Overexpression of myosin-IIB in the brain of a rat model of streptozotocin-induced diabetes

Luciana Karen Calábria a, Gabriel Costa Nunes da Cruz b, Rafael Nascimento a, Washington João Carvalho a, Neire Moura de Gouveia d, Fernanda Vieira Alves a, Fabiana Barcelos Furtado a, Hellen Cristina Ishikawa-Ankerhold c, Marcelo Valle de Sousa b, Luiz Ricardo Goulart a,1, Fousen Salmen Espindola a,⁎

a Institute of Genetics and Biochemistry, Federal University of Uberlândia, Campus Umuarama, 38400-902, Uberlândia-MG, Brazil
b Brazilian Center for Protein Research, Department of Cell Biology, University of Brasília, Campus Darcy Ribeiro, 70910-900, Brasília-DF, Brazil
c Institute for Cell Biology, Ludwig Maximilians University Munich, Schillerstr. 42, 80336, Munich, Germany

ARTICLE INFO
Article history:
Received 21 October 2010
Received in revised form 13 January 2011
Accepted 13 January 2011

Keywords:
Diabetes mellitus
Hyperglycemia
Brain
Calcium
Calmodulin
Myosin-IIB

ABSTRACT
The Ca2+/calmodulin complex interacts with and regulates various enzymes and target proteins known as calmodulin-binding proteins (CaMBPs). This group of proteins includes molecular motors such as myosins. In this study, we show that non-muscle myosin-IIB is overexpressed in the brains of diabetic rats. We isolated CaMBPs from the brains of non-diabetic rats and rats with streptozotocin-induced diabetes and purified them by immobilized-calmodulin affinity chromatography. The proteins were eluted with EGTA and urea, separated by SDS-PAGE, digested and submitted to peptide mass fingerprinting analysis. Thirteen intense bands were found in both types of brains, two were found exclusively in non-diabetic brains and four were found exclusively in diabetic brains. A large fraction of the eluted proteins contained putative IQ motifs or calmodulin-binding sites. The results of the myosin-IIB affinity chromatography elution, western blot and RT-PCR analyses suggest that myosin-IIB protein and mRNA are expressed at high levels in diabetic brains. This is the first study that shows increased expression of CaMBPs in diabetic and non-diabetic brain tissue through a comparative proteomic analysis, and it opens up a new approach to studying the relationship between the expression of myosins in the brain, hyperglycemia and intracellular calcium regulation.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction
Diabetes mellitus is a common metabolic disorder characterized by hyperglycemia that affects the central nervous system, causing alterations in neurotransmission, electrophysiological abnormalities, structural changes and moderate disturbances in learning and memory [1–5]. Hyperglycemia causes an acute rise in cytosolic calcium concentrations due to increased calcium influx into cells. In certain cells, hyperglycemia causes the release of intracellular calcium stores as well. Hyperglycemia has also been associated with decreased calcium efflux [6]. The combination of increased calcium influx and decreased calcium efflux leads to a sustained elevation of the basal level of cytosolic calcium that may adversely affect cell functions, including Ca2+/binding proteins such as calmodulin and its target proteins, which are capable of decoding very small changes in the intracellular concentration of calcium [7].

Calmodulin is an ubiquitous, highly conserved acidic Ca2+/binding protein [8]. Binding of Ca2+ releases Mg2+ from calmodulin and causes the protein to undergo a conformational change that increases its binding affinity for a number of target proteins. Because Ca2+ binds to calmodulin, a small change in the level of cytosolic calcium leads to a large change in the level of active proteins such as Ca2+/calmodulin-binding proteins [9,10].

Calmodulin-binding proteins can be classified into three categories based on their calcium ion dependence: Ca2+-dependent, Ca2+-independent and Ca2+-inhibited [8,9]. Calmodulin binds to the target proteins via an IQ motif in a Ca2+-independent manner [11], as has been observed for the light chains of different myosin classes [12].

Myosins constitute a large family of actin-based motor proteins [13] that contains more than 18 classes and multiple members. Myosin classes I, II, V, VI, and IX are clearly present in vertebrate neurons. Isoforms of myosin-II are expressed in most non-muscle cells and exhibit differences in their biological properties, tissue distribution and intracellular localization, indicating that each isoform might