Exposure to TNF-α but not IL-1β impairs insulin-dependent phosphorylation of protein kinase B and p70S6k in mouse C2C12 myogenic cells

K. Grzelkowska-Kowalczyk, W. Wieteska-Skrzeczyńska

Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Nowoursynowska 159, 02-776 Warsaw, Poland

Abstract

Tumor necrosis factor (TNF)-α is a proinflammatory cytokine considered to play an important role in muscle catabolism, but little is known about the mechanisms of its action. The aim of the present study was therefore to examine the effect of TNF-α pretreatment on glucose uptake and protein synthesis as well as the cellular content and phosphorylation of protein kinase B (PKB), p70S6k, Mitogen Activated Protein (MAP) kinase and p90rsk in mouse C2C12 myotubes stimulated with insulin. To determine whether interleukin (IL)-1β might be involved in the catabolic action of TNF-α, the effects of IL-1β were also tested. Experiments were performed on mouse C2C12 myoblasts subjected to differentiation in the presence of increasing concentrations of TNF-α (0.1-100 ng/ml) or IL-1β (5-50 ng/ml) for 5 or 6 days. Insulin (100 nmol/l) markedly stimulated glucose uptake in C2C12 myotubes (202.6% of control). This effect was profoundly attenuated by pretreatment with TNF-α at a concentration of 1 ng/ml (122.2% of control) and completely abolished by higher cytokine concentrations. Pretreatment of cells with TNF-α at a concentration of 1 ng/ml was also effective in diminishing the effect of insulin on protein synthesis, whereas higher cytokine concentrations prevented hormonal stimulation of protein synthesis in C2C12 myotubes. Pretreatment with TNF-α caused a significant decrease in PKB protein content. Insulin-mediated activation of protein kinase B was significantly diminished in cells differentiated in the presence of TNF-α. Treatment of C2C12 cells with insulin led to the gel mobility retardation of p70S6k indicating its phosphorylation and activation. In cells differentiated in the presence of TNF-α an approximately 2-fold decrease of insulin-mediated p70S6k phosphorylation was noted. Six-day differentiation of myogenic cells in the presence of TNF-α did not affect the protein content of p42MAPK, p44MAPK, p90rsk and phosphorylation of p42MAPK. Neither glucose uptake nor protein synthesis stimulated by insulin were affected significantly by pretreatment with IL-1β. Preincubation of myogenic cells with IL-1β did not modify either the protein content of PKB and p70S6k or the insulin-stimulated phosphorylation of these kinases.

In conclusion: i) high concentrations of TNF-α, but not IL-1β, present in the extracellular environment during myoblast differentiation prevent the stimulatory action of insulin on glucose uptake and protein synthesis; ii) insulin resistance induced by TNF-α in C2C12 myogenic cells could be associated with the decreased insulin-mediated phosphorylation of PKB and p70S6k, but not with the basal phosphorylation of p42MAPK.

Key words: glucose transport, insulin resistance, protein synthesis, signaling pathways, tumor necrosis factor-α

Correspondence to: K. Grzelkowska-Kowalczyk, e-mail: k_grzel_kow@poczta.fm, tel./fax: (48 22) 847 24 52