with profound insulin-sensitizing, anti-inflammatory, and anti-atherogenic properties. Plasma levels of adiponectin are reduced in insulin resistant states such as obesity, type 2 diabetes and cardiovascular disease. However, the mechanism(s) by which adiponectin concentrations are decreased during disease development is unclear. Studies have shown that endothelin-1 (ET-1), a vasoconstrictor peptide, affects adipocyte glucose metabolism and secretion of adipokines such as leptin, resistin, and adiponectin. The goal of our study was to determine the mechanism by which ET-1 decreases adiponectin secretion. 3T3-L1 adipocytes were treated for 24 h with ET-1 (10 nM) and then stimulated with vehicle or insulin (100 nM) for a period of 1–2 h. Chronic ET-1 (24 h) treatment significantly decreased basal and insulin-stimulated adiponectin secretion by 66% and 47%, respectively. Inhibition of phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis by the PLCβ inhibitor, U73122, or exogenous addition of PIP_2:histone carrier complex (1.25:0.625 μM) ameliorated the decrease in basal and insulin-stimulated adiponectin secretion observed with ET-1. However, treatment with exogenous PIP_2:histone carrier complex and the actin depolymerizing agent latrunculin B (20 μM) did not reverse the ET-1-mediated decrease in adiponectin secretion. In conclusion, we demonstrate that ET-1 inhibits basal and insulin-stimulated adiponectin secretion through PIP_2 modulation of the actin cytoskeleton.

Keywords: Phosphatidylinositol 4; 5-Bisphosphate (PIP_2); Endothelin-1; Adiponectin; Latrunculin B; Actin cytoskeleton

The incidence of obesity and type 2 diabetes and the associated risk factors (cardiovascular diseases, dyslipidemia, and the metabolic syndrome) continue to increase in the US. Obesity and type 2 diabetes are associated with insulin resistance, but the pathological link between them is not fully understood. One link may be adipose tissue, traditionally recognized as an energy storage organ, but now also recognized as an endocrine organ. It secretes various "adipokines" such as leptin, resistin, and adiponectin that have been linked to the development of insulin resistance.

Adiponectin, a 30 kDa multimeric protein produced exclusively by white adipose tissue, is one of the most abundant plasma proteins. However, in insulin resistant states such as obesity and type 2 diabetes, its expression and circulating levels are decreased [1]. Decreased adiponectin expression and secretion have been positively correlated with a decrease in insulin sensitivity [2]. Recently, adiponectin has been recognized to have not only insulin sensitizing properties but anti-atherogenic properties as well. Adiponectin levels are decreased in patients with coronary heart disease [3] and it has been suggested that it modulates the endothelial inflammatory state associated with coronary heart diseases [4]. Therefore, adiponectin may provide a link between obesity and insulin resistance and understanding the regulation of adiponectin secretion and gene expression is crucial to our increased knowledge of the etiology of insulin resistance.

Adiponectin expression and secretion from white adipose tissue is regulated by a variety of factors including

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tumor necrosis factor-α (TNF-α) [5], interleukin-6 [6], β-adrenergic agonists [7], and insulin [8]. However, other factors can also regulate adiponectin secretion, including the vasoconstrictor peptide endothelin-1 (ET-1).

ET-1, a 21 amino acid peptide produced by endothelial cells of the vasculature, has mitogenic and vasoconstrictor properties [9]. Circulating levels of ET-1 are elevated in insulin resistant states such as type 2 diabetes [10], cardiovascular disease [11], renal disease [12], and endothelial dysfunction [13]. ET-1 induces insulin resistance in rat adipocytes [14] and rat arterial smooth muscle cells [15]. In 3T3-L1 adipocytes, it leads to heterologous desensitization of insulin signaling by inhibiting insulin-stimulated GLUT4 translocation [16], thus producing a state of insulin resistance. A recent study has demonstrated that ET-1 impairs insulin-stimulated GLUT4 translocation in 3T3-L1 adipocytes via a phosphatidylinositol 4,5-bisphosphate (PIP_2)/actin-dependent mechanism [17,18].

ET-1 also affects adipose tissue protein secretion. ET-1 acutely stimulates leptin secretion from Ob-Luc cells and 3T3-L1 adipocytes [19] and alters resistin secretion from 3T3-L1 adipocytes [20]. Clarke et al., demonstrated that ET-1 acutely stimulates and chronically inhibits adiponectin secretion from 3T3-L1 adipocytes [21]. Since PIP_2 can affect protein secretion, we tested whether PIP_2-modulation of actin polymerization accounts for ET-1's effect on adiponectin secretion.

Materials and methods

Materials. Endothelin-1, latrunculin B, and insulin were purchased from Sigma Chemical (St. Louis, MO). All cell culture reagents were obtained from Invitrogen (Grand Island, NY). Adiponectin antibody was purchased from Affinity Bioreagents (Golden, CO). Phosphatidylinositol 4,5-bisphosphate [PIP_2 cat no. P-4516] and histone carrier were purchased from Echelon Biosciences (Salt Lake City, UT). All other reagents, obtained from commercial sources were of analytical grade.

Fibroblast differentiation. 3T3-L1 mouse fibroblasts were purchased from American Type Culture Collection (ATCC-Manassas, VA) and were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS at 37 °C in an atmosphere of 10% CO_2. Two days after confluence was reached, differentiation was induced by incubating the cells for three days in DMEM containing 10% fetal bovine serum (FBS), 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 250 nM dexamethasone, and 4 μg/ml insulin. Medium was then replaced with DMEM containing 10% FBS and 4 μg/ml insulin for three additional days. Adipocytes were used 9–12 days post-differentiation. Adipogenesis was monitored by morphological examination of the cells for the accumulation of lipid droplets with Oil Red O (0.2%) staining.

3T3-L1 adipocyte treatments. Adipocytes were treated with 10 nM ET-1 for 24 h. For inhibitor treatments, 3T3-L1 adipocytes were pretreated with 1 μM ET_A receptor antagonist, BQ-610 and 20 μM PLCβ inhibitor, U-73122 or respective vehicle for 30 min and then incubated with ET-1 (10 nM) for 24 h. After 24 h, cells were serum-starved for 3 h, treated with 20 μM latrunculin B (60 min) or with PIP_2:histone complex (30 min), and stimulated with insulin (100 nM) or vehicle for 1 h. Media were taken at designated times and adiponectin in the media was measured by using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) (10%) and immunoblotting. Briefly, proteins separated by SDS–PAGE were electrophoretically transferred to nitrocellulose membranes. Membranes were blocked in TBS-T containing 7% non-fat dried milk, incubated overnight with murine adiponectin polyclonal antibody (1:1000) (Affinity Bioreagents, Golden, CO), washed, and incubated with horseradish peroxidase-conjugated secondary antibody (1:2000). Blots were developed using ECL chemiluminescence reagents (Amersham Biosciences, England). The intensities of the resultant bands (optical pixel density) were determined using the Bio-Rad Fluor-S Multimager System.

Adiponectin gene expression. RNA from 3T3-L1 adipocytes was extracted with TRIzol (Invitrogen, Carlsbad, CA). One microgram total RNA was reverse transcribed using iScript cDNA Synthesis kit with 100 U of Superscript II Reverse Transcriptase (Bio-Rad, Hercules, CA). Adiponectin mRNA expression was measured by quantitative real-time PCR (iCycler iQ Real-Time PCR detection System, Bio-Rad, Hercules, CA). Two microliter of each RT reaction was amplified in a 30 μL PCR containing 200 μM of each primer and SYBR Green Super Mix (Bio-Rad, Hercules, CA). Samples were incubated in the iCycler for an initial denaturation at 95 °C for 3.0 min followed by 40 PCR cycles. Each cycle consisted of 95 °C for 10 s and 58 °C for 1 min. The following oligonucleotide primers were used: adiponectin (Accession No. U37222) AGTTTGAGAGTCCTGAGTATTATCC (sense), CTGTTATTGCTA CGATGTAAGAGT (antisense); 36B4 (Accession No.NM007475) AAGCGCGTCCTGGCATTGTCT (sense) and CGCAGGGGGCAGC AGCAGTGGT (antisense). SYBR Green I fluorescence emission was measured after each cycle. Adiponectin mRNA levels were normalized to 36B4 expression. Amplification of specific transcripts was confirmed initially by sequencing and subsequently by producing melting curve profiles during each real-time PCR run (cooling the sample to 55 °C and heating to 95 °C with continuous measurement of fluorescence).

Whole-cell immunofluorescence and phalloidin staining. 3T3-L1 adipocytes after 8 day of differentiation were detached from culture dishes with 0.25% trypsin and reseeded in chamber slides. Cells were allowed to recover for 24 h before treatments. Following treatment, adipocytes were fixed for 20 min at 25 °C in 2% paraformaldehyde/Tris-buffered saline (TBS) (PIP_2 labeling) or 4% paraformaldehyde/0.2% Triton X-100/PBS (actin labeling). For labeling of PIP_2 after fixation, cells were incubated in 0.1% Triton X-100/TBS for 20 min at 25 °C, blocked for 60 min with 5% donkey serum in TBS and incubated for 1 h with 1:100 dilution of mouse anti-PI 4,5-P_2 antibody (Assay Designs, Ann Arbor, MI). For labeling of actin after fixation, cells were incubated with Alexa-fluor 488 conjugated phalloidin for 20 min at 25 °C. Samples were examined with a Nikon microscope. Images were made with Q imaging camera and Q capture Pro Software. All microscopic and camera settings were identical within experiments and representative images are shown. All images were processed and fluorescence intensity was measured in Adobe Photoshop.

Statistical analysis. Data from all experiments are expressed as means ± standard error of the mean (SEM). Statistical analyses were performed using one-way ANOVA and differences between groups determined by the Tukey–Kramer multiple comparisons test ($P < 0.05$).

Results

Chronic ET-1 treatment inhibits adiponectin secretion

3T3-L1 adipocytes were treated with ET-1 (10 nM) for 24 h and then were stimulated with vehicle or insulin (10 nM) for 1 h. ET-1 inhibited basal and insulin-stimulated adiponectin secretion by 66% and 47%, respectively (Fig. 1). No changes in intracellular adiponectin concentrations or cell viability were observed during chronic treatment with ET-1 (data not shown). ET-1 treatment reduced insulin-stimulated adiponectin secretion to a basal level supporting a differential regulation of constitutive and regulated adiponectin-containing compartments, as previously suggested by Bogan et al. [22].

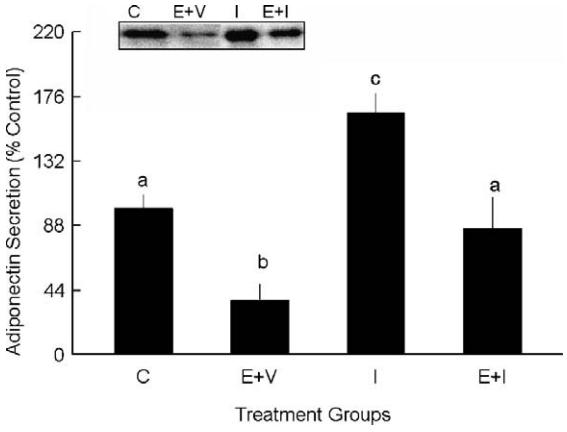

Fig. 1. Chronic ET-1 inhibits adiponectin secretion. 3T3-L1 adipocytes were treated with endothelin-1 (E) (10 nM) for 24 h. After serum starvation, adipocytes were stimulated with vehicle (v) or insulin (I) (10 nM) for 1 h. Adiponectin secretion was measured from the media using SDS–PAGE and immunoblotting. Data are expressed as means ± SEM of 3 separate experiments. Values with different letters are significantly different ($P < 0.05$). A representative blot is shown at the upper portion of the graph.

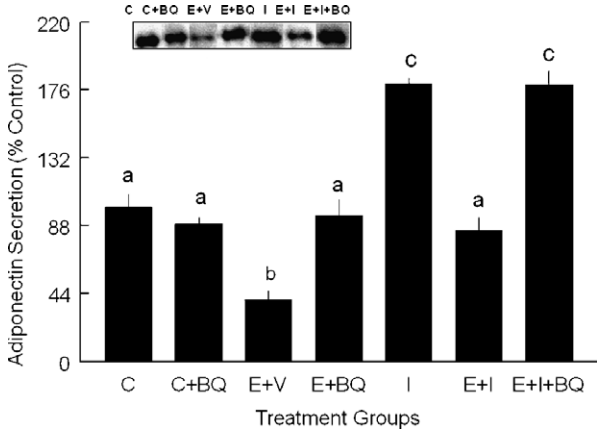

Fig. 2. BQ-610 prevents ET-1-mediated decrease in adiponectin secretion. 3T3-L1 adipocytes were pretreated with the ET_A receptor inhibitor BQ-619 (BQ) (1 μM) for 30 min and then treated with endothelin-1 (E) (10 nM) for 24 h. After serum starvation, adipocytes were stimulated with vehicle (v) or insulin (I) (10 nM) for 1 h. Adiponectin secretion was measured from the media using SDS–PAGE and immunoblotting. Data are expressed as means ± SEM of 3 separate experiments. Values with different letters are significantly different ($P < 0.05$). A representative blot is shown at the upper portion of the graph.

BQ-610 prevents ET-1-mediated decrease in adiponectin secretion

3T3-L1 adipocytes possess only the ET_A receptor and ET-1's effect on leptin secretion, resistin secretion, and GLUT4 translocation in 3T3-L1 adipocytes are mediated through this receptor [17,22–24]. Therefore, we determined if ET-1's inhibitory effect on adiponectin secretion was ET_A receptor-mediated. Cells were treated with or without ET-1 in the presence or absence of the ET_A receptor antagonist, BQ-610. Incubation with BQ-610 prevented the ET-1-mediated decrease in basal and insulin-stimulated adiponectin secretion completely (Fig. 2). In the absence of ET-1, BQ-610 had no effect on basal or insulin-stimulated adiponectin secretion (Fig. 2).

Chronic ET-1 treatment does not effect adiponectin gene expression

After observing that ET-1 inhibits basal and insulin-stimulated adiponectin secretion, it was important to determine if this effect was transcriptional or posttranscriptional. Therefore, 3T3-L1 adipocytes were serum-starved for 3 h then treated with ET-1 (100 nM) for 1–24 h. In a separate series of experiments, 3T3-L1 adipocytes were treated with ET-1 (0.01–100 nM) for 24 h. Adiponectin gene expression was analyzed by real-time RT-PCR. ET-1 did not affect adiponectin gene expression in a time- or concentration-dependent manner (Fig. 3). These studies demonstrate that ET-1 inhibition of adiponectin secretion is through posttranscriptional regulation, and suggest that chronic ET-1 signaling may have a pronounced effect on adiponectin packaging and/or vesicular trafficking.

Inhibition of PIP_2 hydrolysis and PIP_2 addition prevent/restore decreased adiponectin secretion due to chronic ET-1 treatment

Studies have shown that chronic ET-1 stimulation of phospholipase Cβ (PLCβ) leads to loss of plasma membrane PIP_2 [17]. Consistent with the studies done by Strawbridge et al. [17], we demonstrated that immunofluorescent PIP_2 labeling was greatly diminished in ET-1 treated cells as compared to controls (Fig. 4A). Exogenous addition of PIP_2 via carrier-mediated delivery restored PIP_2 in ET-1 treated cells (Fig. 4A).

We then evaluated whether inhibition of PIP_2 hydrolysis or addition of exogenous PIP_2 prevents/restores the ET-1-mediated decrease in adiponectin secretion. Treatment of 3T3-L1 adipocytes with the PLCβ inhibitor, U-73122 (10 μM) prevented the decrease in basal adiponectin secretion observed after chronic treatment with ET-1 (Fig. 4B). This effect was also observed with insulin-stimulated adiponectin secretion. We also demonstrated that carrier-mediated delivery of exogenous PIP_2 (1.25 μM) restores the decrease in basal and insulin-stimulated adiponectin secretion observed with ET-1 while carrier (histone) alone had no effect (Fig. 4C). These data suggest that the presence of adequate concentrations of PIP_2 in the plasma membrane are critical for the trafficking of adiponectin-containing secretory vesicles.

Cortical F-actin polymerization is an important step in adiponectin secretion

PIP_2 regulates actin dynamics in 3T3-L1 adipocytes [17,23,24]. A decrease in PIP_2 levels at the plasma

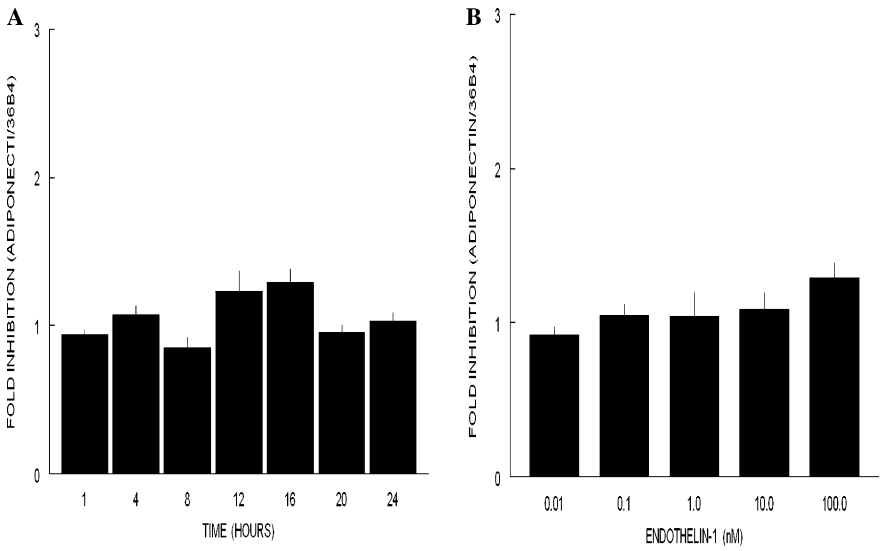

Fig. 3. Endothelin-1 does not regulate adiponectin gene expression in a time- or concentration-dependent manner. 3T3-L1 adipocytes were serum-starved for 3 h and then treated with ET-1 (100 nM) for 1–24 h (A). In a separate series of experiments, 3T3-L1 adipocytes were treated with ET-1 (0.01–100 nM) for 24 h (B). Adiponectin gene expression was analyzed by real-time RT-PCR. Bars represent means ± SEM of 3 separate experiments and are shown as fold inhibition (adiponectin/36B4).

membrane results in a decrease in F-actin polymerization [17,25]. Chronic ET-1 treatment results in a decrease in cortical F-actin as shown by phalloidin staining in fixed cells (Fig. 5A). To determine whether chronic ET-1 treatment inhibits adiponectin secretion by depolymerization of the actin network, it was necessary to first demonstrate that adiponectin secretion is an actin-mediated event. 3T3-L1 adipocytes were pretreated with the actin depolymerizing toxin latrunculin B (20 μM) for 1 h and then stimulated with vehicle or insulin for 1 h. Latrunculin B significantly inhibited basal and insulin-stimulated adiponectin secretion by 63% and 40%, respectively (Fig. 5B). Interestingly, insulin was able to partially stimulate adiponectin secretion even when F-actin remains impaired by latrunculin B. These studies suggest that the actin network is not a barrier but is necessary for constitutive adiponectin secretion; however, regulated secretion of adiponectin is independent of intact cortical F-actin, at least in part. As mentioned previously, studies have demonstrated that PIP_2 stimulates actin polymerization, which is important for the movement of specific vesicles to the cell surface [17,23]. We determined whether the significant restoration in basal adiponectin secretion by PIP_2 was dependent on F-actin by evaluating the effect of PIP_2 in conjunction with latrunculin B (Fig. 5C). Consistent with PIP_2 being associated with F-actin regulation, replenishment of PIP_2 did not restore basal adiponectin secretion in the presence of latrunculin B (Fig. 5C). These studies suggest that ET-1 inhibits constitutive adiponectin secretion by depolymerizing the actin network indirectly through PIP_2 depletion. However, insulin-stimulated adiponectin secretion appears to be independent of F-actin, at least in part.

Discussion

Even though adiponectin was only recently discovered, studies have demonstrated that it is directly involved in a number of disease states. Originally, it was observed that adiponectin plasma concentration and gene expression are reduced in obesity and type 2 diabetes [1]. Recently, it has also been demonstrated that adiponectin has inflammatory-modulating activities, and clinical studies have demonstrated inverse associations between adiponectin levels and serum markers of inflammation [26]. Adiponectin involvement in a variety of disease states suggests that a number of factors may be involved in the regulation of adiponectin secretion. It is our contention that one of these factors is ET-1.

Studies have demonstrated that ET-1 has a dramatic impact on adipocyte physiology. ET-1 inhibits adipocyte differentiation [27], reduces lipoprotein lipase activity, impacts glucose metabolism in adipocytes [28], and regulates synthesis and release of adipokines including leptin [19], resistin [20], and adiponectin [21]. These findings indicate that ET-1 can directly and indirectly modulate adipocyte functions. Therefore, dysregulation of adipocyte functions by ET-1 may disrupt whole body energy homeostasis and progressively lead to a number of disorders, including type 2 diabetes and cardiovascular disease.

Our studies demonstrate that ET-1 inhibits basal and insulin-stimulated secretion of adiponectin secretion in 3T3-L1 adipocytes. Its effect on adiponectin secretion is not based on modulation of gene transcription but is mediated by effects on vesicular trafficking by depleting plasma membrane PIP_2. Chronic ET-1 treatment leads to PIP_2 depletion from the plasma membrane [17,18] which correlates with an ET-1-induced decrease in adiponectin

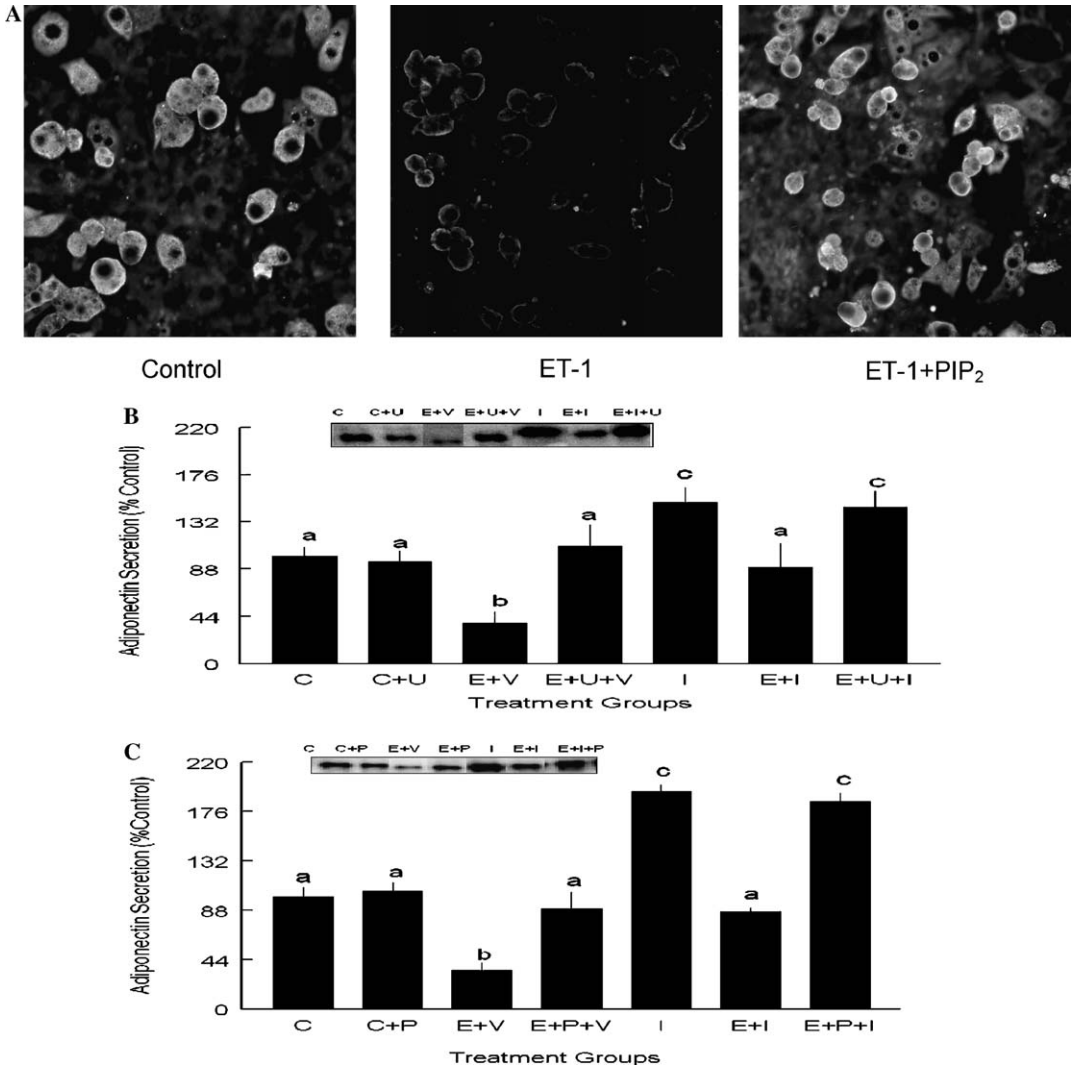

Fig. 4. (A) PIP_2 replenishment restores PIP_2 decreased by ET-1. 3T3-L1 adipocytes were incubated with ET-1 (10 nM) for 24 h, serum-starved for 3 h, and PIP_2:histone complex was added back for 1 h and PIP_2 labeling detected in the cells by immunofluorescence. (B) U73122 prevents ET-1-mediated decrease in adiponectin secretion. 3T3-L1 adipocytes were pretreated with the PLCβ inhibitor, U73122 (U) (10 μM) for 30 min and then treated with endothelin-1 (E) (10 nM) for 24 h. After serum starvation, adipocytes were stimulated with vehicle (v) or insulin (I) (10 nM) for 1 h. (C) PIP2 addition restores the decrease in adiponectin secretion observed with ET-1. 3T3-L1 adipocytes were treated with endothelin-1 (E) (10 nM) for 24 h. After serum starvation, PIP_2:histone complex (P) (1.25: .625 μM) was added for 30 min and then stimulated with vehicle (v) or insulin (I) for 1 h. Images and data are expressed as means ± SEM from 3 separate experiments. Values with different letters are significantly different ($P < 0.05$). Representative blots are shown at the upper portion of the graphs.

secretion. Prevention of ET-1-mediated PIP_2 hydrolysis by the PLCβ inhibitor, U-73122 or PIP_2 replenishment restores adiponectin secretion.

The mechanism by which PIP_2 regulates adiponectin secretion likely involves the actin cytoskeleton. Mature adipocytes possess a layer of cortical actin, primarily at the plasma membrane. Cortical F-actin may be a barrier for vesicles to fuse to the plasma membrane [29] or can be a scaffold necessary for vesicular trafficking [17]. PIP_2 modulates actin dynamics in 3T3-L1 adipocytes [23] and its loss leads to decreased polymerization of cortical F-actin [17,25]. While studies have demonstrated cortical F-actin to be important for regulated insulin-stimulated translocation of GLUT4 [17,23,35], our studies demonstrate that the actin cytoskeletal integrity is also crucial for constitutive secretion of adiponectin. Maintenance of this cortical F-actin is dependent on PIP_2, which is present at the plasma membrane in basal states [30].

Adiponectin is primarily located in the Golgi and its intracellular trafficking is dependent on GGA-coated vesicles [31]. Its path from the Golgi to the plasma membrane is not known. Our study demonstrates that F-actin is important for adiponectin secretion; most likely functioning as a scaffold for adiponectin-containing GGA-coated vesicles to fuse to the plasma membrane. The failure of PIP_2 added back to restore ET-1-mediated adiponectin secretion in the presence of latrunculin B highlights the importance of F-actin in adiponectin secretion. A recent study by Clarke et al., demonstrating that adiponectin is secreted in an rab11/ARF6-dependent manner [32] further

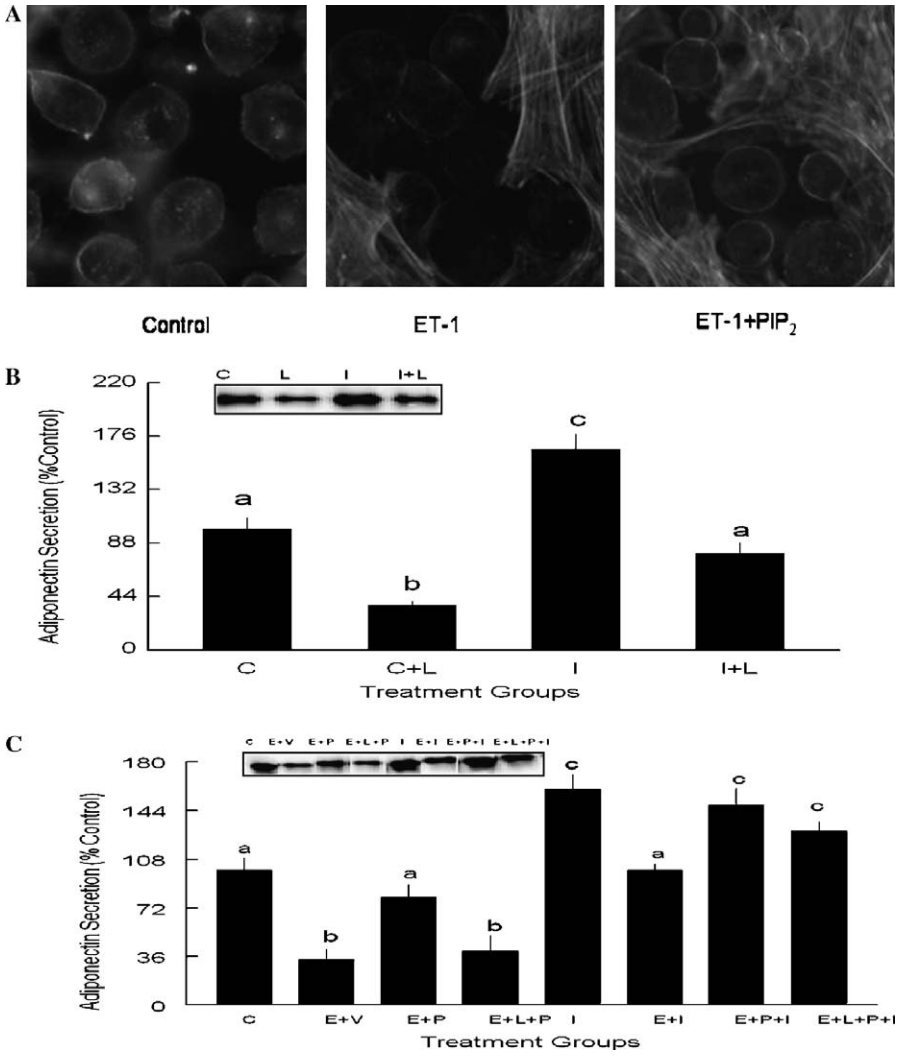

Fig. 5. (A) Immunofluorescence detection of F-actin in cells. (B) Latrunculin B inhibits adiponectin secretion. 3T3-L1 adipocytes were pretreated with latrunculin B and then stimulated with vehicle or insulin for 1 h. (C) PIP_2 reversed the ET-1-mediated decrease adiponectin secretion when actin remained impaired with latrunculin B. 3T3-L1 adipocytes were treated with endothelin-1 (E) (10 nM) for 24 h. After serum starvation, pretreated with latrunculin B (L) for 1 h and then treated with PIP2:histone complex (P) (1.25:0.625 μM) for 30 min and stimulated with vehicle (v) or insulin (100 nM) (I) for 1 h. Images and data are expressed as means ± SEM from of 3 separate experiments. Values with different letters are significantly different ($P < 0.05$). Representative blots are shown at the upper portion of the graphs.

supports our theory that F-actin provides a "road" for adiponectin secretion, as ARF6 has been shown to regulate F-actin polymerization [33].

The majority of adiponectin is sorted into a compartment which is secreted constitutively, and the remainder is sorted into a regulated compartment [22]. Insulin stimulates adiponectin secretion from this regulated compartment [34] in a PI3K- and Akt-dependent manner [5]. The ability of insulin to stimulate adiponectin secretion in latrunculin B treated cells suggests that the regulated compartment of adiponectin-containing vesicles is F-actin independent. Although latrunculin B treatment depolymerizes F-actin, it does not affect insulin-stimulated Akt phosphorylation in 3T3-L1 adipocytes [35]. Studies have shown that insulin-mediated F-actin polymerization and PI3K activation are independent of each other in 3T3-L1 adipocytes [36], suggesting that PI3K/Akt activation can mediate trafficking of vesicles irrespective of actin cytoskeleton integrity. Consistent with this, in our studies insulin was able to stimulate adiponectin secretion even when F-actin was impaired by latrunculin B, probably due to insulin-stimulated PI3K and Akt activity. These observations are supported by Eyster et al., who showed that constitutively active PKB/Akt signals GLUT4 translocation in the absence of the an intact actin cytoskeleton [37], which further suggests that insulin stimulates adiponectin secretion by enhancing mobilization of the adiponectin-containing vesicles of the regulated compartment. This regulated secretion is Akt-dependent and is not affected by actin depolymerization. These studies indicate a differential regulation of constitutive and regulated secretion of adiponectin. Previous studies have shown that chronic ET-1 treatment impairs Akt-1 activity but not Akt-2 activity in 3T3-L1 adipocytes [17]. Chronic ET-1 treatment did not

impair the ability of insulin to stimulate adiponectin secretion, suggesting the possibility of insulin-stimulated adiponectin secretion to be Akt-2-dependent.

While our understanding of regulation of adiponectin secretion and its decrease in insulin resistant states is limited, findings presented in this paper demonstrate that membrane lipids and the actin cytoskeleton play an important role in the regulation of adiponectin secretion. Not only do these studies indicate one mechanism by which adiponectin secretion is regulated, they also point to the fact that vascular signals such as ET-1 are capable of dysregulating its secretion, suggesting a vascular-adipocyte axis.

Acknowledgments

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References

[1] C. Weyer, T. Funahashi, S. Tanaka, K. Hotta, Y. Matsuzawa, R.E. Pratley, P.A. Tataranni, Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia, J. Clin. Endocrinol. Metab. 86 (2001) 1930–1935.

[2] K. Hotta, T. Funahashi, N.L. Bodkin, H.K. Ortmeyer, Y. Arita, B.C. Hansen, Y. Matsuzawa, Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys, Diabetes 50 (2001) 1126–1133.

[3] M. Kumada, S. Kihara, S. Sumitsuji, T. Kawamoto, S. Matsumoto, N. Ouchi, Y. Arita, Y. Okamoto, I. Shimomura, H. Hiraoka, T. Nakamura, T. Funahashi, Y. Matsuzawa, Association of hypoadiponectinemia with coronary artery disease in men, Arterioscler. Thromb. Vascul. Biol. 23 (2003) 85–89.

[4] N. Ouchi, S. Kihara, Y. Arita, K. Maeda, H. Kuriyama, Y. Okamoto, K. Hotta, M. Nishida, M. Takahashi, T. Nakamura, S. Yamashita, T. Funahashi, Y. Matsuzawa, Novel modulator for endothelial adhesion molecules : adipocyte-derived plasma protein adiponectin, Circulation 100 (1999) 2473–2476.

[5] J. Kamon, T. Yamauchi, S. Muto, S. Takekawa, Y. Ito, Y. Hada, W. Ogawa, A. Itai, M. Kasuga, K. Tobe, T. Kadowaki, A novel IKKbeta inhibitor stimulates adiponectin levels and ameliorates obesity-linked insulin resistance, Biochem. Biophys. Res. Commun. 323 (2004) 242–248.

[6] S. Engeli, M. Feldpausch, K. Gorzelniak, F. Hartwig, U Heintze, J. Janke, M. Mohlig, A.F. Pfeiffer, F.C. Luft, A.M. Sharma, Association between adiponectin and mediators of inflammation in obese women, Diabetes 52 (2003) 942–947.

[7] M.L. Delporte, T. Funahashi, M. Takahashi, Y. Matsuzawa, S.M. Brichard, Pre- and post-translational negative effect of beta-adrenoceptor agonists on adiponectin secretion: in vitro and in vivo studies, Biochem. J. 367 (2002) 677–685.

[8] M. Fasshauer, J. Klein, S. Neumann, M. Eszlinger, R. Paschke, Hormonal regulation of adiponectin gene expression in 3T3-L1 adipocytes, Biochem. Biophys. Res. Commun. 290 (2002) 1084–1089.

[9] S. Kakui, K. Mawatari, T. Ohnishi, Y. Niwa, N. Tanoue, N. Harada, A. Takahashi, K. Izume, Y. Nakaya, Localization of the 31-amino-acid endothelin-1 in hamster tissue, Life Sciences 74 (2004) 1435–1443.

[10] G. Ak, S. Buyukberber, A. Sevinc, H.M. Turk, M. Ates, R. Sari, H. Savli, A. Cigli, The relation between plasma endothelin-1 levels and metabolic control, risk factors, treatment modalities, and diabetic microangiopathy in patients with Type 2 diabetes mellitus, J. Diabet. Complications 15 (2001) 150–157.

[11] A. Kurita, T. Matsui, T. Ishizuka, B. Takase, K. Satomura, Significance of plasma nitric oxide/endothelial-1 ratio for prediction of coronary artery disease, Angiology 56 (2005) 259–264.

[12] Y. Nakayama, K. Tomita, [Endothelin and kidney disease], Nippon Rinsho 62 (Suppl 9) (2004) 655–660.

[13] J.T. Parissis, S. Korovesis, E. Giazitzoglou, P. Kalivas, D. Katritsis, Plasma profiles of peripheral monocyte-related inflammatory markers in patients with arterial hypertension. Correlations with plasma endothelin-1, Int. J. Cardiol. 83 (2002) 13–21.

[14] Y.C. Chou, J.C. Perng, C.C. Juan, S.Y. Jang, C.F. Kwok, W.L. Chen, J.C. Fong, L.T. Ho, Endothelin-1 inhibits insulin-stimulated glucose uptake in isolated rat adipocytes, Biochem. Biophys. Res. Commun. 202 (1994) 688–693.

[15] Z.Y. Jiang, Q.L. Zhou, A. Chatterjee, E.P. Feener, M.G. Myers, M.F. White, G.L. King, Endothelin-1 modulates insulin signaling through phosphatidylinositol 3-kinase pathway in vascular smooth muscle cells, Diabetes 48 (1999) 1120–1130.

[16] K.I. Ishibashi, T. Imamura, P.M. Sharma, J. Huang, S. Ugi, J.M. Olefsky, Chronic endothelin-1 treatment leads to heterologous desensitization of insulin signaling in 3T3-L1 adipocytes, J. Clin. Invest. 107 (2001) 1193–1202.

[17] A.B. Strawbridge, J.S. Elmendorf, Phosphatidylinositol 4,5-bisphosphate reverses endothelin-1-induced insulin resistance via an actin-dependent mechanism, Diabetes 54 (2005) 1698–1705.

[18] A.B. Strawbridge, J.S. Elmendorf, Endothelin-1 impairs glucose transporter trafficking via a membrane-based mechanism, J. Cell. Biochem. 97 (2006) 849–856.

[19] Y. Xiong, H. Tanaka, J.A. Richardson, S.C. Williams, C.A. Slaughter, M. Nakamura, J.L. Chen, M. Yanagisawa, Endothelin-1 stimulates leptin production in adipocytes, J. Biol. Chem. 276 (2001) 28471–28477.

[20] Q. Zhong, C.Y. Lin, K.J. Clarke, R.J. Kemppainen, D.D. Schwartz, R.L. Judd, Endothelin-1 inhibits resistin secretion in 3T3-L1 adipocytes, Biochem. Biophys. Res. Commun. 296 (2002) 383–387.

[21] K.J. Clarke, Q. Zhong, D.D. Schwartz, E.S. Coleman, R.J. Kemppainen, R.L. Judd, Regulation of adiponectin secretion by endothelin-1, Biochem. Biophys. Res. Commun. 312 (2003) 945–949.

[22] J.S. Bogan, H.F. Lodish, Two compartments for insulin-stimulated exocytosis in 3T3-L1 adipocytes defined by endogenous ACRP30 and GLUT4, J. Cell. Biol. 146 (1999) 609–620.

[23] M. Kanzaki, M. Furukawa, W. Raab, J.E. Pessin, Phosphatidylinositol 4,5-bisphosphate regulates adipocyte actin dynamics and GLUT4 vesicle recycling, J. Biol. Chem. 279 (2004) 30622–30633.

[24] M.A. Bittner, R.W. Holz, Phosphatidylinositol-4,5-bisphosphate: actin dynamics and the regulation of ATP-dependent and -independent secretion, Mol. Pharmacol. 67 (2005) 1089–1098.

[25] G. Chen, P. Raman, P. Bhonagiri, A.B. Strawbridge, G.R. Pattar, J.S. Elmendorf, Protective effect of phosphatidylinositol 4,5-bisphosphate against cortical filamentous actin loss and insulin resistance induced by sustained exposure of 3T3-L1 adipocytes to insulin, J. Biol. Chem. 279 (2004) 39705–39709.

[26] N. Ouchi, S. Kihara, T. Funahashi, T. Nakamura, M. Nishida, M. Kumada, Y. Okamoto, K. Ohashi, H. Nagaretani, K. Kishida, H. Nishizawa, N. Maeda, H. Kobayashi, H. Hiraoka, Y. Matsuzawa, Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue, Circulation 107 (2003) 671–674.

[27] H. Hauner, T. Petruschke, F.A. Gries, Endothelin-1 inhibits the adipose differentiation of cultured human adipocyte precursor cells, Metabolism 43 (1994) 227–232.

[28] I. Usui, T. Imamura, J.L. Babendure, H. Satoh, J.C. Lu, C.J. Hupfeld, J.M. Olefsky, G protein-coupled receptor kinase 2 mediates endothelin-1-induced insulin resistance via the inhibition of both

Galphaq/11 and insulin receptor substrate-1 pathways in 3T3-L1 adipocytes, Mol. Endocrinol. 19 (2005) 2760–2768.
[29] J.T. Lawrence, M.J. Birnbaum, ADP-ribosylation factor 6 regulates insulin secretion through plasma membrane phosphatidylinositol 4,5-bisphosphate, Proc. Natl. Acad. Sci. US 100 (2003) 13320–13325.
[30] S. Huang, L. Lifshitz, V. Patki-Kamath, R. Tuft, K. Fogarty, M.P. Czech, Phosphatidylinositol-4,5-bisphosphate-rich plasma membrane patches organize active zones of endocytosis and ruffling in cultured adipocytes, Mol. Cell. Biol. 24 (2004) 9102–9123.
[31] L. Xie, D. Boyle, D. Sanford, P.E. Scherer, J.E. Pessin, S. Mora, Intracellular trafficking and secretion of adiponectin is dependent on GGA coated vesicles, J. Biol. Chem. (2005).
[32] M. Clarke, M.A. Ewart, L.C. Santy, R. Prekeris, G.W. Gould, ACRP30 is secreted from 3T3-L1 adipocytes via a Rab11-dependent pathway, Biochem. Biophys. Res. Commun. 342 (2006) 1361–1367.
[33] S. Klein, M. Franco, P. Chardin, F. Luton, Role of Arf6 GDP/GTP cycle and Arf6 GAP in actin remodeling and intracellular transport, J. Biol. Chem. (2006).
[34] P.E. Scherer, S. Williams, M. Fogliano, G. Baldini, H.F. Lodish, A novel serum protein similar to C1q, produced exclusively in adipocytes, J. Biol. Chem. 270 (1995) 26746–26749.
[35] M. Kanzaki, J.E. Pessin, Insulin-stimulated GLUT4 translocation in adipocytes is dependent upon cortical actin remodeling, J. Biol. Chem. 276 (2001) 42436–42444.
[36] Z.Y. Jiang, A. Chawla, A. Bose, M. Way, M.P. Czech, A phosphatidylinositol 3-kinase-independent insulin signaling pathway to N-WASP/Arp2/3/F-actin required for GLUT4 glucose transporter recycling, J. Biol. Chem. 277 (2002) 509–515.
[37] C.A. Eyster, Q.S. Duggins, A.L. Olson, Expression of constitutively active Akt/protein kinase B signals GLUT4 translocation in the absence of an intact actin cytoskeleton, J. Biol. Chem. 280 (2005) 17978–17985.