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Hepatocyte nuclear factors 1α/4α and forkhead box A2 regulate the solute carrier 2A2 (*Slc2a2*) gene expression in the liver and kidney of diabetic rats

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ABSTRACT

Aims: Solute carrier 2a2 (*Slc2a2*) gene codifies the glucose transporter GLUT2, a key protein for glucose flux in hepatocytes and renal epithelial cells of proximal tubule. In diabetes mellitus, hepatic and tubular glucose output has been related to *Slc2a2*/GLUT2 overexpression; and controlling the expression of this gene may be an important adjuvant way to improve glycemic homeostasis. Thus, the present study investigated transcriptional mechanisms involved in the diabetes-induced overexpression of the *Slc2a2* gene.
Main methods: Hepatocyte nuclear factors 1α and 4α (HNF-1α and HNF-4α), forkhead box A2 (FOXA2), sterol regulatory element binding protein-1c (SREBP-1c) and the CCAAT-enhancer-binding protein (C/EBPβ) mRNA expression (RT-PCR) and binding activity into the *Slc2a2* promoter (electrophoretic mobility assay) were analyzed in the liver and kidney of diabetic and 6-day insulin-treated diabetic rats.
Key findings: Slc2a2/GLUT2 expression increased by more than 50% ($P<0.001$) in the liver and kidney of diabetic rats, and 6-day insulin treatment restores these values to those observed in non-diabetic animals. Similarly, the mRNA expression and the binding activity of HNF-1α, HNF-4α and FOXA2 increased by 50 to 100% ($P<0.05$ to $P<0.001$), also returning to values of non-diabetic rats after insulin treatment. Neither the *Srebf1* and *Cebpb* mRNA expression, nor the SREBP-1c and C/EBP-β binding activity was altered in diabetic rats.
Significance: HNF-1α, HNF-4α and FOXA2 transcriptional factors are involved in diabetes-induced overexpression of *Slc2a2* gene in the liver and kidney. These data point out that these transcriptional factors are important targets to control GLUT2 expression in these tissues, which can contribute to glycemic homeostasis in diabetes.

Introduction

Solute carrier 2a2 (*Slc2a2*) gene codifies the protein GLUT2, a high capacity and low affinity glucose transporter, expressed in cells with high glucose fluxes (Thorens, 1996). In hepatocytes, glucose influx during the absorptive period and glucose efflux in the post absorptive period are carried out by GLUT2. In the kidney, GLUT2, located in basolateral membrane of epithelial cells of the proximal tubule, operates glucose efflux to the interstitium (Thorens, 1996), participating in renal glucose reabsorption. Additionally, GLUT2 in epithelial renal cells provides the efflux of glucose generated by gluconeogenesis, which contributes to maintaining glycemia in the fasting state (Gerich et al., 2001). Furthermore, in diabetic rats, GLUT2 may translocate to the brush border membrane of proximal tubular cells, contributing to increasing glucose reabsorption (Marks et al., 2003).

In the Fanconi–Bickel syndrome, characterized by a mutation of the *SLC2A2* gene, GLUT2-mediated glucose transport is reduced, increasing intracellular glucose and favoring glycogen accumulation in the liver and kidney (Santer et al., 1998), which points out to the role of this transporter in hepatic and renal glucose fluxes. In diabetes mellitus (DM), the *Slc2a2* expression is increased in the liver (Yamamoto et al., 1991) and kidney (Vestri et al., 2001), resulting in increased GLUT2 protein, which allows increased glucose fluxes in these tissues. Besides, the *Slc2a2* overexpression in the kidney is an important pathophysiological feature of DM, playing an important role in diabetic nephropathy (Kim and Ahn, 1998; Schaan et al., 2005; Freitas et al., 2005, 2007).

Hepatocyte nuclear factors (HNFs), transcriptional factors originally described in the liver, have been described in other tissues, where they have been linked to the control of several genes related to metabolism (Hayashi et al., 1999). HNF-1α, HNF-4α and FOXA2 (former HNF-3β)

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Abbreviations

Genes and respective proteins were abbreviated in accordance with the HUGO Gene Nomenclature Committee (HGNC) mouse genome informatics (MGI) and rat genome nomenclature committee (RGNC) as follows

Slc2a2	solute carrier 2a2 gene
GLUT2	glucose transporter 2 protein
Hnf1a	hepatocyte nuclear factor 1 homeobox A gene
HNF-1α	hepatocyte nuclear factor-1α protein
Foxa2	forkhead box A2 gene
FOXA2	forkhead box A2 protein
Hnf4a	hepatocyte nuclear factor 4a gene
HNF-4α	hepatocyte nuclear factor-4α protein
Srebf1	sterol regulatory element binding transcription factor 1 gene
SREBP-1c	sterol regulatory element binding protein-1c
Cebpb	CCAAT/enhancer binding protein b gene
C/EBP-β	CCAAT/enhancer binding protein-β
Actb	beta-actin gene
ACT-β	beta-actin protein

are related to the control of the genes of enzymes (PEPCK and G6Pase), hormones (glucagon and insulin), and glucose transporters (*Slc2a2*, *Slc5a2* and *Slc5a1*) (Duncan et al., 1994; Cha et al., 2000, 2001; Parrizas, 2002; Gautier-Stein et al., 2006; Freitas et al., 2008). Interestingly, some specific types of DM, known as maturity-onset diabetes of the young (MODY), are characterized by mutations in HNF-1α (MODY 3), HNF-1β (MODY 5) and HNF-4α (MODY 1) (Fajans et al., 2001), with variable impairment of glucose metabolism in pancreatic B cells, the liver and kidney.

The sterol regulatory element binding proteins (SREBP) are key regulators of nutritional homeostasis, and the SRBP-1c was reported to enhance the *Slc2a2* gene expression (Fleischmann and Iynedjian, 2000). Besides, from the family of CCAAT-enhancer-binding proteins (C/EBPs) transcriptional factors, also related to the control of metabolic genes (Croniger et al., 1998), the C/EBPβ has been described as an enhancer of the *Slc2a2* expression in hepatocytes (Kim and Ahn, 1998).

Since the activity of the described transcriptional factors can be modulated according to distinct metabolic conditions (Croniger et al., 1998; Cha et al., 2000; Osborne, 2000; Cha et al., 2001; Parrizas, 2002), diabetes is a disease which might interfere with the expression and/or activity of these transcriptional factors; therefore, altering the *Slc2a2* expression. We hypothesized that HNF-1α, HNF-4α, FOXA2, SREBP-1c and C/EBPβ transcriptional factors play a role in the expression of the *Slc2a2* gene in the liver and kidney of diabetic rats, which was not previously shown. To test this hypothesis in the liver and kidney of diabetic rats treated or not with insulin, we investigated both the mRNA expression of these transcriptional factors and their binding activity into their specific binding sites of the *Slc2a2* gene promoter.

Materials and methods

Experimental animals

Male Wistar rats (weighing ~260 g) were individually caged in an environment maintained at controlled temperature (23 ± 2 °C) and lighting (from 6:00 AM to 6:00 PM), and allowed free access to water and standard rodent chow diet (Nuvilab CR-1, Nuvital, Curitiba, Brazil). One week later, the animals were fasted overnight and rendered diabetic (D) by a single intravenous injection (penis vein) of alloxan (38 mg/kg body weight). Non-diabetic control (ND) rats were injected with saline in the same volume. Animals with polyuria (>25 mL/day) and glycosuria, without ketonuria were maintained in the protocol as D. Diabetes-induced effects were firstly evaluated in 20-day diabetic rats (20-day D), comparing to ND rats. Besides, 20-day D rats were treated with placebo or insulin for up to 6 days, as described below. Treatments were subcutaneous NPH insulin (Biohulin N, Biobras, Belo Horizonte, BR), 2 U in the morning (8:30 AM) and 4 U in the afternoon (5:30 PM), or saline, as placebo, at the same volume and frequency. Evaluations were performed after 1, 4 and 6 days of treatment, and placebo-treated groups were referred to as DP1, DP4 and DP6, and insulin-treated groups as I1, I4 and I6. After confirmation that results from DP1, DP4 and DP6 animals did not differ, data were pooled in one single DP group. Twenty-four-hour urine was collected in the last 24 h before the end of the experiment. Animals were killed between 9:00 and 10:00 AM. For diabetes induction and final experimental procedures, animals were anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). Experimental procedures were approved by the Ethical Committee for Animal Research of the Institute of Biomedical Sciences, of the University of São Paulo (protocol #075/08).

Protein and RNA extraction

Renal out-cortices and liver samples were harvested and immediately frozen in liquid nitrogen for further procedures as previously described (Tomie Furuya et al., 2005; Freitas et al., 2007). Plasma membrane protein enriched fraction was obtained by differential centrifugation (Tomie Furuya et al., 2005; Freitas et al., 2007), and the protein concentration of the samples was determined by the Bradford method (Bio-Rad protein assay, Bio-Rad, USA). Total RNA was extracted using TRIZOL reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) as described by manufacturer instructions.

Western blotting analysis of GLUT2

Total proteins were resolved by electrophoresis and transferred to nitrocellulose membrane for further immunodetection, using an antiserum raised against the COOH-terminus of rat GLUT2 (Vestri et al., 2001; Freitas et al., 2005; Tomie Furuya et al., 2005). The blots were quantified by densitometry, and normalized by the densitometric value of the respective lane in the post-transferring Commassie-stained gel (Ferguson et al., 2005). Results were finally normalized setting the mean of control (non-diabetic) values in each gel as 100.

RT-PCR analysis for Slc2a2, Hnf1a, Foxa2, Hnf4a, Srebf1 and Cebpb mRNAs

Reverse transcription was done with 2 μg of total RNA using ImProm-II® reverse transcriptase and random primers, according to manufacturer's instructions (Promega, Madison, WI, USA). RT-PCR assays were done in quadruplicate using recombinant GoTaq® Green Master Mix (Promega, Madison, WI, USA) and 10 pmol of each primer in a master mix of 50 μL. The cycle numbers for each gene were defined after titration using 18 and 40 cycles and were within the logarithmic phase of amplification. The number of cycles and annealing temperature for each gene were shown in Table 1. Additionally, the primers used for each gene and the size of the amplified fragment were also informed in Table 1. PCR products were resolved on 1.2% EtBr-agarose gels and the band intensities were determined by digital scanning followed by quantification using Scion Image analysis software (Scion Corp., Frederick, MD). Results were normalized, after normalization by the respective *Actb* values, setting the mean of non-diabetic rats as 100.

Electrophoretic mobility shift assay (EMSA)

Nuclear protein extraction from renal cortex and liver samples was performed as previously described (Andrews and Faller, 1991). A double-stranded oligonucleotides corresponding to a sequence of rat *Slc2a2* promoter that contains the binding sites of HNF-1α