Biochemical and Biophysical Research Communications 424 (2012) 753–757

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Globular adiponectin activates Akt in cultured myocytes

Akm A. Sattar *, Rifat Sattar

Division of Endocrinology, Department of Internal Medicine, Wayne State University School of Medicine, Detroit, MI 48201, USA

ARTICLE INFO

Article history:
Received 29 June 2012
Available online 16 July 2012

Keywords:
Adiponectin
Akt
AMPK
Insulin
Phosphorylation
Wortmannin

ABSTRACT

The serine/threonine kinase Akt plays an important role in insulin-mediated glucose uptake. Adiponectin (Adp) is known to sensitize this process. The purpose of the current study is to investigate if Adp activates Akt independently from insulin; and if Adp synergizes with insulin on Akt phosphorylation in the rat skeletal muscle L6 cells. Differentiated L6 cells were serum-starved and exposed to various concentrations (0–100 nM) of recombinant globular Adp (gAdp) and/or insulin for different time periods at 37 °C. Phosphorylation of Akt was monitored by Western blot using an antiserum against $pSer^{473}$ or $pThr^{308}$ Akt. The results demonstrate that gAdp activates Akt in dose- and time-dependent manners. When L6 cells were treated with sub-maximal concentrations of both insulin (10 nM) and gAdp (10 nM) for 10 min neither synergistic nor additive activation of Akt was observed. Similar non-synergistic or non-additive effect of gAdp on insulin-induced Akt activation was also observed in mouse C2C12 myocytes and rat vascular smooth muscle PAC cells. Moreover, pretreatment of the L6 cells with wortmannin (100 nM) for 20 min significantly reduced gAdp (100 nM) induced and insulin (100 nM) induced Akt activation by ~80 and ~70%, respectively. These data suggest that adiponectin stimulates Akt activation via the wortmannin sensitive pathway in L6 cells; and that its effects on Akt phosphorylation are not additive to those of insulin.

1. Introduction

Type 2 diabetes (T2D) is a growing public health problem characterized by an initial reduction in glucose uptake by skeletal muscle and adipose tissue due to insulin resistance caused by obesity and a complex interaction between multiple environmental and genetic factors [1–5]. Insulin resistance is compounded, later in the disease process, by a reduction in insulin secretion by pancreatic β-cells in response to increasing blood glucose [6]. Adiponectin (Adp) is a recently discovered adipokine whose levels, paradoxically, are decreased in obesity despite the increase in adipocyte mass [7–9]. Adp induces vascular smooth muscle cell differentiation [10] and also improves endothelial dysfunction elevated by FFAs level [11]. Adp suppresses triglyceride accumulation, increases fatty acid oxidation and activates the AMP kinase (AMPK) in skeletal muscles [12], improving insulin signaling [13]. Adp also suppresses glucose production and activates AMPK in liver [13]. Hence, Adp is an insulin sensitizer in skeletal muscles. Adp is involved in the regulation of whole-body energy metabolism [14]; its effects include the control of AMPK activity in skeletal muscles, liver and adipose tissues, as well as other mechanisms mediated by the control of transcription factor expression [15–17].

Akt plays a central role in cell signaling downstream of growth factors, cytokines, and other cellular stimuli. Aberrant loss or gain of Akt activation underlies insulin resistance and pathophysiological properties of T2D [18–20]. Adp binding to its receptors, AdipoR1 and AdipoR2, leads to AMPK activation, which, in turn, suppresses S6 Kinase phosphorylation of the serine residues of the insulin receptor substrate 1 (IRS-1) thereby enhancing its tyrosine phosphorylation and sensitization of insulin action [21]. Adp was also shown to stimulate the new blood vessel growth by promoting cross-talk between AMP-activated protein kinase and Akt signaling within endothelial cells [22]. It has also been reported that although gAdp alone does not promote phosphorylation of Akt it potentiates insulin induced Akt phosphorylation in C2C12 cells [23]. Recent study demonstrates a lack of AMPK involvement and implicates Akt and ERK in adiponectin signaling, leading to protection against apoptosis and stimulation of insulin gene expression and secretion in pancreatic beta cells [24]. Given the beneficial actions of Adp on insulin resistance, promotion of pharmacological strategies to restore or increase plasma adiponectin levels or adiponectin receptor expression could help reduce insulin resistance in disorders associated with obesity and T2D.

Abbreviations: gAdp, globular adiponectin; T2D, type-2 diabetes; Akt, protein kinase B; AMPK, AMP kinase; IR, insulin receptor; IRS-1, insulin receptor substrate 1; AdipoR1/R2, adiponectin receptors 1 and 2; L6, rat skeletal muscle cells; C2C12, mouse skeletal muscle cells; PAC1, rat pulmonary aorta cells; FBS, fetal bovine serum; BSA, bovine serum albumin.

* Corresponding author. Address: Division of Endocrinology, Department of Internal Medicine, Wayne State University school of Medicine, 421 E. Canfield Avenue, Detroit, MI 48201-1928, USA. Fax: +1 313 577 8615.

E-mail address: asattar@med.wayne.edu (A.A. Sattar).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.07.027

The purpose of the current study was to investigate if gAdp activates Akt independently from insulin; and if gAdp synergizes with insulin on Akt phosphorylation in the rat skeletal muscle L6 cell line.

2. Materials and methods

2.1. Materials

Minimum essential medium, Ham's eagle medium and penicillin–streptomycin were purchased from GIBCO BRL Life Technologies, Inc. Fetal bovine serum (FBS) was purchased from Hyclones and insulin-free bovine serum albumin (BSA) purchased from Fluka. Tissue culture laboratory ware was purchased from Falcon. Rat gAdp was purchased from Bio-Vision, CA. Human biosynthetic insulin was kindly supplied by Eli Lilly and Co. Antibodies to β-actin, Akt, Thr^{308} phosphorylated Akt and Ser^{473} phosphorylated Akt were purchased from Santa-Cruz or Abcam. Antibody to the active Ser^{474} phosphorylated Akt was purchased from Abcam. Electrophoretic reagents were obtained from Bio-Rad. The chemiluminescence detection reagent kit was from Perkin Elmer. All other chemicals were reagent grade and purchased from Sigma.

2.2. Cell line and cell culture

Conditions for culturing Rat L6, PAC1, and C2C12 cells were cultured essentially as described in earlier studies [25–27]. Rat L6 cells and C2C12 cells were differentiated to myotubes by culturing the cells in DMEM containing a low fetal bovine serum (2% FBS) for 5–8 days after initiation of differentiation and as described [28].

2.3. Adp and insulin stimulation

The L6 cells were cultured and differentiated in 6 well tissue culture plates. The cells were rinsed and incubated in a serum-free DMEM containing 0.1% insulin-free BSA for 8 h prior to Adp and/or insulin treatment. This step was necessary to reduce basal Akt phosphorylation. For dose response studies, Adp or insulin was added to the cells for 10 min at 37 °C, at final concentrations of 0.1, 1, 5, 10, 50 or 100 nM. Media lacking Adp and/or insulin (0 nM) served as a vehicle control. Cells were then placed on ice and rinsed twice with ice-cold phosphate buffer saline, pH 7.5 (PBS). The cells were lysed on ice in a solubilizing buffer containing 150 mM NaCl, 50 mM Tris–HCl, pH 7.4, 1% Triton X-100, 0.2% sodium deoxycholate, 0.2% sodium dodecylsulfate (SDS), 1 mM sodium ethylenediaminetetraacetate, 1 mM phenylmethylsulfonyl fluoride, 1 mM NaF, 5 μg/ml aprotinin, 5 μg/ml leupeptin, and 1 mM $NaVO_4$. Lysates were centrifuged at 10,000×*g* for 2 min and supernatants were stored at −20 °C until determination of protein concentration and Western immunoblot analysis.

2.4. Immunodetection of pAkt

Samples from control and Adp or/and insulin-treated cells were diluted with 5× sample buffer (0.5 M Tris–HCl, pH 6.8,

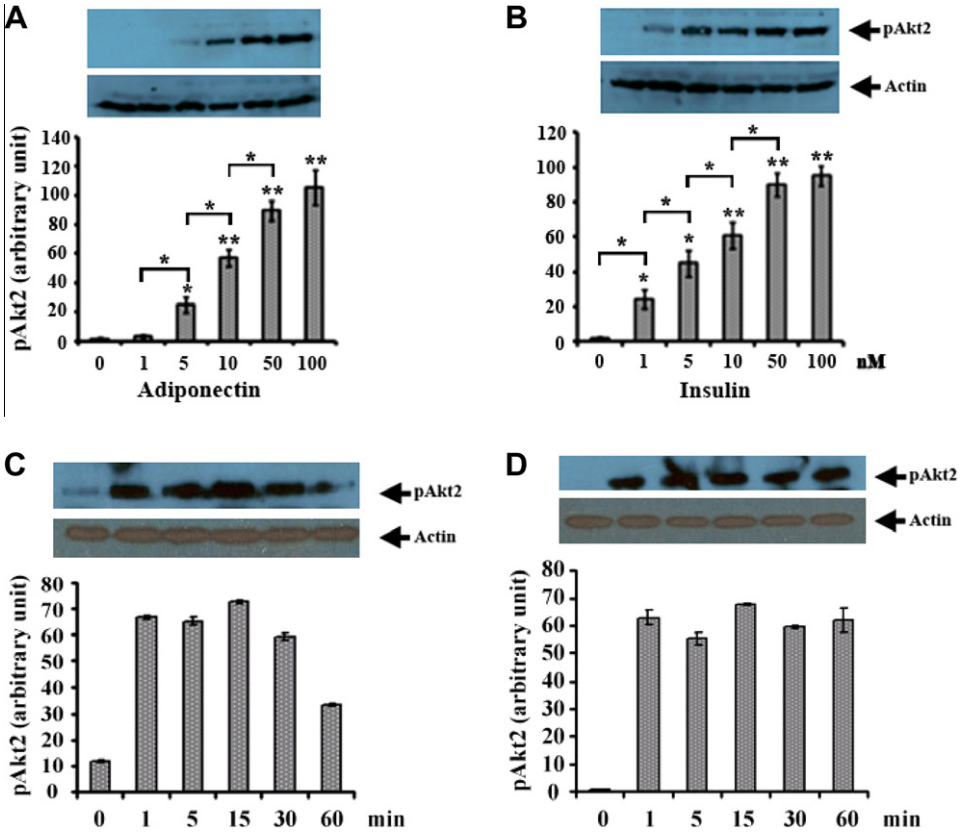

Fig. 1. Dose and time course of the effects of adiponectin and insulin on Akt phosphorylation in L6 cells. Dose-dependent adiponectin (A) and insulin (B) stimulated phosphorylation of Akt in L6 cells. The cells were serum-starved for 8 h, the medium was replaced, and the cells were incubated with the indicated concentrations of adiponectin or insulin for 10 min at 37 °C. The cells were then solubilysed and the extracts were subjected to SDS–PAGE followed by transferring to a nitrocellulose membrane and were analyzed by immunoblotting with an anti $pSer^{474}$Akt or an anti actin antisera. The arrowheads indicate the phosphorylated Akt bands or actin bands. The Western blot (upper panel) is representative of three similar experiments. The blots from 3 experiments were scanned and means ± SEM of pAkt band densities are shown (lower panel). $^{*}P < 0.05$; $^{**}P < 0.01$ vs. control (vehicle) or between the indicated groups. Time course of the effects of adiponectin (C) and insulin (D) on Akt phosphorylation in L6 cells. The cells were serum-starved for 8 h and incubated with 50 nM adiponectin or insulin for the indicated time periods at 37 °C. The Western blots were performed and analyzed as in Fig. 1A and B. Upper panels: Representative blots. Lower panels: means ± SEM of pAkt band densities calculated from three experiments.