Heat stress acutely stimulates insulin-independent glucose transport and 5′ adenosine monophosphate-activated protein kinase in rat skeletal muscle

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Introduction: Skeletal muscle is the major tissue responsible for whole-body glucose utilization. Heat stress stimulates the expression of heat shock protein (HSP) that has been implicated in regulating glucose metabolism in skeletal muscle (Kurucz *et al.*, 2002; Gupte *et al.*, 2009). It is notable that the amount of HSP72 protein is increased several hours after heat stress, but little is known about the acute change in glucose metabolism before an increase in HSP72. We have hypothesized that heat stress rapidly activates glucose transport, the rate limiting step of glucose utilization, prior to an increase in HSP72 in skeletal muscle. Thus, the purpose of the present study was to investigate the short-term effect of heat stress on glucose transport and related signaling events using isolated rat skeletal muscle.

Methods: All protocols for animal use and euthanasia were reviewed and approved by the Kyoto University Graduate School of Human and Environmental Studies and Kyoto University Radioisotope Research Center in Japan. Male Sprague-Dawley rats weighting 150 g were killed by cervical dislocation without anesthesia, and epitrochlearis muscle was isolated. Muscle was then incubated in MEM containing 0.01 % bovine serum albumin, $NaHCO_3$ 2.2 g/l, Mannitol 5 mM, $CaCl_2$ 2.54 mM and 10% fetal bovine serum, insulin 50 U/ml (Gupte *et al.*, 2011) and antifoam SI 0.005% in the absence or presence of heat stress (42°C, 10 or 30 min).

Results: Heat stress for 30 min increased both hspa1a and hspa1b mRNA ($P < 0.05$), but not HSP72 protein. However, heat stress for 10 and 30 min robustly increased the rate of 3-O-methylglucose (3MG) transport activity ($P < 0.05$), and the stimulatory effect of 3MG transport was blocked by cytochalasin B. Heat stress for 10 and 30 min also decreased ATP, phosphocreatine, and glycogen concentrations ($P < 0.05$). Correspondingly, heat stress for 10 min increased the phosphorylation level of 5′ adenosine monophosphate-activated protein kinase (AMPK) α Thr172, and both AMPKα1 and AMPKα2 activities ($P < 0.05$). On the other hand, heat stress did not change the phosphorylation level of CaMKII Thr286 or Akt Ser473.

Discussion: We demonstrated that acute heat stress activates glucose transport prior to an increase in HSP72 protein in skeletal muscle. Blockade of glucose transport by cytochalasin B indicated that glucose is taken up into muscle cells via glucose transporter (GLUT) 4. We also demonstrated that acute heat stress decreases the energy status and stimulates the “cellular energy sensor” AMPK, a signaling intermediary leading to insulin-independent GLUT4 translocation in skeletal muscle (Kahn *et al.*, 2005). We propose that heat stress acutely stimulates GLUT4 translocation and glucose transport at least in part by activating AMPK in skeletal muscle.

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