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Hyperglycemia is associated with enhanced gluconeogenesis in a rat model of permanent cerebral ischemia

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ABSTRACT

Hyperglycemia is common after acute stroke. In the acute phase of stroke (within 24 h), rats with permanent cerebral ischemia developed higher fasting blood glucose and insulin levels in association with up-regulation of hepatic gluconeogenic gene expression, including phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, and fructose-1,6-bisphosphatase. In addition, hepatic gluconeogenesis-associated positive regulators, such as FoxO1, CAATT/enhancer-binding proteins (C/EBPs), and cAMP responsive element-binding protein (CREB), were up-regulated. For insulin signaling transduction, phosphorylation of insulin receptor (IR), insulin receptor substrate-1 (IRS1) at the tyrosine residue, Akt, and AMP-activated protein kinase (AMPK), were attenuated in the liver, while negative regulators of insulin action, including phosphorylation of p38, c-Jun N-terminal kinase (JNK), and insulin receptor substrate-1 (IRS1) at the serine residue, were increased. In addition, the brains of rats with stroke exhibited a reduction in phosphorylation of IRS1 at the tyrosine residue and Akt. Circulating cortisol, glucagon, C-reactive protein (CRP), monocyte chemoattractant protein 1 (MCP-1), and resistin levels were elevated, but adiponectin was reduced. Our data suggest that cerebral ischemic insults might modify intracellular and extracellular environments, favoring hepatic gluconeogenesis and the consequences of hyperglycemia.

1. Introduction

Hyperglycemia during acute illness has been associated with increased mortality (Dungan et al., 2009). In contrast, glucose control improves clinical outcomes, particularly in hospitalized patients with acute myocardial infarctions, patients undergoing coronary bypass surgery, and intensive care unit patients requiring ventilator support (Furnary et al., 2003; Malmberg et al., 2005; van den Berghe et al., 2006). A high proportion of patients with acute stroke may develop hyperglycemia, even in the absence of a pre-existing diagnosis of diabetes (Capes et al., 2001; Kent et al., 2001; McCormick et al., 2008). Both human and animal studies suggest that stress-induced hyperglycemia after acute stroke is not a benign occurrence, and can be associated with a high risk of mortality (Capes et al., 2001; Kent et al., 2001; McCormick et al., 2008). These studies also highlight the importance of stress hyperglycemia as a pathologic factor in stroke progression, and imply that lowering blood glucose levels after ischemic stroke may improve outcome. However, the evolution of post-stroke hyperglycemia is unknown. In view of the importance of blood glucose on the prognosis of acute stroke, there is a need for further elucidation of post-stroke hyperglycemia.

The underlying mechanisms of stroke-associated hyperglycemia appear to be multifactorial, and are currently not well understood. In our previous study, we reported that cerebral ischemia in rats induce insulin resistance in association with increased production of adipose tissue-derive pro-inflammatory cytokines (Wang et al., 2011). Further, in other experiments, post-stroke glucose intolerance was shown to be accompanied by decreased insulin receptor (IR) phosphorylation, and increased phosphoenolpyruvate carboxykinase and glucose-6-phosphatase mRNA expression in the liver (Harada et al., 2009, 2011). It is known that hepatic gluconeogenesis is controlled by insulin, counter-regulatory hormones, and

Abbreviations: AMPK, AMP-activated protein kinase; CRP, C-reactive protein; CREB, cAMP responsive element-binding protein; C/EBP, CAATT/enhancer-binding protein; IR, insulin receptor; IRS1, insulin receptor substrate-1; JNK, c-Jun N-terminal kinase; MCP-1, monocyte chemoattractant protein 1.

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pro-inflammatory cytokines through complex intracellular signaling pathways (He et al., 2009; Pandey et al., 2009; Chen et al., 2012; Jitrapakdee, 2012). In the presence of chronic inflammation and insulin resistance, as occurs in obesity, increased hepatic gluconeogenesis develops and contributes to obesity-related diabetes (Grimble, 2002; Hotamisligil, 2006). Accordingly, we hypothesized that in acute cerebral ischemia, insulin resistance and adipose inflammation might up-regulate hepatic gluconeogenesis and become an important determinant of post-stroke hyperglycemia. To extend the scope of previous research, this study aimed to determine whether or not alterations in blood glucose levels in a rat model of cerebral ischemia is associated with hepatic gluconeogenesis, and so, which upstream regulatory molecules are involved. In addition, ischemic injury has been shown to result in a disturbance in the insulin signaling pathway in neuron cells in *in vitro* studies (Sun et al., 2010), and impaired insulin action in the brain can lead to a disorder in peripheral glucose metabolism and insulin resistance (Brüning et al., 2000; Obici et al., 2002). Therefore, the present study also examined the changes in insulin signaling pathways, if any, in the brain after acute cerebral ischemia.

2. Materials and methods

2.1. Animals and induction of cerebral ischemia

The Animal Experimental Committee of Taichung Veterans General Hospital approved the protocol of this animal study. Adult male Sprague-Dawley rats (300–350 g) were anesthetized with chloral hydrate (400 mg/kg ip). The body temperature of each rat was maintained at 37.0 ± 0.5 °C with a heating pad. Focal ischemic infarcts in the right lateral cerebral cortex were produced by clamping the two common carotid arteries and the right middle cerebral artery, as described previously (Wang et al., 2011). In animals undergoing sham operations, all surgical procedures were the same as above, but no arterial occlusion was performed.

2.2. Experimental design of the study

All rats were randomly allocated into two study groups. In the first group, animals were divided into sham-operated and ischemia subgroups (n = 8/subgroup). In the second group, animals were divided into four subgroups: sham with normal saline; sham with insulin; ischemia with normal saline; and ischemia with insulin (n = 8/subgroup), in which intraperitoneal insulin (10 U/kg) or normal saline injection was administrated 15 min prior to sacrifice.

2.3. Blood and tissue sample collection

All animals were sacrificed at day 1 after ischemic surgery and food was withdrawn for 12 h before obtaining blood and tissue samples. After anesthesia with chloral hydrate (400 mg/kg ip), blood was collected through intra-arterial catheterization of the left femoral artery. In addition, the liver and brain were rapidly dissected and stored in liquid nitrogen until analysis. The blood levels of cortisol, glucagon, resistin, C-reactive protein (CRP; all from R&D Systems, Minneapolis, MN, USA), monocyte chemoattractant protein 1 (MCP-1; PeproTech, Rocky Hill, NJ, USA), adiponectin (Chemicon, Temecula, CA, USA), and insulin (Mercodia, Winston-Salem, NC, USA) were measured by ELISA, following the instructions provided by the manufacturers.

2.4. Blood glucose measurement

To determine the effect of cerebral ischemia on blood glucose, another set of animals were fasted overnight before ischemic and sham surgery (n = 6/subgroup). After surgery, blood glucose was monitored in the tail veins hourly for 24 h using a hand-held Accucheck glucometer (Roche Diagnostics, Indianapolis, IN, USA).

2.5. Quantification of cerebral infarction

The brains were quickly removed and chilled in cold phosphate-buffered saline (PBS) for 5 min, and 2-mm coronal slices were cut using a tissue slicer. The slices were immersed in a PBS solution containing 2% triphenyltetrazolium chloride (TTC) at 37 °C for 30 min, after which sections were fixed in 10% phosphate-buffered formalin for 45 min.

2.6. RNA isolation and quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR)

Total cellular RNAs were extracted from the liver tissues using a TriZol RNA isolation reagent (Invitrogen, Carlsbad, CA, USA) and subjected to complementary DNA synthesis. Quantitative real-time PCR was performed on ABI StepOne™ (Applied Biosystems, Foster City, CA, USA). Relative gene expression was determined by the ΔΔCT method. Primers used for amplifications were as follows: phosphoenolpyruvate carboxykinase,5′-CAGGAAGTGAGGAAGTTT GTGG and 5′-ATGACACCCTCCTCCTGCAT; glucose-6-phosphatase, 5′-CCATCTGGTTCCACATTCAAGAG and 5′-CAGTGTCCAGGACCCACC AATAC; fructose-1,6-bisphosphatase,5′-CCATCATAATAGAGCCCGA GAAGA and 5′-CTTTCTCCGAAGCCTCATTAGC; and β-actin,5′-AAGTC CCTCACCCTCCCAAAAG and 5′-AAGCAATGCTGTCACCTTCCC.

2.7. Western blot

Total proteins were extracted from the cerebral cortical and liver tissues (100 mg) using tissue protein extraction reagents (T-PER, Pierce Biotechnology, Rockford, IL). Protein extracts were resolved by SDS–polyacrylamide gel electrophoresis, and transferred onto a blotting membrane. The membranes were incubated with antibodies against IR, phosphorylated IR (Tyr-1150/1151), insulin receptor substrate-1 (IRS1), Akt, phosphorylated Akt (Ser-473), AMP-activated protein kinase (AMPK), p38, CAATT/enhancer-binding protein-α (C/EBP-α; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), FoxO1, phosphorylated IRS1 (Ser-307), phosphorylated AMPK (Thr-172; Cell Signaling, Beverly, MA, USA), c-Jun N-terminal kinase (JNK; R&D Systems, Minneapolis, MN, USA), phosphorylated p38 (Thr-180/Tyr-182), phosphorylated JNK (Thr-183/Tyr-185; BD Biosciences, San Diego, CA, USA), phosphorylated IRS1 (Tyr-896), cAMP responsive element-binding protein (CREB), phosphorylated CREB (Ser-133), C/EBP-β (Epitomics, Burlingame, CA, USA), and phosphorylated C/EBP-β (Thr-188/235; Abcam, Cambridge, MA, USA). Then, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody. Specific protein bands were visualized by enhanced chemiluminescence and were quantified by densitometry. β-tubulin (Sigma–Aldrich, St. Louis, MO, USA) was used as protein loading control.

2.8. Statistical analysis

Data are expressed as the mean ± standard deviation. For comparison between two groups, the statistical significance was determined by Student's t-test. Comparisons among several groups were compared statistically by ANOVA, followed by the Bonferroni multiple comparison test. A $p < 0.05$ was considered statistically significant.