Models of insulin action on metabolic and growth response genes

M. Alexander-Bridges,¹ C. Buggs, L. Giere, M. Denaro, B. Kahn, M. White, V. Sukhatme and N. Nasrin¹
¹Diabetes Unit, Massachusetts General Hospital, Harvard Medical School, Howard Hughes Medical Institute, Boston, MA 02114, USA

Abstract

In ongoing studies aimed at elucidating the mechanism of insulin action on the expression of genes that modulate glucose utilization and cell growth, we have focused on the inductive effect of insulin on transcription of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the early growth response gene, Egr-1. Insulin acutely stimulates the expression of both genes in 3T3 adipocytes; however, in primary adipocytes, chronic insulin exposure has opposing effects on the expression of these genes. GAPDH mRNA is decreased in the epididymal fat cells of diabetic animals and is increased over control levels when insulin is replaced, while Egr-1 mRNA levels are increased in diabetic animals. These observations, coupled with the finding that insulin-stimulated Egr-1 gene transcription is impaired in a Chinese hamster ovarian (CHO) cell line that displays normal metabolic responses but impaired ability to regulate DNA synthesis, support the conclusion that insulin regulation of Egr-1, a growth response gene, and GAPDH, a metabolic response gene, are mediated by distinct pathways. We present evidence that supports the role of protein phosphorylation in mediating the effect of insulin on activation of Egr-1 and GAPDH gene transcription. (Mol Cell Biochem 109: 99–105, 1992)

Key words: phosphorylation, IRE-A, Egr-1, GAPDH, gene transcription, insulin

Introduction

Insulin induces glyceraldehyde-3-phosphate dehydrogenase, GAPDH, mRNA levels 8-fold in cultured 3T3-L1 adipocytes and 10-fold in fat or liver tissue isolated from rats fasted then refed a high-carbohydrate, low-fat diet overnight [1]. In contrast, expression of the GAPDH gene is not regulated in preadipocytes [2]. Insulin markedly stimulates expression of the early growth response gene, Egr-1, in fibroblasts, epithelial cells, and H35 hepatoma cells [3], which suggests that the signal transduction pathway involved in mediating the growth effect of insulin is intact in all three cell types.

In primary adipocytes, we show that GAPDH mRNA levels are markedly decreased in streptozotocin-treated diabetic animals and induced above basal levels upon replacement of insulin. In contrast, Egr-1 gene expression is induced during the induction of diabetes and is decreased below control values with chronic insulin therapy. This result supports the conclusion that insulin is not the predominant factor regulating expression of this gene in vivo.

Address for offprints: M. Alexander-Bridges, Wellman 306, Blossom Street, Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA, 02114, USA
This observation raised the question whether the effect of insulin on the transcription of Egr-1 and metabolic genes is mediated via distinct pathways. This hypothesis was supported by the fact that Chinese hamster ovarian (CHO) cells which express an insulin receptor mutant capable of stimulating glycogen synthesis, a metabolic event, but not DNA synthesis [4], a growth-related event, also showed impaired insulin-stimulated Egr-1 gene transcription. Mutations in the juxtamembrane region of the receptor that impair tyrosine kinase activity and intracellular signalling, as measured by phosphorylation of pp 185 [5], also inhibit Egr-1 gene transcription. This result suggests that the ability of insulin to stimulate Egr-1 gene transcription is linked to its ability to stimulate protein phosphorylation.

The role of phosphorylation in mediating the effect of insulin on the GAPDH gene could not be studied in CHO cells because insulin regulation of GAPDH gene expression is limited to lipogenic tissues. A detailed description of the mechanism of insulin action on gene transcription will require that the trans-acting factors that regulate gene expression be defined, characterized, and cloned. Towards this end, we have identified a cis-acting sequence, IRE-A, in the upstream region of the GAPDH gene that confers insulin-responsive gene transcription to marker genes. Binding of a trans-acting factor, IRP-A, to this motif is enhanced by insulin. Insulin is known to regulate the activity of its target enzymes by promoting phospho-dephospho interconversions. For the most part, insulin has been shown to regulate the activity of metabolic enzymes by promoting a net decrease in phosphorylation; glycogen synthase is an example. We posed the question whether IRP-A binding activity would be enhanced by phosphatase treatment. We found that DNA binding was undetectable when the protein was dephosphorylated. This result suggests that acute regulation of IRP-A activity may be mediated by a net increase in protein phosphorylation.

Methods

Isolation of epididymal fat cell RNA

Fat cells were isolated as previously described [6] and total RNA extracted using the guanidium isothiocyanate method [6]. Total RNA was matched by optical density and ethidium staining and subjected to Northern analysis. Northern analysis of cellular RNA was performed using a randomly primed Egr-1 cDNA probe [3] and a GAPDH-cDNA [2]. The autoradiographs were subjected to densitometry.

Mutant receptor lines

Stable lines of CHO cells transfected with i) normal human insulin receptors (HIR), ii) mutant HIR, or iii) the neomycin-resistance gene alone were grown to confluence in Ham's F12 medium supplemented with 10% fetal bovine serum, glutamine (2mM), and G418 (400 μg/ml). The cells were incubated in serum-free Ham's F12 medium containing 10 mM Hepes and 0.1% crystalline bovine serum albumin overnight then stimulated with 15% FBS or 10 nM insulin for 30 min. Total RNA was isolated as previously described [2] and matched by optical density and ethidium bromide staining.

Isolation of nuclear extracts

3T3 adipocytes were grown to confluence and differentiated as previously described [2]. Nuclear extracts were isolated from differentiated 3T3-L1 cells exposed to insulin for 1 h (I) using a modification of the procedure of Dignam et al. [1].

UV cross-linking

Body-labelled bromouridinated IRE-A was prepared for UV cross-linking experiments as previously described [7]. A specific primer was used to synthesize the IRE-A [1] binding motif in the presence of 50 μM dCTP, dGTP, 5-bromo-2'-deoxyuridine triphosphate, and 5 μM [α32P]-dATP. Nuclear extract (10 μg) was incubated with 1 ng of body-labelled probe in a buffer containing 20% glycerol (V/V), 150 mM NaCl, 5 mM MgCl2, 0.2 mM EDTA, 0.5 mM DTT, 20 mM Hepes (pH 8.0) in a total volume of 20 μl. The mixture was incubated at 0°C for 90 min then irradiated with a Fotodyne UV lamp (maximum emission wave length 310 nm) for 15 min. At the end of the incubation, the mixture was brought up to 10 mM CaCl2 and the DNA was digested for 30 min at 37°C with 1 μg of DNAAse I. The mixture was subjected to electrophoresis on a 12.5% SDS polyacrylamide gel. The gel was dried and subjected to autoradiography.