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BRAIN RESEARCH

Research Report

Expression of hexokinase isoforms in the dorsal root ganglion of the adult rat and effect of experimental diabetes

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ABSTRACT

The effect of streptozotocin (STZ)-induced diabetes on expression and activity of hexokinase, the first enzyme and rate-limiting step in glycolysis, was studied in sensory neurons of lumbar dorsal root ganglia (DRG). The DRG and sciatic nerve of adult rats expressed the hexokinase I isoform only. Immunofluorescent staining of lumbar DRG demonstrated that small–medium neurons and satellite cells exhibited high levels of expression of hexokinase I. Large, mainly proprioceptive neurons, had very low or negative staining for hexokinase I. Intracellular localization and biochemical studies on intact DRG from adult rats and cultured adult rat sensory neurons revealed that hexokinase I was almost exclusively found in the mitochondrial compartment. Duration of STZ-diabetes of 6 or 12 weeks diminished hexokinase activity by 28% and 30%, respectively, in lumbar DRG compared with age matched controls ($P<0.05$). Quantitative Western blotting showed no effect of diabetes on hexokinase I protein expression in homogenates or mitochondrial preparations from DRG. Immunofluorescent staining for hexokinase I showed no diabetes-dependent change in small–medium neuron expression in DRG, however, large neurons became positive for hexokinase I ($P<0.05$). Such complex effects of diabetes on hexokinase I expression in the DRG may be due to glucose-driven up-regulation of expression or the result of impaired axonal transport and perikaryal accumulation in the large neuron sub-population. Because hexokinase is the rate-limiting enzyme of glycolysis these results imply that metabolic flux through the glycolytic pathway is reduced in diabetes. This finding, therefore, questions the role of high glucose-induced metabolic flux as a key driving force in reactive oxygen species generation by mitochondria.

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Abbreviations: AGE, advanced glycation end product; CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglia; ERK, extracellular regulated kinase; G-6-P, glucose 6-phosphate; GDNF, glial cell line-derived neurotrophic factor; HSP60, heat shock protein 60; 4-HNE, 4-hydroxy-2-nonenal; MNCV, motor nerve conduction velocity; NGF, nerve growth factor; NT-3, neurotrophin-3; ROS, reactive oxygen species; SCb, slow transport component b; STZ, streptozotocin; TCA, tricarboxylic acid; VDAC, voltage-dependent anion channel

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1. Introduction

Diabetic sensory neuropathy in humans is associated with a spectrum of structural changes in peripheral nerve that includes axonal degeneration, microangiopathy, paranodal demyelination and loss of myelinated and unmyelinated fibers — the latter probably the result of a dying-back of distal axons (Malik et al., 2005; Yagihashi, 1997). In the streptozotocin (STZ)-diabetic rat and Bio-Breeding (BB) rat animal models of type I diabetes, similar structural abnormalities in peripheral nerve have been observed (Mizisin et al., 1999; Sima and Sugimoto, 1999; Yagihashi, 1997). The Diabetes Control and Complications Trial (DCCT) concluded that control of hyperglycemia is still the ideal means of preventing appearance of complications in diabetes, such as peripheral neuropathy (DCCT, 1990). However, such a goal remains an unrealized ideal and research continues to focus on biochemical transducers downstream from hyperglycemia that may directly induce neuropathic sensory nerve damage. Brownlee and colleagues have proposed that high cellular concentrations of glucose lead to mitochondrial dysfunction and the generation of damaging levels of reactive oxygen species (ROS) which then mediate cell degeneration (Nishikawa et al., 2000b). One component of such a theory presupposes that high glucose concentrations are converted by glycolysis into high levels of pyruvate that then enter the tricarboxylic acid cycle (TCA) within the mitochondrial matrix and provide high levels of reduced electron donors for electron transport. The inability of mitochondrial oxidative phosphorylation to efficiently deal with this high level of electron donation may lead to excessive superoxide production and oxidative stress (Nishikawa et al., 2000a,b).

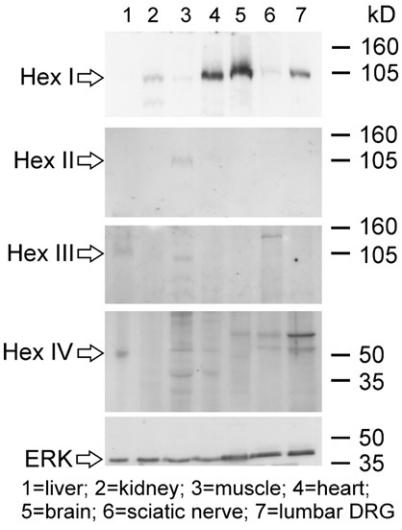

Fig. 1 – Brain, lumbar DRG and sciatic nerve express hexokinase I. Tissues from adult rats were subjected to Western blotting and probed for all the isoforms of hexokinase (I–IV). The following tissues were assessed: 1. liver, 2. kidney, 3. muscle (soleus), 4. heart (ventricle), 5. brain (cortex), 6. sciatic nerve and 7. lumbar (L4/5) DRG. Hexokinase I, a 100 kDa band, was the main isoform detected in brain and DRG, with weak expression in sciatic nerve. There was also weak expression of hexokinase IV (glucokinase) in DRG. ERK was also probed to confirm the level of protein loading.

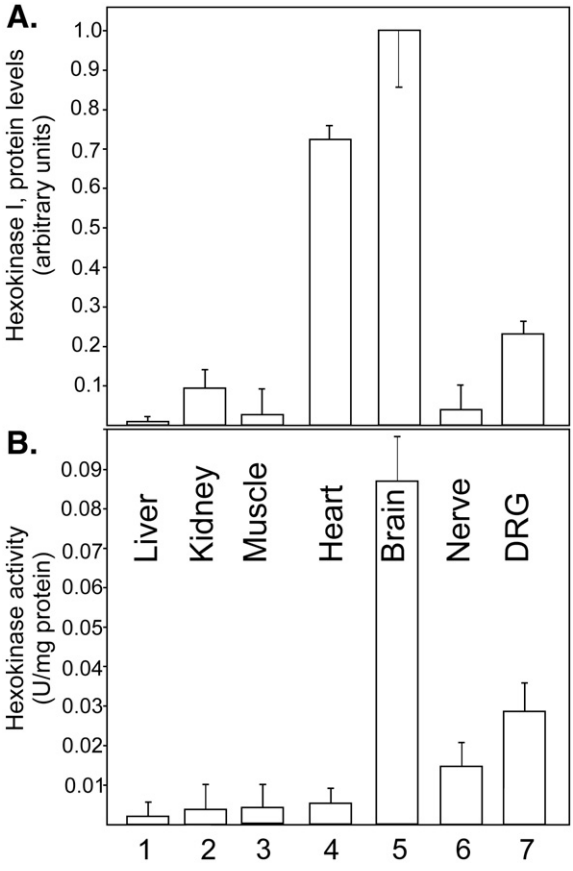

Fig. 2 – Expression and activity levels of hexokinase in peripheral and nervous tissues. Protein expression levels of hexokinase I in a range of adult tissues are presented in panel A. samples are: 1. liver, 2. kidney, 3. muscle (soleus), 4. heart (ventricle), 5. brain (cortex), 6. sciatic nerve and 7. lumbar (L4/5) DRG. Presented as arbitrary units relative to total ERK levels. In panel B are shown the total hexokinase activity levels (U/mg total protein). All values are means ± SEM (n = 3 replicate determinations).

Hexokinases serve as the gateway through which glucose enters glycolysis, by catalyzing the phosphorylation of glucose to yield glucose 6-phosphate (G-6-P), the initial and rate-limiting step of the glycolytic pathway (Wilson, 1995, 2003). In mammals, four distinct hexokinase isozymes exist, designated types I, II, III and IV, with the latter commonly known as glucokinase (Wilson, 2003). The hexokinase I–III isozymes are 100 kDa molecules that display internal sequence repetition, and the N- and C-terminal halves have extensive sequence similarity to each other and to other members of the hexokinase family, in contrast, glucokinase has a molecular weight of approximately 50 kDa (Wilson, 1995). Based upon the kinetics of hexokinase isoform activities it is believed that hexokinase I functions primarily in a catabolic role, introducing glucose into glycolytic metabolism with the primary purpose of generating ATP. This is consistent with the ubiquitous expression of hexokinase I in tissues. In addition, hexokinase I is expressed at particularly high levels in the brain, which is