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Chromium modulates expressions of neuronal plasticity markers and glial fibrillary acidic proteins in hypoglycemia-induced brain injury

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ABSTRACT

Aims: This experiment investigated if chromium (Cr) as Cr-histidinate (CrHis) and Cr29 picolinate (CrPic) have a protective role in rats with hypoglycemia-induced brain injury, assessed by neuronal plasticity and regeneration potential.

Main methods: Male Sprague–Dawley rats were prospectively divided into 2 groups: control and hypoglycemic (induced by insulin administration, 15 U/kg, i.p., n = 56). Hypoglycemic rats were then received randomly 1) none, 2) dextrose (on the day of sampling), 3) CrHis, or 4) CrPic. Cr-chelates were delivered via drinking water (providing 8 μg elemental Cr per day) for one week prior to the hypoglycemia induction. The expressions of neuroplasticity markers [neural cell adhesion molecule (NCAM), growth-associated protein-43 (GAP-43), glial fibrillary acidic protein (GFAP)], glucose transporters (GLUT), and nuclear transcription proteins [nuclear factor-kappa (NF-κB), nuclear factor (erythroid-derived 2)-like 2 (Nrf2), and 4-hydroxyl nonenal (HNE)] were determined using Western blot.

Key findings: Hypoglycemia caused increases in the expressions of GLUT-1, GLUT-3, GFAP, NF-κB and HNE and decreases in the expression of NCAM's, GAP-43 and Nrf2 in the hippocampus, cerebellum, and cortex. Cr-chelates suppressed expressions of GLUTs, GFAP, NF-κB and HNE expressions and enhanced expressions of NCAM, GAP-43 and Nrf2, which were more notable for CrHis than for CrPic.

Significance: In conclusion, hypoglycemia leads to cerebral injury and Cr-chelates, particularly CrHis have protective and regeneration potential in cerebral tissues through modulating neuroplasticity markers and nuclear transcription proteins as well as facilitating glucose transporters.

Introduction

Inadvertent and recurrent episodes of severe hypoglycemia, which are occasionally seen in diabetic patients receiving insulin therapy, may result in a transient or a persistent alteration in brain function. Hypoglycemia (<3 mmol/l) causes nerve cell injury through largely unknown mechanisms and leads to abnormal behavior, memory loss, seizures, and coma. In association with hypoglycemia, the glutamate–glutamine cycle and the altered capacity of brain cells to generate reducing equivalents in the presence of oxidative stress are postulated to link to brain dysfunction. The brain plasticity and the mechanisms controlling plasticity are central to learning and memory as well as recovery of the function of injured brain (Singh et al., 2003). The recovery of nervous system following injury and the roles of neurotrophic factors (Li et al., 1998; Kobayashi et al., 1999), and neural cell adhesion molecule (NCAM) in brain injury (Cotman et al., 1998) have been investigated. The growth-associated protein-43 (GAP-43) is also reported to involve in the plasticity mechanisms, with respect to the preservation of neurons and regeneration (Skene, 1989; Benowitz and Routtenberg, 1997). Recurrent hypoglycemic insult may affect the ability of astrocytes, which provide alternative fuel to neurons during glucose deprivation (Papadopoulos et al., 1997). Glucose deprivation causes a progressive and almost a complete loss of glutathione in the astrocytes, which is associated with lipid peroxidation and membrane rigidity.

Chromium (Cr), as a part of glucose tolerance factor (GTF), low molecular-mass chromium-binding oligopeptides (LMCr), and chromodulin potentiate insulin action and improve glucose utilization through cellar signal transduction and glucose transporters (GLUT) (Yamamoto et al., 1987; Vincent, 2000). Moreover, Cr alleviates cerebral

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oxidative stress in diabetes resulting from hyperglycemia through modulating nuclear transcription factors including nuclear factor-kappa (NF-κB), nuclear factor (erythroid-derived 2)-like 2 (Nrf2), and 4-hydroxyl nonenal (HNE) in brain (Rains and Jain, 2011; Sahin et al., 2012). Decreased availability of glucose induces energy crisis in the brain, consequently alters electrical activity and increases calcium concentrations in neuronal cells. Neurotoxic-reactive oxygen species also appear in hypoglycemic brain. The protective effect of astrocytes in hypoglycemic conditions prompted us to study the effects of Cr supplementation on neuronal plasticity and regeneration potential through neuroplasticity markers [NCAM, GAP-43, glial fibrillary acidic protein (GFAP)], GLUT's, and nuclear transcription proteins (NF-κB, Nrf2, and HNE) in hypoglycemia-induced brain injury.

Materials and methods

Experimental protocol and groups

Male Sprague–Dawley rats (n = 70, 8 wk-old) weighing 200–220 g were reared at a room temperature (22 ± 2 °C) with 55 ± 5% humidity and subjected to a 12/12 h light/dark cycle. The rats were offered ad libitum consumption of a standard diet. The experimental protocol was approved by the Ethical Committee of Firat University. All procedures were conducted in strict compliance with relevant laws, the Animal Welfare Act, Public Health Services Policy, and guidelines established by the Institutional Animal Care and Use Committee of the University.

Prospectively, the rats were divided randomly into 2 groups: rats injected with saline (positive control, n = 14) and rats induced-hypoglycemia (negative control, hypoglycemic, n = 56). Hypoglycemic rats were then received either no agent, dextrose on the day of sampling, or Cr-chelates [Cr-histidinate (CrHis), or Cr-picolinate (CrPic)] for a period of one week prior to the hypoglycemia induction. Eight μg elemental Cr was delivered daily via drinking water in the form of Cr-His (Cr, 25.22%) or CrPic (Cr, 12.43%) (Nutrition 21, Inc., Purchase, NY, USA). Hypoglycemia was induced by injecting insulin as described by Suh et al. (2005). Briefly, rats were fasted overnight, and given an intra-peritoneal injection (15 U/kg BW) of regular insulin (Novolin-R; Novo Nordisk, Clayton, NC). Rats in the control group received an equal volume of isotonic saline solution. Blood samples (50 μl) were obtained from the tail vein at 0, 0.5, 1, 2, 6, 12, and 24 h after the insulin injection and glucose levels were measured using a portable glucometer (ACCU-Check Active, Roche Diagnostics, Mannheim, Germany). Since the extent of brain injury upon severe hypoglycemia is correlated with the duration of electroencephalogram (EEG) iso-electricity, hypoglycemia was terminated after 30 min of EEG generating a repeatable brain injury of a moderate severity by administering a 25% glucose infusion (i.v.) for 3 h (1.5 ml/h) to maintain blood glucose level between 5 and 10 mmol/l.

Laboratory analyses

At the end of the experiment, after sampling blood, rats were sacrificed by cervical dislocation to obtain brain. Blood samples were centrifuged at 3000 ×*g* for 10 min and sera were separated. Serum insulin (Linco Research Inc., St. Charles, MO, USA) concentrations were determined using an ELISA commercial kit (EL_x-800, Bio-Tek Instruments Inc., City, VT). After digesting with a mixture of concentrated HNO_3 (65% Merck, Darmstadt, Germany) and H_2O_2 (30% Merck) in a Microwave Digestion System (Berghoff, Eningen, Germany), brain tissue and sera were analyzed for Cr content in a graphite furnace atomic absorption spectrophotometer (AAnalyst 800, Perkin-Elmer Corp., Norwalk, CT, USA) as described by Dogukan et al. (2009).

The brain malondialdehyde (MDA) content was measured by high performance liquid chromatography (HPLC, Shimadzu, Tokyo, Japan) using a Shimadzu UV–vis SPD-10 AVP detector and a CTO-10 AS VP column in a mobile phase consisting of 30 mM KH_2PO_4 and methanol (82.5 + 17.5, v/v; pH 3.6) at a flow rate of 1.2 ml/min. Column effluents were monitored at 250 nm and the injection volume was 20 μl. The brain homogenate (10%, w/v) was prepared in 10 mM phosphate buffer (pH 7.4), centrifuged at 13,000 ×*g* for 10 min at 4 °C, and the supernatant was collected and stored at −80 °C for MDA analysis (Ulu et al., 2012).

For Western blot analyses protein extraction was performed by homogenizing the hippocampus, cerebellum and cortex of the brain in 1 ml ice-cold hypotonic buffer A, containing 10 mM HEPES (pH 7.8), 10 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.1 mM EDTA, and 0.1 mM phenylmethylsulfonyl-fluoride (PMSF). The homogenates were added with 80 μl of 10% Nonidet P-40 (NP-40) solution and then centrifuged at 14,000 ×*g* for 2 min. The precipitates were washed once with 500 μl of buffer A plus 40 μl of 10% NP-40, centrifuged, re-suspended in 200 μl of buffer C [50 mM HEPES (pH 7.8), 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mM PMSF, 20% glycerol], and re-centrifuged at 14,800 ×*g* for 5 min. The supernatants were collected for determinations of GAP-43, NCAM, GFAP, GLUT-1, GLUT-3, NF-κB, Nrf2, HNE, and insulin according to the method described by Sahin et al. (2012). Equal amounts of protein (50 μg) were electrophoresed and subsequently transferred onto a nitrocellulose membrane (Schleicher and Schuell Inc., Keene, NH, USA). Antibodies against GAP-43, NCAM, GFAP, GLUT-1, GLUT-3, NF-κB, Nrf2, HNE and insulin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) were diluted (1:1000) in the same buffer containing 0.05% Tween-20. Protein loading was controlled using a monoclonal mouse antibody against β-actin (A5316; Sigma). Bands were analyzed densitometrically using an image analysis system (Image J; National Institute of Health, Bethesda, USA).

Statistical analysis

Data were expressed as relative to the control (%) and analyzed using one-way ANOVA. In the analyses for plasma glucose and insulin concentration, the repeated statement was added in the general linear model. The group differences were attained by the Fisher's multiple comparison test (SPSS, Version 17.0, Chicago, IL). Statistical significance was declared at $p < 0.05$.

Results

Serum glucose–insulin concentrations and cerebral insulin protein expression in hypoglycemia-induced brain injury

Serum glucose concentration decreased by 47.5% in hypoglycemic rats as compared to control rats (Table 1). Dextrose treatment did not

Table 1
Effect of chromium on serum and brain metabolites in hypoglycemic rats.*

Groups[1]	Variables				
	Glucose (mg/dl)	Insulin (μU/ml)	Glucose: insulin	Brain Cr (ng/g)	Brain MDA (nmol/mg protein)
Control	114.40^{a}	2.09^{c}	55.75^{a}	18.51^{bc}	0.09^{d}
Hypoglycemic (H)	60.01^{c}	2.56^{a}	39.43^{c}	16.75^{cd}	0.15^{bc}
H + dextrose	71.02^{b}	2.41^{b}	45.63^{b}	15.32^{d}	0.34^{a}
H + Cr-histidinate	60.33^{c}	2.53^{a}	39.77^{c}	21.62^{a}	0.16^{bc}
H + Cr-picolinate	59.97^{c}	2.55^{a}	40.36^{c}	20.33^{ab}	0.18^{b}
Pooled SE	0.77	0.02	1.33	0.89	0.02
ANOVA	p<				
Group	0.0001	0.0001	0.0001	0.0001	0.0001
Time	0.0001	0.0001	0.0001	–	–
Group × time	0.0001	0.0001	0.0001	–	–

* Different superscripts among the rows significantly differ, $p < 0.05$. The initial glucose and insulin concentrations were 105.37 ± 1.07 mg/dl and 2.09 ± 0.01 μU/ml, respectively.

[1] Dextrose was administered on the day of sampling, whereas Cr-chelates were delivered via drinking water to provide 8 μg elemental Cr daily for one week prior to the hypoglycemia induction by insulin injection.