

学位論文要約

論文題目 Effects of acute heat stress on glucose metabolism and 5' adenosine monophosphate-activated protein kinase in skeletal muscle
(急性的な熱刺激が骨格筋糖代謝とAMPキナーゼに及ぼす影響)

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Introduction

Heat stress (HS) has recently been implicated in the regulation of whole-body glucose homeostasis, and indications are that the upregulation of heat shock protein (HSP) 72 in skeletal muscle plays an important role in the mechanism leading to metabolic enhancement. Although HS is a potent stimulator of HSP72 mRNA expression, it does not lead to a rapid increase in the amount of HSP72 protein in skeletal muscle. We hypothesized that, prior to an increase in the level of HSP72 protein, HS activates glucose metabolism by rapidly stimulating 5' adenosine monophosphate-activated protein kinase (AMPK), which is a metabolite-sensing kinase that is involved in mechanisms leading to insulin-independent glucose transport in skeletal muscle.

Skeletal muscle is the major site of whole-body glucose disposal, and the process of glucose transport across the cellular membrane is a rate-limiting step of glucose metabolism in skeletal muscle. To test our hypothesis, we investigated the short-term (< 30 min) effect of HS on the levels of HSP72 mRNA and protein, and we examined the change in glucose transport activity together with underlying signaling mechanisms including AMPK.

Materials and Methods

Male Sprague-Dawley rats weighing 150–160 g (aged 5 weeks) were used. Rats were fasted overnight before experiments. The rats were killed by cervical dislocation without anesthesia, and epitrochlearis muscles of each side were gently and rapidly (< 1–2 min) dissected from nearly all the animals. Both ends of each muscle were immediately tied with sutures, and muscles were mounted on an incubation apparatus with the resting tension set to 0.5 g. To recover from *post mortem* AMPK activation induced during dissection, the muscles were preincubated in 7 mL of alpha minimum essential medium supplemented with 0.01% bovine serum albumin, 2.2 g/L NaHCO₃, 5

mmol/L mannitol, 2.54 mol/L CaCl₂, 10% fetal bovine serum, 50 μU/mL insulin, 0.005% Antifoam SI and 1% penicillin/streptomycin for 60 min maintained at 35°C. Muscles were then randomly assigned to the experimental groups. For HS treatment, muscles were then incubated in 7 mL of fresh medium maintained at 42°C for 10 or 30 min. When present, inhibitors were added during preincubation and incubation. Then, the muscles were used either fresh for glucose transport measurement or frozen in liquid nitrogen for subsequent analysis.

Results

We found that HS for 30 min led to a significant increase in the amount of HSP mRNA (Hspa1a and Hspa1b). In contrast, the same HS did not lead to a change in the amount of HSP72 protein. HS for 10 and 30 min significantly increased glucose transport, to a level comparable to that achieved by a maximally effective stimulation of insulin (1 μmol/L, 30 min). HS for 10 and 30 min did not change the amount of glucose transporter (GLUT) 4 mRNA or GLUT4 protein. Wortmannin, a phosphatidylinositol 3-kinase inhibitor, did not inhibit HS-stimulated glucose transport. We also found that basal and HS-stimulated glucose transport was totally inhibited by cytochalasin B, an inhibitory ligand of glucose transporters including GLUT4. HS for 10 and 30 min decreased adenosine triphosphate, phosphocreatine, and glycogen concentrations. Correspondingly, HS for 10 min increased the phosphorylation level of AMPKα Thr¹⁷² and acetyl-CoA carboxylase Ser⁷⁹. Similarly, the α-isoform-specific AMPK activity assay revealed that 10 min HS increased AMPKα1 and AMPKα2 activity. Dorsomorphin (Compound C), an ATP-competitive inhibitor of AMPK, blocked HS (for 10 and 30 min)-stimulated glucose transport completely. On the other hand, HS did not affect the phosphorylation status of insulin receptor signaling (insulin receptor subunit β, Akt) or Ca²⁺/calmodulin-dependent protein kinase II (CaMKII).

Discussion

We found novel findings in relation to the metabolic effects of HS in skeletal muscle. First, HS acutely increased insulin-independent glucose transport prior to an increase in the level of HSP72 protein. Second, blockade of glucose transport by cytochalasin B indicates that glucose transport occurs through translocation of GLUT4. Third, HS reduced the muscle energy status and stimulated AMPK, and correspondingly, HS-stimulated glucose transport was blocked by AMPK inhibitor. Lastly, HS did not activate insulin receptor signaling or CaMKII. Collectively, these results provide evidence that HS acts directly on skeletal muscle and acutely activates glucose

metabolism, at least in part, through AMPK activation.

Energy deprivation is a strong stimulator of AMPK in skeletal muscle. In isolated rat skeletal muscle incubated *in vitro*, both AMPK α 1 and AMPK α 2 are activated by energy-decreasing stimuli, including exercise (contraction), hypoxia, chemical inhibition of oxidative phosphorylation, and hyperosmolarity (Hayashi et al. 2000), all of which lead to potent activation of insulin-independent glucose transport. Although the precise mechanism of reduced energy status by HS is unclear, HS increased mitochondrial enzyme activity in rats (Chen et al. 1999) and oxygen consumption along with fatty acid oxidation in L6 muscle cells (Gupte et al. 2009). Thus, HS may act on energy status through an acceleration in the metabolic rate and an increase in the energy requirements rather than by inducing mitochondrial dysfunction and an inadequate energy supply.

We demonstrated the effects of HS on fast-glycolytic epitrochlearis muscle but did not examine other muscles. Considering that the intracellular energy status is a critical regulator of AMPK activity, a range of muscle types, including slow-oxidative muscles, may respond to HS, although the magnitude of the response may vary. We used an isolated rat skeletal muscle preparation to eliminate potential influence by HS-induced alteration of muscle blood flow and humoral factors. Our preparation made it possible to control the confounding components and to evaluate the direct effect of acute HS on skeletal muscle.

There are some reports that indicate the clinical relevance of HS for enhancing glucose metabolism in humans. For example, Hooper et al. (1999) treated subjects with T2DM by immersing them in a hot water tub up to their shoulders for 30 min, six days a week for three weeks. They found that fasting blood glucose and hemoglobin A1c levels decreased significantly. Subsequent rodent studies also indicated the therapeutic usefulness of HS. Chung et al. (2008) found that a single exposure to HS is sufficient to increase the level of HSP72 protein in mouse skeletal muscle, and weekly treatment of HS for 16 weeks improved high-fat-diet-induced hyperinsulinemia and hyperglycemia in mice. Similarly, Gupte et al. (2009) showed that weekly HS treatment for 12 weeks improved glucose tolerance and insulin sensitivity in rats that were fed a high-fat diet, and this was associated with increased levels of HSP72 protein and enhanced insulin signaling in skeletal muscle. By contrast, our findings strongly suggest that a single exposure to HS can influence glucose metabolism acutely, prior to an increase in the amount of HSP72 protein. To our knowledge, no study has examined changes in muscle glucose metabolism during (or immediately after) exposure to HS in humans. We believe that our results provide a theoretical rationale for conducting future studies to

investigate the acute effects of HS using various heat modalities in humans.

Conclusions

We have demonstrated for the first time that during the acute period after HS before an increase in the protein levels of HSP72, AMPK activity increases rapidly with decreased energy status, and insulin-independent glucose transport in skeletal muscle is activated. We propose that HS is an acute stimulus that promotes glucose metabolism, independently of increased HSP72 protein in skeletal muscle. Although further research is warranted, acute heat treatment in skeletal muscle might be potentially useful modality for the improvement of glucose homeostasis.

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