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Transcriptional Regulation of Hexokinase II in Denervated Rat Skeletal Muscle

Jared P. Jones, Brian R. Roberts, Edward B. Tapscott, and G. Lynis Dohm
Department of Biochemistry, East Carolina University School of Medicine, Greenville, North Carolina 27858

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Hexokinase II protein is augmented in denervated skeletal muscle; therefore, we determined if hexokinase II gene transcription rates and mRNA levels are increased with denervation. The right hindlimb skeletal muscles of male rats were denervated while the left hindlimbs were sham operated. Seventy-two h following surgery, rats were sacrificed and the gastrocnemius and soleus muscles were harvested for nuclear and RNA isolation. Nuclear run-on and ribonuclease protection analyses indicated that denervation increased hexokinase II transcription rates and mRNA levels 42% and 88%, respectively ($p<0.05$). Total hexokinase activity rose 23% in denervated gastrocnemius muscle. In conclusion, the increase in hexokinase II gene transcription and mRNA may account for the increase in hexokinase II protein and the subsequent rise in total hexokinase activity in denervated rat skeletal muscle.

The first two steps of glucose utilization in skeletal muscle involve the transport of glucose across the plasma membrane by GLUT4 and GLUT1 and subsequent phosphorylation of glucose by hexokinase. The predominant hexokinase isozyme within skeletal muscle is hexokinase II (6), and its activity is vital to glucose metabolism by maintaining a downhill concentration gradient so that the facilitative transport of glucose across the plasma membrane may occur.

Denervation causes severe insulin resistance in skeletal muscle (11) which is compensated, in part, by an increase in basal glucose transport due to augmented concentrations of GLUT1 protein (5). Hexokinase II protein is likewise increased in denervated skeletal muscle (12) and may play a role in the compensatory mechanism to increase glucose disposal in denervated skeletal muscle. In this study, we determined whether or not the increased expression of hexokinase II protein in denervated skeletal muscle is, in part, transcriptionally mediated.

MATERIALS AND METHODS

Animals. Eighteen male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were housed in a light (12-h light-dark cycle) and temperature (22°C) controlled room and had free access to lab chow and water. Anesthetized rats (pentobarbital sodium, 4.8 mg/100g b.w., ip) had their right hindlimb skeletal muscles denervated via sciatic resection while their left hindlimbs were sham operated. Rats (384 ± 2 g) were sacrificed seventy-two h following surgery, and the gastrocnemius and soleus muscles from three rats were harvested and pooled to form one sample. Nuclear isolation and transcriptional run-on analyses were performed as previously described (10). Approximately 100 mg of mixed gastrocnemius muscle tissue from each hindlimb were quick-frozen to the temperature of liquid nitrogen for RNA isolation using TRIzol reagent (Gibco-BRL) as previously described (10). Ten to twenty μg of total RNA were subjected to ribonuclease protection analysis using Ambion's ribonuclease protection kit (RPA II, Austin, TX) according to manufacturer's instructions. The size of the protected RNA fragment for hexokinase II was 247-bp. The synthesis of the radioactive ([α-^{32}P]UTP, 800 Ci/mmol, Dupont, NEN) antisense RNA (324-bp) was done using Ambion's T7 MAXscript in vitro transcription kit (Austin, TX). Results from nuclear run-on and ribonuclease protection analyses were visualized by phoshorimaging and quantitated using Imagequant software (Molecular Dynamics, Sunnyvale, CA). Total hexokinase activity in mixed gastrocnemius muscles was determined from prepared muscle homogenates (8) using a modified method as previously described (7). Values are represented as means ± S.E. To determine significant differences between treatment means, a Student's *t* test was performed.

RESULTS AND DISCUSSION

Hexokinase II transcription rates (Fig. 1) and mRNA levels (Fig. 2) are increased 42% and 88%, respectively, in denervated rat skeletal muscle ($p<0.05$). Previous research has shown hexokinase II activity to account for most of the total hexokinase activity in skeletal muscle (6). Furthermore, Weber et al. (12) demonstrated that increases in skeletal muscle hexokinase II protein as a result of electrical stimulation or denervation correlated with increases in total hexokinase activity. In the present study, total hexokinase activity was increased 23% (Fig. 3) in denervated gastrocnemius muscles ($p<0.05$).

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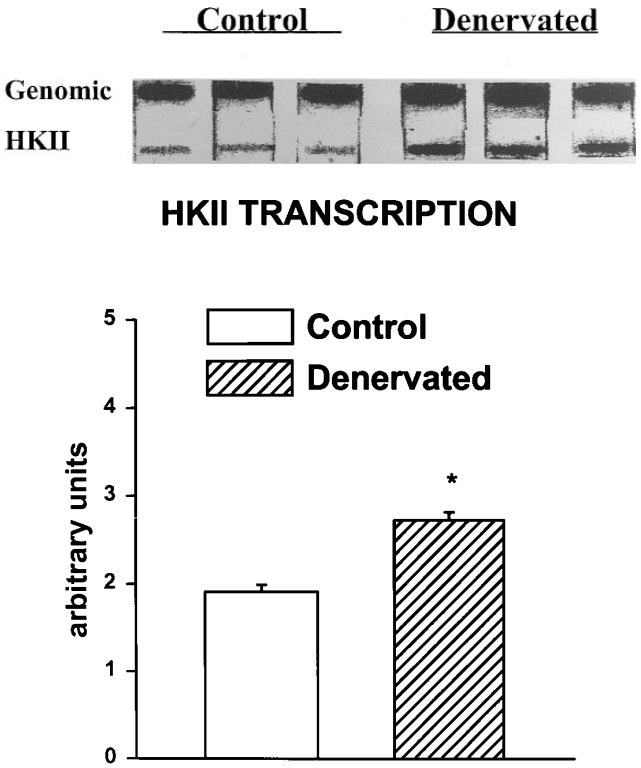

FIG. 1. Transcriptional run-on analysis and determination of the relative levels of hexokinase II transcription from soleus and gastrocnemius muscles of sham operated (control) and denervated rat hindlimbs. A phosphorimage is presented showing hybridization of the *in vitro* transcribed RNA to the hexokinase II cDNA (2 μg) and to rat skeletal muscle genomic DNA (0.1 μg). Hybridized transcripts were quantitated by Imagequant software (Molecular Dynamics, Sunnyvale, CA). A summary of values for hexokinase II transcription (n = 6/group) is presented as means ± S.E. *Significantly ($P < 0.05$) different from control.

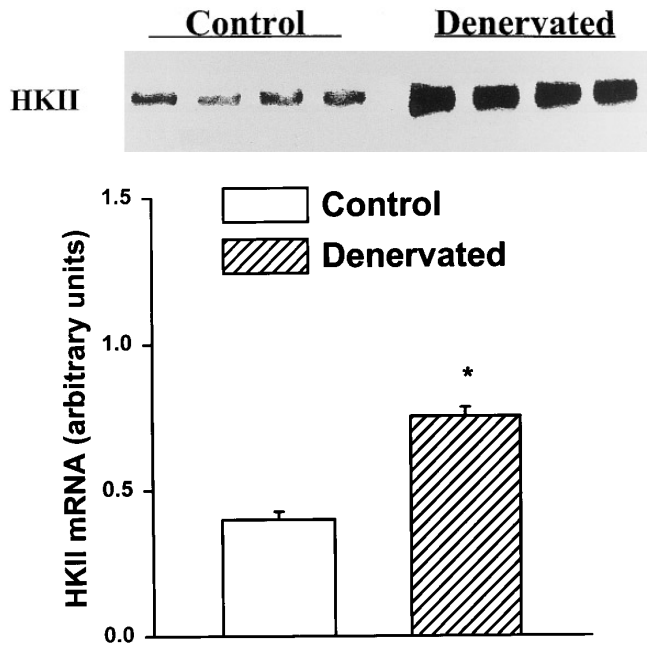

FIG. 2. Ribonuclease protection analysis of RNA isolated from gastrocnemius muscle of sham operated (control) and denervated rat hindlimbs. A phosphorimage is presented of a ribonuclease protection assay for hexokinase II mRNA followed by a summary of hexokinase mRNA values (n = 6/group) presented as means ± S.E. *Significantly ($P<0.05$) different from control.

Glucose uptake involves the coupling of transport with phosphorylation; therefore, hexokinase II activity is important to glycemic control. Recent evidence suggested that decreased hexokinase activity in noninsulin-dependent diabetes mellitus patients may contribute to their poor glycemic control (2). The overexpression of hexokinase II in skeletal muscle of transgenic mice has been shown to improve basal and insulin-stimulated glucose uptake (4).

The transcriptional mechanisms evoked by denervation are not clearly understood but may involve cAMP. The 5′-flanking region of the rat hexokinase II gene contains a cAMP response element (9), and muscle cAMP concentrations increase in denervated skeletal muscle (3). However, denervation also causes nerve damage, cessation of neurogenic factors, and the elimination of electrical activity of the muscle; all of which may affect the regulation hexokinase II transcription.

In summary, hexokinase II gene transcription is increased with a concomitant rise in hexokinase II mRNA levels and total hexokinase activity in denervated rat skeletal muscle. We hypothesize that the increased expression of hexokinase II protein in denervated rat skeletal muscle is transcriptionally mediated. Furthermore, the increased concentrations of hexokinase II protein elevate the total hexokinase activity in denervated skeletal muscle.

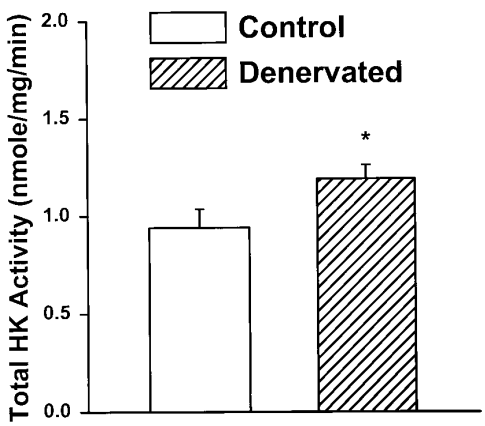

FIG. 3. A summary of total hexokinase activity from mixed gastrocnemius muscles of sham operated (control) and denervated rat hindlimbs (n = 6/group) is presented as means ± S.E. *Significantly ($P<0.05$) different from control.

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