Impacts of β agonists and pro-inflammatory cytokines on basal and insulin-stimulated glucose metabolism in rat soleus.

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Abstract

Recent studies suggest that catecholamines and pro-inflammatory cytokines help regulate muscle growth and metabolism at sub-stress levels. Our objective was to determine acute effects of β1 and β2 adrenergic agonists, TNFα, and IL-6 on muscle glucose uptake and oxidation under basal and insulin-stimulated conditions. Soleus muscles from adult Sprague-Dawley rats were separated into 25-45mg strips and incubated in KHb spiked with insulin, racaptamine (β1 agonist), zilpertol (β2 agonist), TNFα, or IL-6. Glucose uptake was determined from cellular accumulation of [3H]-2-deoxyglucose after 20min. Glucose oxidation of [14C]-glucose was determined after 2h. Phospho-Akt-totol Akt (p-Akt/Akt) was determined after 1h. Compared to muscle incubated in un-spiked (basal) media, incubation with insulin increased (P<0.05) glucose uptake by ~47%, oxidation by ~32%, and p-Akt/Akt by ~238%. Muscle incubated with β2 agonist exhibited ~20% less (P<0.05) glucose uptake but ~32% greater (P<0.05) oxidation than basal. Moreover, β2 agonist+insulin increased (P<0.05) glucose oxidation and p-Akt/Akt over insulin alone. β1 agonist incubations did not differ from basal for any output. Likewise, β1 agonist+insulin incubations did not differ from insulin alone. Glucose oxidation was ~25% and ~33% greater (P<0.05), respectively, in TNFα and IL-6 incubations compared to basal, yet uptake and p-Akt/Akt did not differ. Glucose uptake, oxidation, and p-Akt/Akt were similar among TNFα+insulin, IL-6, insulin, and insulin alone. In addition, glucose oxidation in TNFα+insulin and IL-6+insulin incubations did not differ from TNFα or IL-6 alone. These results show that acute β2 stimulation had opposite effects on muscle glucose uptake and oxidation, and that acute β1 stimulation had no evident metabolic impact. Moreover, β2 stimulation was synergistic with insulin, as glucose oxidation and Akt phosphorylation were greater with both agents together than either individually. Lastly, acute cytokine stimulation increased glucose oxidation independently of insulin or Akt phosphorylation. Together, our findings demonstrate that adrenergic and inflammatory mediators can have insulin-associated or insulin-independent effects on glucose metabolism and that these effects may differ for glucose uptake and oxidation.

Introduction

Skeletal muscle makes up <1/2 of total body mass but accounts for the majority of insulin-stimulated glucose utilization. Muscle glucose metabolism can be regulated by traditional "stress" factors of the adrenergic and inflammatory systems. However, it is unclear whether these factors act directly on metabolic pathways or indirectly through mediation of insulin action. In this study, we performed in vitro incubations of soleus muscle strips in order to better understand the impact of stress components on muscle glucose metabolism. We hypothesized that inflammatory and adrenergic factors illicit different effects on glucose uptake vs. oxidation and these effects may be insulin-associated or insulin-independent.

Materials & Methods

- 20-50mg strips of rat soleus muscle (4 strips/rat, 8 rats/condition)
- Culture Conditions
  - Basal: No additive
  - Insulin: 5mU/ml Humulin R
  - β1 agonist: 10μM Ractopamine HCl
  - β2 agonist: 0.5μM Zilpertol HCl
  - IL6: 1ng/ml rhIL-6
  - TNFα: 20ng/ml porcine TNFα
- Incubation Periods
  1.) Pre-incubation: 1hr, KHB, 5mM glucose
  2.) Wash: 20min, KHB, 0mM glucose
  3.) Tracer KHB (below):
- Glucose Uptake
  a.) 20min incubation, KHB, 1mM [3H]-2-deoxyglucose & 39mM [1-14C] mannitol
  b.) Strips were lysed with 0.5ml NaOH, mixed with 15ml UltimaGold scintillation fluid
  c.) Glucose uptake rate determined from [3H] 2-deoxyglucose Specific Activity in lysate (less ECF)
    Extracellular fluid volume determined from [1-14C] mannitol Specific Activity in lysate
- Glucose Oxidation
  a.) 2hr incubation, KHB, 5mM [14C-U] glucose
  b.) 14CO₂ produced by muscle strips released by 2N HCl and captured in 1M NaOH
      NaOH mixed with 15ml UltimaGold scintillation fluid
  c.) Glucose oxidation rate determined from 14CO₂ Specific Activity in NaOH
- Akt Phosphorylation
  a.) Muscle strips incubated in spiked KHB media (no tracer) for 1 hour
  b.) Phosphorylated Akt (pAkt) and total Akt determined by western immunoblot

![Figure 1: A] Individual rubber gaskets that are combined to seal the six-well plate during oxidation studies.

![Figure 2: A] Glucose metabolism in primary muscle strips incubated with adrenergic and pro-inflammatory factors. Rates of glucose uptake (A, n=10) and glucose oxidation (B,n=9) determined from [3H]-2-deoxyglucose and [1-14C]-glucose, respectively, are presented as mean + SEM. Means with differing superscripts are different (P < 0.05). Akt phosphorylation (C,n=8) during 1-h incubation in adrenergic and pro-inflammatory are presented as fold change from basal + SEM. Means with differing superscripts are different (P < 0.05).

- **β2 agonist alone had opposite effects on glucose uptake (decreased) vs. glucose oxidation (increased)**
- **β2 agonist & insulin synergistically enhanced glucose oxidation more than either factor alone**
- **Cytokines impaired insulin-stimulated Akt phosphorylation but increased glucose oxidation directly, effectively “replacing” insulin (acutely)**

Implications

Metabolic changes due to acute stress may initially be beneficial to energy production by muscle and may include compensation for reduced insulin action. Adrenergic stimulation appears to boost insulin’s metabolic actions. Together, our findings show that components of the stress response may help to maintain glucose metabolism during the initial onset of stress.

Acknowledgements

Supported by University of Nebraska-Lincoln Research Council Interdisciplinary Grant #737 & NE Center for Prevention of Obesity Diseases Pilot Project Grant (Parent NIH P20GM104320).