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Original Contribution

Oxidant stress-induced loss of IRS-1 and IRS-2 proteins in rat skeletal muscle: Role of p38 MAPK

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ABSTRACT

Oxidative stress is characterized as an imbalance between the cellular production of oxidants and the cellular antioxidant defenses and contributes to the development of numerous cardiovascular and metabolic disorders, including hypertension and insulin resistance. The effects of prolonged oxidant stress in vitro on the insulin-dependent glucose transport system in mammalian skeletal muscle are not well understood. This study examined the in vitro effects of low-level oxidant stress (60–90 μM, H_2O_2) for 4 h on insulin-stimulated (5 mU/ml) glucose transport activity (2-deoxyglucose uptake) and on protein expression of critical insulin signaling factors (insulin receptor (IR), IR substrates IRS-1 and IRS-2, phosphatidylinositol 3-kinase, Akt, and glycogen synthase kinase-3 (GSK-3)) in isolated soleus muscle of lean Zucker rats. This oxidant stress exposure caused significant (50%, $p<0.05$) decreases in insulin-stimulated glucose transport activity that were associated with selective loss of IRS-1 (59%) and IRS-2 (33%) proteins, increased (64%) relative IRS-1 Ser^{307} phosphorylation, and decreased phosphorylation of Akt Ser^{473} (50%) and GSK-3β Ser^{9} (43%). Moreover, enhanced (37%) phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK) was observed. Selective inhibition of p38 MAPK (10 μM A304000) prevented a significant portion (29%) of the oxidant stress-induced loss of IRS-1 (but not IRS-2) protein and allowed partial recovery of the impaired insulin-stimulated glucose transport activity. These results indicate that in vitro oxidative stress in mammalian skeletal muscle leads to substantial insulin resistance of distal insulin signaling and glucose transport activity, associated with a selective loss of IRS-1 protein, in part due to a p38 MAPK-dependent mechanism.

Defects in the expression and functionality of critical elements of the canonical insulin signaling cascade, including the insulin receptor (IR), IR substrates 1 and 2 (IRS-1 and IRS-2), phosphatidylinositol 3-kinse (PI3-kinase), Akt, and glycogen synthase kinase-3 (GSK-3), are known to cause insulin resistance in mammalian skeletal muscle, and these impairments in insulin action are associated with the development of prediabetes and type 2 diabetes (reviewed in Refs. [1–4]). One condition contributing to the multifactorial etiology of insulin resistance is oxidative stress, the imbalance between the cellular production of reactive oxygen species and the antioxidant defenses in cells [5–9]. Previous investigations, using either cultured cell lines or isolated mammalian skeletal muscle, have demonstrated that in vitro exposure to a low-level oxidant stress (H_2O_2) leads to diminished insulin-stimulated glucose transport activity [10–15], attributed to dysfunctions in the normal engagement of the insulin signaling pathway [11–17]. Additional investigations using 3T3-L1 adipocytes and rat hepatoma cells have shown that exposure to this type of oxidant stress leads to a reduction in the protein expression of IRS-1 protein [16,17], possibly by modulation of IRS-1 serine phosphorylation [16,17]. However, the impact of this type of in vitro oxidative stress on the expression of critical insulin signaling factors in mammalian skeletal muscle is not currently known.

Several investigations have focused on the potential roles of various stress-activated serine kinases in the development of oxidative stress-associated insulin resistance (see Ref. [5]). We have shown recently that short-term (2 h) exposure to a low-level oxidant stress in vitro in mammalian skeletal muscle is associated with decreased insulin suppression of the activity of the serine kinase GSK-3 and that selective inhibition of GSK-3 rescues about 20% of the impaired insulin action on glucose transport activity [15]. Another stress-activated serine kinase, p38 mitogen-activated protein kinase (p38 MAPK), is also thought to play a role in the development of insulin resistance [6,7,18–20]. Blair et al. [12] and Maddux et al. [14], in experiments using L6 myocytes, have shown that exposure to a low level of oxidant stress leads to activation of the serine kinase p38

Abbreviations: 2-DG, 2-deoxyglucose; GSK-3, glycogen synthase kinase-3; KHB, Krebs–Henseleit buffer; IR, insulin receptor; IRS-1, insulin receptor substrate-1; p38 MAPK, p38 mitogen-activated protein kinase; PI3-kinase, phosphatidylinositol 3-kinase.

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MAPK. Moreover, we have shown that this same in vitro oxidant stress in isolated rat skeletal muscle leads to engagement of p38 MAPK [15,21]. Interestingly, the activation of p38 MAPK is inappropriately upregulated in fat cells [18] and skeletal muscle [22] of type 2 diabetic patients, and in cultured fat cells p38 MAPK overactivity is associated with reduced expression of GLUT-4 protein [18]. Using a model of insulin resistance in 3T3-L1 adipocytes, Carlson and colleagues [19] have shown that selective pharmacological inhibition of p38 MAPK improves insulin-stimulated glucose transport activity, an effect associated with the increased expression of GLUT-4, but not IRS-1, protein. However, the specific role of p38 MAPK in the etiology of the oxidant-induced insulin resistance in mammalian skeletal muscle is not well understood.

In the context of the foregoing information, the primary aim of this investigation was to assess the effect of in vitro exposure of skeletal muscle from the lean Zucker rat to a low-grade oxidant stress (60–90 μM H_2O_2) for up to 4 h on the protein expression of critical elements of the canonical insulin signaling cascade, including IR-β, IRS-1 and IRS-2, PI3-kinase, Akt, and GSK-3, on GLUT-4 protein expression and on insulin-dependent glucose transport activity. Moreover, an important associated aim was to address the specific role of p38 MAPK, which is engaged by oxidants in insulin-sensitive cell lines and tissues [5,12,14,15,21], in the induction of insulin resistance by this oxidant intervention in this mammalian skeletal muscle preparation.

Materials and methods

Animals

The University of Arizona Animal Use and Care Committee approved all procedures used in this study. Female lean (Fa/–) Zucker rats, ages 7–9 weeks, were obtained from Harlan (Indianapolis, IN, USA) 1 week in advance of experiments, and they were used when they had a body weight of 150–170 g. Animals were housed in a temperature-controlled room (20–22°C) with a 12:12-h light:dark cycle (lights on from 7:00 AM to 7:00 PM) at the University of Arizona Central Animal Facility. The animals had free access to chow (Teklad 7001, Madison, WI, USA) and water. At 5:00 PM on the evening before each experiment, the animals were restricted to 4 g of chow, which was consumed immediately. Experiments began at 8:00 AM the next morning.

Skeletal muscle incubations and oxidant exposure

The animals were deeply anesthetized using an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt), and both soleus muscles were dissected and prepared for incubation. Each muscle was split into two strips (~25–35 mg each). The muscles were then incubated for 2 or 4 h at 37°C in 3 ml of oxygenated (95% O_2–5% CO_2) Krebs–Henseleit buffer (KHB) supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% BSA (radioimmunoassay grade; Sigma Chemical), in the absence or presence of a maximally effective concentration of insulin (5 mU/ml Humulin; Eli Lilly, Indianapolis, IN, USA) and without or with 100 mU/ml glucose oxidase (MP Biomedicals, Solon, OH, USA), which produced the oxidant hydrogen peroxide at a concentration of 60–90 μM [15,21]. The incubation medium was changed after each hour of treatment. In some experiments, the selective p38 MAPK inhibitor A304000 (kindly provided by Abbott Laboratories, Abbott Park, IL, USA) was added to the incubation medium.

Determination of glucose transport activity

After the initial 2- or 4-h period of incubation, the muscles were rinsed for 10 min at 37°C in 3 ml of KHB containing 40 mM mannitol, 0.1% BSA, and insulin, glucose oxidase, and inhibitor, if present previously. The muscles were then transferred to 2 ml of KHB containing 1 mM 2-deoxy-[1,2-^{3}H]glucose (2-DG) (300 μCi/mmol; Sigma Chemical), 39 mM [U-^{14}C]mannitol (0.8 mCi/mmol; ICN Radiochemicals, Irvine, CA, USA), 0.1% BSA, and insulin, glucose oxidase, and inhibitor, if present previously. After this final 20-min incubation at 37°C, the muscles were removed, trimmed of excess fat and connective tissue, and quickly frozen between aluminum blocks cooled in liquid nitrogen. The specific intracellular accumulation of 2-DG was determined as described previously [23].

Signaling protein expression and functionality

Some muscles were frozen after the initial incubation period, weighed, and stored at −80°C until analysis. These muscles were homogenized in 8 volumes of ice-cold lysis buffer (50 mM Hepes, 150 mM NaCl, 20 mM Na pyrophosphate, 20 mM β-glycerophosphate, 10 mM NaF, 2 mM Na_3VO_4, 2 mM EDTA, 1% Triton X-100, 10% glycerol, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 μg/ml aprotinin, 10 μg/ml leupeptin,

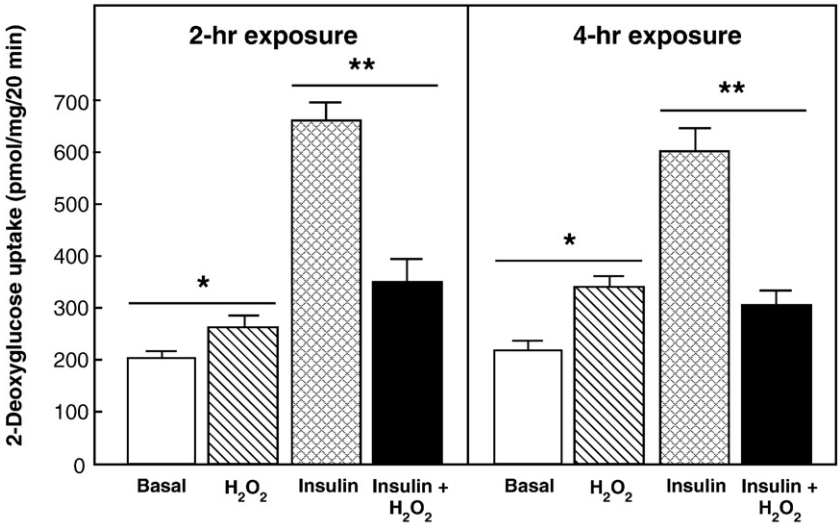

Fig. 1. Effects of an oxidant stress on glucose transport activity in mammalian skeletal muscle. Basal and insulin-stimulated (5 mU/ml) rates of 2-deoxyglucose uptake were determined in soleus muscle preparations from lean Zucker rats after 2- and 4-h exposures to an in vitro oxidant stress (60–90 μM H_2O_2). Values are means ± SE for six to nine muscles per group. *$p<0.05$ vs basal in the absence of H_2O_2. **$p<0.05$ vs insulin-stimulated in the absence of H_2O_2.