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Molecular mechanism(s) of burn-induced insulin resistance in murine skeletal muscle: Role of IRS phosphorylation

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Abstract

Hyperglycemia, glucose intolerance and elevated insulin levels frequently occur in burned patients; however, the mechanism(s) for this insulin resistance has not been fully elucidated. One possible mechanism could involve alterations in the phosphorylation of serine 307 of the insulin receptor substrate-1 (IRS-1) via activation of stress kinase enzymes, including SAPK/JNK. In the present study we examined the time course of the effect of burn injury to mice on: levels of IRS-1 protein, phosphorylation of serine 307 of IRS-1, SAPK/JNK kinase levels and activity and Akt kinase activity in hind limb skeletal muscle. Burn injury produced a reduction in hind limb muscle mass 24 h after injury, and, which persisted for 168 h. At 24 h after injury, there was a dramatic (~9-fold) increase in phosphorylation of IRS-1 serine 307 followed by a more moderate elevation thereafter. Total IRS-1 protein was slightly elevated at 24 h after injury and decreased to levels below sham treated animals at the later times. Burn injury did not appear to change total SAPK/JNK protein content, however, enzyme activity was increased for 7 days after injury. Akt kinase activity was decreased in skeletal muscle following burn injury; providing a biochemical basis for burn-induced insulin resistance. These findings are consistent with the hypothesis that burn-induced insulin resistance may be related, at least in part, to alterations in the phosphorylation of key proteins in

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the insulin signaling cascade, including IRS-1, and that changes in stress kinases, such as SAPK/JNK produced by burn injury, may be responsible for these changes in phosphorylation.

Keywords: Burn injury; Insulin resistance; Insulin receptor substrate-1; SAPK/JNK; Akt kinase

Introduction

Hyperglycaemia and insulin resistance are common in severely burned patients, even if they have not previously had diabetes. Insulin resistance, defined as the pathological state in which target cells fail to respond to ordinary levels of circulating insulin (Kahn and Flier, 2000; Matthaei et al., 2000; Carlson, 2003), plays a major role as an underlying mechanism of the metabolic disorders in burn patients (Schwacha and Chaudry, 2002).

Insulin, produced by the pancreas, is the major anabolic hormone whose action is essential for growth and development, glucose homeostasis, fat and protein metabolism (Virkamaki et al., 1999; Le Roith and Zick, 2001: Zick, 2001). Circulating insulin interacts with its cognate receptor, which is a transmembrane tyrosine kinase with an $\alpha_2\beta_2$ configuration. Insulin binding to the α subunits leads to a conformational change and stimulation of receptor kinase activity through autophosphorylation of Tyrosine residues in the β subunits. The activated insulin receptor kinase (IRK) then phosphorylates specific Tyrosine residues of substrate proteins, such as Shc, Gab-1, CbI/CAP and the family of insulin receptor substrate (IRS) proteins, which serve as docking sites for downstream effectors.

Molecular mechanisms of insulin resistance are complicated and may differ in different conditions and tissues. There is evidence that Serine/Threonine phosphorylation of IRS proteins plays a role in the development of chronic insulin resistance in type 2 diabetes and obesity, but little is known about its role in acute insulin resistance after burn injury. Recent work suggests that induced insulin resistance may be in part due to phosphorylation-based negative-feedback, which may uncouple the insulin receptor or insulin receptor docking proteins from its upstream and downstream signaling pathway, terminating signal transduction and altering insulin action (Zick, 2001; Tanti et al., 1994; Hotamisligil et al., 1996; De Fea and Roth, 1997; Rui et al., 2001). The IRS proteins are major targets for this phosphorylation-based, negative-feedback control of insulin signaling.

In this report, we investigated the phosphorylation of IRS-1 at the serine 307 position (phospho-IRS-1 Ser307) following burn injury to mice, and the relationships between this phosphorylation and the levels of stress-activated protein kinase/Jun N-terminal kinase (SAPK/JNK) and Akt kinase enzyme in hind limb muscle.

Materials and methods

Antibodies and reagents

Antibodies to IRS-1 and phospho-IRS-1 Ser307 were purchased from Upstate Laboratories (Lake Placid, NY, USA). Antibodies to SAPK/JNK (#9252) and phospho-SAPK/JNK Thr183/Tyr185 (#9251), SAPK/JNK kinase assay kits (#9810), p38 MAP kinase assay kits (#9820), p44/42 MAP kinase assay