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Acute exposure of primary rat soleus muscle to zilpaterol HCl (β2 adrenergic agonist), TNFα, or IL-6 in culture increases glucose oxidation rates independent of the impact on insulin signaling or glucose uptake

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ABSTRACT

Recent studies show that adrenergic agonists and inflammatory cytokines can stimulate skeletal muscle glucose uptake, but it is unclear if glucose oxidation is similarly increased. Thus, the objective of this study was to determine the effects of ractopamine HCl (β1 agonist), zilpaterol HCl (β2 agonist), TNFα, and IL-6 on glucose uptake and oxidation rates in unstimulated and insulin-stimulated soleus muscle strips from adult Sprague-Dawley rats. Effects on phosphorylation of Akt (phospho-Akt), p38 MAPK (phospho-p38), and p44/42 MAPK (phospho-p44/42) was also determined. Incubation with insulin increased ($P < 0.05$) glucose uptake by ~47%, glucose oxidation by ~32%, and phospho-Akt by ~238%. Insulin also increased ($P < 0.05$) phospho-p38, but only after 2 h in incubation. Muscle incubated with β2 agonist alone exhibited ~20% less ($P < 0.05$) glucose uptake but ~32% greater ($P < 0.05$) glucose oxidation than unstimulated muscle. Moreover, co-incubation with insulin + β2 agonist increased ($P < 0.05$) glucose oxidation and phospho-Akt compared to insulin alone. Conversely, β1 agonist did not appear to affect basal or insulin-stimulated glucose metabolism, and neither β agonist affected phospho-p44/42. TNFα and IL-6 increased ($P < 0.05$) glucose oxidation by ~23% and ~33%, respectively, in the absence of insulin. This coincided with increased ($P < 0.05$) phospho-p38 and phospho-p44/42 but not phospho-Akt. Furthermore, co-incubation of muscle with insulin + either cytokine yielded glucose oxidation rates that were similar to insulin alone, despite lower ($P < 0.05$) phospho-Akt. Importantly, cytokine-mediated increases in glucose oxidation rates were not concomitant with greater glucose uptake. These results show that acute β2 adrenergic stimulation, but not β1 stimulation, directly increases fractional glucose oxidation in the absence of insulin and synergistically increases glucose oxidation when combined with insulin. The cytokines, TNFα and IL-6, likewise directly increased glucose oxidation in the absence of insulin, but were not additive in combination with insulin and in fact appeared to disrupt Akt-mediated insulin signaling. Rather, cytokines appear to be acting through MAPKs to elicit effects on glucose oxidation. Regardless, stimulation of glucose oxidation by these key stress factors did not rely upon greater glucose uptake, which may promote metabolic efficiency during acute stress by increasing fractional glucose oxidation without increasing total glucose consumption by muscle.

1. Introduction

Skeletal muscle comprises about 40% of total body mass in humans, yet it accounts for greater than 85% of the body's insulin-stimulated glucose utilization [1,2]. The role of insulin in metabolic regulation is well-understood, but muscle metabolism can be influenced by additional factors including catecholamines and cytokines [3–5]. Catecholamines (i.e. epinephrine, norepinephrine) are released into circulation by the adrenal medulla and act by binding to a large class of G protein-coupled adrenergic receptors (α_{1A}, α_{1B}, α_{1C}, α_{2A}, α_{2B}, α_{2D}, β1, β2, and β3) [6] located throughout the body. Thus, the impact that adrenergic stimulation has on a specific tissue is a function of the specific receptor type or types that it expresses. In skeletal muscle, β2 adrenergic receptors are the most highly-expressed isoform, but β1 receptors and to a lesser extent β3 and α_{1D} receptors are also present [7,8]. Growth studies in animals [9] have led to the development of isoform-specific β adrenergic growth promoters that are used as feed additives in the livestock industry to increase meat yield per animal [10]. In addition, reports show that β agonists are commonly used by athletes to boost muscle growth and athletic performance, despite being restricted by the

Abbreviations: phospho-Akt, phosphorylated Akt; phospho-p38, phosphorylated p38 MAPK; phospho-p44/42, phosphorylated p/44/42 MAPK

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World anti-Doping Agency [11]. However, far less is known about the effects of β agonists on muscle metabolism. The seemingly complex effects of inflammatory cytokines on metabolism are likewise only beginning to be understood. Inflammation is known to cause insulin resistance [12], yet recent studies show that two major inflammatory cytokines, TNFα and IL-6, may stimulate glucose metabolism in muscle independent of their actions on insulin signaling [4,13,14]. Insulin increases glucose uptake in skeletal muscle through a well-characterized signaling cascade that begins with binding of its transmembrane tyrosine kinase receptor followed by sequential activation of the downstream targets, IRS1, PI3K, and Akt via phosphorylation [15]. This canonical pathway ultimately stimulates translocation of glucose transporter, Glut4, to the cell membrane where it is imbedded and facilitates greater glucose passage into the muscle cell [16]. Phosphorylation of Akt appears to be a critical step in most insulin-regulated events, and thus the ratio of phosphorylated Akt to total Akt is considered to be a reliable indicator of insulin signaling [17]. In addition to the canonical PI3K/Akt-mediated signal transduction pathway, mitogen-activated protein kinases (MAPKs) have also been identified as activators of Glut4-mediated glucose uptake [18,19]. Insulin-stimulated glucose oxidation rates have long been presumed to be proportional with glucose uptake rates [20], but we postulate that this relationship may not be maintained with the additional influence of adrenergic or inflammatory factors. Moreover, it is unclear whether these factors would regulate skeletal muscle glucose metabolism through direct effects or by altering insulin signaling. Thus, the objective of this study was to determine the respective impacts of β1 and β2 adrenergic agonists, TNFα, and IL-6 on glucose uptake and oxidation rates in intact soleus muscle strips isolated from adult rats. Furthermore, we sought to determine whether these effects were insulin-associated or insulin-independent by incubating muscle strips with each factor alone or in combination with insulin.

2. Materials and methods

2.1. Animals and tissue isolation

The following experiments were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln. Animal studies were performed at the University of Nebraska-Lincoln Animal Science Complex, which is accredited by the American Association for Accreditation of Laboratory Animal Care.

Soleus muscles collected tendon-to-tendon from adult Sprague-Dawley rats (females 252.86 ± 14.93 g, males 378 ± 15.72 g) after decapitation under heavy isoflurane anesthesia were used to measure glucose uptake (n = 10), glucose oxidation (n = 9), and protein expression (n = 8). Males and females were spread evenly across groups. Isolated soleus muscles were washed in ice-cold phosphate buffered saline (PBS; pH 7.4), and each muscle was dissected longitudinally (tendon to tendon) into 25–45 mg strips. For all experiments, muscle strips were pre-incubated for 1 h at 37 °C in gassed (95% O_2, 5% CO_2) Krebs-Henseleit bicarbonate buffer (KHB, pH 7.4; 0.1% bovine serum albumin; Sigma-Aldrich, St. Louis, MO) spiked with respective treatment (Table 1) and 5 mM D-glucose (Sigma-Aldrich, St. Louis, MO). Treatment concentrations were consistent with those previously described [4,21–25]. Muscle strips were then washed for 20 min in treatment-spiked KHB with no glucose. Pre-incubation and wash media for glucose uptake (but not glucose oxidation) experiments also contained 35 mM and 40 mM mannitol (Sigma-Aldrich), respectively. Glucose uptake and glucose oxidation rates were determined as described below. Protein analysis was performed in soleus strips from parallel incubations.

2.2. Glucose uptake

Glucose uptake rates were determined from intracellular accumulation of [^{3}H]2-deoxyglucose as previously described [26], with some modifications. After being pre-incubated and washed, soleus strips were incubated at 37 °C for 20 min in treatment-spiked KHB containing 1 mM [^{3}H]2-deoxyglucose (300 μCi/mmol) and 39 mM [1-^{14}C] mannitol (1.25 μCi/mmol). Muscle strips were then removed, thrice washed in ice-cold PBS, weighed, and lysed in 2 M NaOH (Sigma-Aldrich) at 37 °C for 1 h. Lysates were vortexed and mixed with UltimaGold scintillation fluid, and specific activity of ^{3}H and ^{14}C was measured by liquid scintillation with a Beckman-Coulter 1900 TA LC counter (Brea, CA). Specific activity of the media was likewise determined in triplicate 10-μl aliquots mixed with 500 μl distilled water and scintillation fluid. Mannitol concentrations were used to estimate the amount of extracellular fluid in each lysate, and intracellular accumulation of 2-deoxyglucose was calculated as total 2-deoxyglucose in the lysate less the extracellular concentration. All radioactive compounds and scintillation fluids were purchased from Perkin-Elmer (Waltham, MA).

2.3. Glucose oxidation

Glucose oxidation rates were determined by oxidation of [^{14}C-U] D-glucose as previously described [27], with some modifications. After being pre-incubated and washed, muscle strips were placed in one side of a sealed dual-well chamber and incubated at 37 °C for 2 h in treatment-spiked KHB containing 5 mM [14C-U] D-glucose (0.25 μCi/mmol). NaOH (2 M) was placed in the adjacent well to capture CO_2. After 2 h, chambers were cooled at −20 °C for 2 min. HCl (2 M; Sigma-Aldrich) was added to the media through a rubber seal on the top of the chamber, and the chambers were then incubated at 4 °C for 1 h to release bicarbonate-bound CO_2 from the media. Finally, chambers were unsealed and each muscle strip was washed and weighed. NaOH was collected from each chamber, mixed with UltimaGold scintillation fluid, and analyzed by liquid scintillation to determine specific activity of captured $^{14}CO_2$. Specific activity of media was determined as described above.

2.4. Western immunoblot

The respective activities of Akt and MAPKs were estimated by the proportions of phosphorylated target protein to total target protein as previously described [28–31], with minor modifications. Target protein concentrations were determined in soleus strips that were incubated in treatment-spiked KHB for 1 or 2 h and then snap-frozen and stored at −80 °C. Each muscle strip was thoroughly homogenized in 200 μl of radioimmunoprecipitation buffer containing manufacturer-recommended concentrations of Protease and Phosphatase Inhibitor (Thermo Fisher, Carlsbad, CA). Homogenates were then sonicated and centrifuged (14,000*g* for 5 min at 4 °C), and supernatant was collected. Total protein concentrations were determined by Pierce BCA Protein Assay Kit (Thermo Fisher). Protein samples (35 μg) were boiled for 5 min at 95 °C in BioRad 4× Laemmli Sample Buffer (BioRad, Hercules, CA) and then separated by SDS-polyacrylamide. Gels were transferred to polyvinylidene fluoride low florescence membranes (BioRad), which were incubated in Odyssey block solution (Li-Cor Biosciences, Lincoln, NE) for 1 h at room temperature and then washed with 1X TBS-T (20 mM Tris-HCl + 150 mM NaCl + 0.1% Tween 20). Membranes were subsequently incubated overnight at 4 °C with one of the following rabbit antibodies diluted in Odyssey block solution + 0.05% Tween-20: anti-Akt (1:1000), anti-phospho-Akt (Ser473) (1:2000), p44/42 MAPK (1:2000), phospho-p44/42 MAPK (Thr202/Tyr204) (1:1000), p38 MAPK (1:1000), or phospho-p38 MAPK (Thr180/Tyr182) (1:1000) (Cell Signaling, Danvers, MA). An IR800 goat *anti*-rabbit IgG secondary antibody (1:10,000 for Akt; 1:5000 for MAPKs; Li-Cor) diluted in Odyssey block solution with 0.05% Tween-20 and 0.01% SDS was applied for 1 h at room temperature. Blots were scanned on an Odyssey Infrared Imaging System and analyzed with Image Studio Lite Software (Li-Cor). For each protein of interest, phosphorylation rates were