Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical Pharmacology 70 (2005) 1309–1311
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Contrasting effects of insulin and cellular differentiation on expression of the novel insulin receptor substrate APS in skeletal muscle

Rustam Rea, Samuel Gray, Richard Donnelly *

Centre for Integrated Systems Biology & Medicine, School of Medical & Surgical Sciences, University of Nottingham Medical School, Derby City General Hospital, Derby DE22 3DT, UK

Received 24 June 2005; accepted 26 July 2005

Abstract

The novel insulin receptor substrate protein APS is highly expressed in insulin-sensitive tissues and plays an important role in insulin-mediated glucose uptake and GLUT4 translocation via the Cbl/CAP pathway. Tyrosine phosphorylation of APS leads to recruitment of c-Cbl and Crk, while overexpression of APS mutant inhibits GLUT4 translocation in response to insulin, but the regulation of APS expression in skeletal muscle has not been previously reported. L6 myoblasts were differentiated in 2% FBS and serum starved for 24 h prior to stimulation for 24 h with either insulin 1 μM ($n = 6$), rosiglitazone 10 μM ($n = 6$), resistin 500 nM ($n = 6$) or the MAP kinase inhibitor PD098059 50 μM ($n = 6$) for 30 min, followed by insulin 1 μM for 24 h. Semi-quantitative real-time RT-PCR was used to determine the expression of APS mRNA relative to the control gene TF2D. APS expression was markedly upregulated by myoblast differentiation (0.55 ± 0.08 versus 1.14 ± 0.08, $p = 0.001$), and this effect was augmented by addition of rosiglitazone 10 μM for 24 h to the differentiated myotubes (1.50 ± 0.09, $p = 0.025$). Insulin caused a 3.1-fold decrease in APS mRNA expression (0.37 ± 0.01 versus 1.14 ± 0.08, $p = 0.001$), an effect that was attenuated by the MAP kinase inhibitor PD098059 (0.80 ± 0.03, $p = 0.001$). Exposure to resistin produced a modest decrease (1.4-fold) in myotube expression of APS (0.8 ± 0.09, $p = 0.025$). In conclusion, this is the first study to show that exposure to insulin markedly reduces the expression of APS in skeletal muscle via a MAP kinase dependent pathway, whereas myocyte differentiation and rosiglitazone increase APS expression. Changes in APS expression may be important in the aetiology and therapeutic reversal of insulin resistance in skeletal muscle.

Keywords: APS; Skeletal muscle; Insulin; Gene expression; Rosiglitazone; Resistin

1. Introduction

Type 2 diabetes is characterised by peripheral (muscle and fat) and hepatic insulin resistance, which in turn leads to a reduction in insulin-stimulated glucose disposal and overproduction of glucose by the liver. The biochemical mechanisms of insulin resistance are complex, but autophosphorylation of the insulin receptor leads to recruitment of insulin receptor substrates which trigger a series of downstream biochemical events. At least nine insulin receptor substrate proteins have so far been identified, including adapter protein with pleckstrin homology (PH) and Src homology 2 (SH2) domains (APS) [1]. APS is a novel insulin receptor substrate, expressed exclusively in insulin sensitive tissues, which plays an important role in insulin mediated glucose uptake and GLUT4 translocation via the Cbl/CAP pathway [2–4] and activation of the G-protein TC10 [5]. That APS expression is restricted to insulin sensitive tissues, such as skeletal muscle, heart and fat suggests a physiological role in insulin signalling, and APS has recently been implicated as a mechanism and therapeutic target in insulin resistance [4].

Very little, if any, work has been undertaken on the regulation of APS mRNA expression in insulin sensitive

Abbreviations: APS, adapter protein with a pleckstrin homology and an Src homology 2 domain; CAP, cbl associated protein; IRS, insulin receptor substrate; PI3, phosphatidylinositol-3; PKC, protein kinase C; PPAR-γ, peroxisome proliferators activated receptor gamma

* Corresponding author. Tel.: +44 1332 724619; fax: +44 1332 724618.
E-mail address: Richard.donnelly@nottingham.ac.uk (R. Donnelly).

0006-2952/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2005.07.027

tissues, particularly skeletal muscle. Thus, the present study investigates the regulation of APS gene expression in skeletal muscle cells.

2. Materials and methods

2.1. Materials

L6 myoblasts, a rat skeletal-muscle-derived cell line were obtained from ECACC (Cambridge, UK). Reagents were obtained as follows: insulin (Sigma, Poole, UK), rosiglitazone (GlaxoSmithKline, Worthing, UK), resistin (R&D Systems Inc., Minneapolis, MN, USA), and PD098059 (Pfizer, NY, USA).

2.2. Cell culture and stimulation

L6 myoblasts were cultured in Dulbecco's Modified Eagle's Medium (Gibco, Paisley, UK) supplemented with 10% fetal bovine serum, glutamine 2 mM and 1% antibiotic/antimycotic. Differentiation was induced by culturing the cells in 2% fetal bovine serum for 5 days. Myotubes were then serum starved for 24 h prior to stimulation for 24 h with insulin 1 μM, rosiglitazone 10 μM, resistin 500 nM or the MAP kinase inhibitor PD098059 50 μM for 30 min prior to incubation with insulin 1 μM for 24 h ($n = 6$ for each of four experimental conditions).

2.3. mRNA extraction and optimisation of RT-PCR

Following stimulation the cells were washed with PBS and mRNA isolated using RNAqueous kit (Ambion, Austin, TX, USA). An amount of 1 μg mRNA was used for reverse transcription with MMLV-Rtase (Gibco, Paisley, UK). Primers for rat APS were obtained from Genosys (Pampisford, UK) and relative concentrations of 100 nM (sense) to 900 nM (antisense) produced the lowest threshold cycle value. Agarose gel electrophoresis of the PCR product confirmed a single band with no primer dimerization. Four different control genes were evaluated, and transcription factor 2D (TF2D) was the most stable under the different experimental conditions.

Thus, semi-quantitative real time PCR analysis was used to determine the expression of APS mRNA relative to TF2D. Each sample was run in triplicate and SYBR Green 1 dye chemistry (Applied Biosystems, Warrington, UK) was used to detect the PCR products in an ABI Prism 7700 Sequence Detector. The following oligonucleotide primers were used:

APS primers:
Sense: 5′-CACCTTCGCCTTTCTCTGAATG-3′,
Antisense: 5′-TCAAACACCGACTGGAACCA-3′.
Transcription factor 2D primers:
Sense: 5′-ATGCTCAGGGCTTGGCC-3′,
Antisense: 5′-CATTGGACTAAAGATGGGAATTCC-3′.

2.4. Statistical analysis

APS expression relative to TF2D for each experimental intervention is expressed as the mean ± S.E.M. Comparisons between different conditions were made using a two-tailed Mann Whitney *U*-test.

3. Results

3.1. APS mRNA expression is markedly increased by differentiation of L6 myoblasts and augmented by rosiglitazone

Differentiated L6 myotubes showed a marked increase in APS expression compared to undifferentiated myoblasts (0.55 ± 0.08 versus 1.14 ± 0.08, $p = 0.001$). This increase in expression was augmented by the addition of rosiglitazone 10 μM for 24 h to the differentiated myotubes (1.5 ± 0.09, $p = 0.025$; Fig. 1).

3.2. Inhibitory effects of resistin and insulin on APS mRNA expression

Co-incubation of differentiated myotubes with resistin 500 nM for 24 h resulted in a 1.4-fold reduction in APS expression (0.80 ± 0.09 versus 1.14 ± 0.08, $p = 0.025$). A more pronounced (3.1-fold) reduction in APS expression was observed in L6 myotubes exposed to insulin 1 μM for 24 h (0.37 ± 0.01 versus 1.14 ± 0.08, $p = 0.001$), an effect that was partially attentuated by preincubation with the MAP kinase inhibitor PD098059 (0.60 ± 0.03, $p = 0.001$) (Fig. 2).

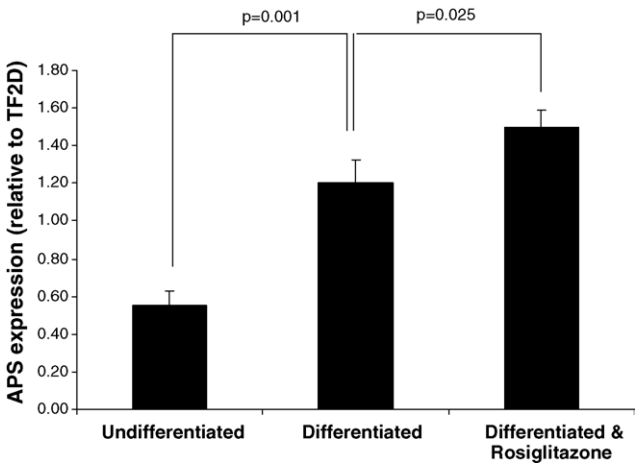

Fig. 1. Expression of APS mRNA relative to the control gene TF2D in undifferentiated L6 myoblasts compared with differentiated myotubes, and myotubes exposed for 24 h to rosiglitazone 10 μM ($n = 6$).

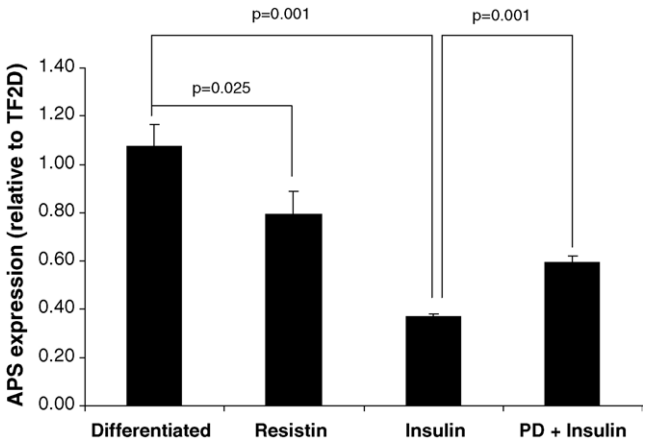

Fig. 2. Expression of APS mRNA relative to TF2D in differentiated L6 myotubes exposed to resistin 500 nM ($n = 6$) and insulin 1 μM ($n = 6$). A further experiment pretreated cells with the MAP kinase inhibitor PD098059 prior to incubation with insulin 1 μM for 24 h.

4. Discussion

These results highlight a number of important new observations. Firstly, the differentiation dependent nature of APS expression in muscle cells, which concurs with previous results in adipocytes, and secondly that APS expression is significantly up-regulated by short-term exposure to rosiglitazone. Cellular differentiation had a major effect on APS expression. Various genes involved in lipid metabolism and energy balance are regulated by PPAR response elements and thiazolidinediones increase PPAR-γ mRNA levels up to three-fold, but to date Cbl-associated protein (CAP) is the only insulin signalling molecule that is known to be directly upregulated by PPARγ agonists [6]. Previous studies have shown the importance of the Cbl-dependent pathway in mediating the effects of rosiglitazone on PI3 kinase and PKC-λ activation, and glucose transport, in adipocytes [7]. Thus, rosiglitazone induced upregulation of APS expression in the present study provides further evidence that thiazolidinediones may potentiate insulin signalling in skeletal muscle in part via effects on the APS/Cbl/CAP complex [3].

Resistin is an adipocyte-secreted hormone which impairs glucose tolerance in normal mice and attenuates insulin stimulated glucose uptake in isolated cells, but the underlying mechanism is unclear [8]. For example, the insulin antagonist effect of resistin occurs without affecting insulin stimulated phosphorylation of the insulin receptor or IRS-1, or the association of IRS-1 with PI3 kinase and the subsequent activation of PI3 kinase and Akt [9]. The present study suggests that resistin may affect glucose uptake in muscle (independently of the PI3 kinase pathway) by reducing expression of APS.

Expression of APS protein has been demonstrated in human skeletal muscle (awaiting publication). In the present study, it is unclear whether the changes in mRNA expression produce corresponding changes in APS protein and function. However, recent work has shown that APS is the key protein recruiting both CAP and c-Cbl to the insulin receptor, and that small changes in APS have the potential to induce large changes in downstream signalling events that mediate the metabolic actions of insulin in skeletal muscle [3]. Thus, it seems likely that the two–four-fold differences in APS gene expression observed in these experiments will be of functional importance in muscle insulin sensitivity.

The marked down-regulation of APS expression by insulin is consistent with the effects of insulin on other insulin receptor substrates. For example, insulin-mediated suppression of IRS-1 and IRS-2 is PI3 kinase dependent, and insulin causes transcriptional suppression of the IRS-2 gene [10]. In the present study, the effect of insulin on APS expression was at least partly attenuated by prior exposure to the MAP kinase inhibitor.

In summary, APS has been identified as an important insulin receptor substrate protein which affects insulin-mediated glucose uptake and GLUT4 translocation. This study is the first to show that APS expression in muscle cells is differentiation dependent, up-regulated by rosiglitazone and down-regulated by insulin and resistin. These effects may be important in understanding the molecular mechanisms of insulin resistance, and the mode of action of insulin senstizer drugs, such as thiazolidinediones.

References

[1] Moodie SA, Alleman-Sposeto J, Gustafson TA. Identification of the APS protein as a novel insulin receptor substrate. J Biol Chem 1999; 274:11186–93.
[2] Liu J, Kimura A, Baumann CA, Saltiel AR. APS facilitates c-Cbl tyrosine phosphorylation and GLUT4 translocation in response to insulin in 3T3-L1 adipocytes. Mol Cell Biol 2002;22:3599–609.
[3] Ahn MY, Katanakis KD, Bheda F, Pillay TS. Primary and essential role of the adaptor protein APS for recruitment of both c-Cbl and its associated protein CAP in insulin signaling. J Biol Chem 2004; 279:21526–32.
[4] Minami A, Iseki M, Kishi K, Wang M, Ogura M, Furukawa N, et al. Increased insulin sensitivity and hypoinsulinemia in APS knockout mice. Diabetes 2003;52:2657–65.
[5] Saltiel AR, Pessin JE. Insulin signaling pathways in time and space. Trends Cell Biol 2002;12:65–71.
[6] Ribon V, Johnson JH, Camp HS, Saltiel AR. Thiazolidinediones and insulin resistance: peroxisome proliferator activated receptor gamma activation stimulates expression of the CAP gene. Proc Natl Acad Sci USA 1998;95:14751–6.
[7] Standaert ML, Kanoh Y, Sajan MP, Bandyopadhyay G, Farese RV. Cbl, IRS-1, and IRS-2 mediate effects of rosiglitazone on PI3K, PKC-λ, and glucose transport in 3T3/L1 adipocytes. Endocrinology 2002; 143:1705–16.
[8] Rea R, Donnelly R. Resistin: an adipocyte derived hormone—has it a role in diabetes and obesity? Diab Obes Metab 2004;6:163–70.
[9] Moon B, Kwan JJ, Duddy N, Sweeney G, Begum N. Resistin inhibits glucose uptake in L6 cells independently of changes in insulin signaling and GLUT4 translocation. Am J Physiol (Endocrinol Metab) 2003;285:E106–15.
[10] Pirola L, Bonnafous S, Johnston AM, Chaussade C, Portis F, van Obberghen E. Phosphoinositide 3-kinase-mediated reduction of insulin receptor substrate-1/2 protein expression via different mechanisms contributes to the insulin-induced desensitization of its signaling pathways in L6 muscle cells. J Biol Chem 2003;278:15641–51.