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SEVERE INSTEAD OF MILD HYPERGLYCEMIA INHIBITS NEUROGENESIS IN THE SUBVENTRICULAR ZONE OF ADULT RATS AFTER TRANSIENT FOCAL CEREBRAL ISCHEMIA

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Abstract—Accumulated evidence suggests that enhanced neurogenesis stimulated by ischemic injury contributes to stroke outcome. However, it is unclear whether hyperglycemia, which is frequently tested positive in patients with acute ischemic stroke, influences stroke-induced neurogenesis. The aim of the present study is to examine the effect of hyperglycemia on stroke-induced neurogenesis in a rat model of transient focal cerebral ischemia. For this purpose, adult male Sprague–Dawley rats (220–250 g) were subjected to 90 min of middle cerebral artery occlusion (MCAO). Glucose was administered during ischemia to produce target blood levels ranging from 4.83 ± 0.94 mM (normoglycemia) to 20.76 ± 1.56 mM. To label proliferating cells in ischemic ipsilateral subventricular zone (SVZ) of lateral ventricles, 5′-bromo-2′-deoxyuridine (BrdU) was injected 24 h after MCAO. Brains were harvested 2 h post-BrdU to evaluate the effects of hyperglycemia on infarct volume and SVZ cell proliferation. Rats that were severely hyperglycemic (19.26 ± 1.48 mM to 20.76 ± 1.56 mM) during ischemia had 24.26% increase in infarct volume ($P < 0.05$) and more serious neurological function deficits ($P < 0.05$). The severe hyperglycemic rats also showed dramatically decreased proliferation of neural stem/progenitor cells (NSPCs) ($P < 0.05$) and down-regulation of the phosphorylation of cyclic-AMP response element-binding protein (pCREB) ($P < 0.05$)and brain-derived neurotrophic factor (BDNF) ($P < 0.05$) in ipsilateral SVZ. But the above-mentioned detrimental effects were not observed in rats that were rendered with mild hyperglycemia (9.43 ± 1.39–10.13 ± 1.24 mM). Our findings indicate that severe instead of mild hyperglycemia exacerbates ischemic injury and inhibits stroke-induced SVZ neurogenesis by a mechanism involving suppression of CREB and BDNF signaling.

Key words: hyperglycemia, cerebral ischemia, neurogenesis, subventricular zone, cAMP-responsive element-binding protein, brain-derived neurotrophic factor.

INTRODUCTION

Stroke, which has exceeded heart disease to become the leading cause of death and adult disability in china, is a serious and debilitating neurological disease with ischemic stroke being the most common form (Liu et al., 2011). Relief of the damage of pre-existing neurons and generation of new neurons in the injured brain are believed to be crucial to functional restoration after ischemic insult onset. To date a wealth of studies has demonstrated the existence of neural stem/progenitor cells (NSPCs) capable of self-renewal and multipotency in the adult central nervous systems of rodents (Reynolds and Weiss, 1992) and humans (Eriksson et al., 1998), restricted in two specific ''neurogenic niches'', subventricular zone (SVZ) of the lateral ventricles (LV) and subgranular zone (SGZ) in the dentate gyrus (DG) of the hippocampus. New neurons generated from SVZ normally migrate via the rostral migratory stream (RMS) to the olfactory bulb to integrate as granule cells and periglomerular interneurons into the olfactory circuit (Gage, 2000). However, differing from normal circumstances, pathological conditions such as cerebral ischemia could stimulate neurogenesis in SVZ and directed migration of newly formed neural progenitor cells toward the infarct area (Jin et al., 2001; Zhang et al., 2001; Yamashita et al., 2006). Furthermore, such injury-induced neurogenesis is supposed to contribute to stroke outcome (Raber et al., 2004; Jin et al., 2010; Sun et al., 2012, 2013, 2015; Wang et al., 2012). There is therefore an urgent need for a better understanding of injury-induced neurogenesis in adult mammals. To our knowledge, adult neurogenesis in the hippocampus is sharply

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Abbreviations: ATP, adenosine triphosphate; BDNF, brain-derived neurotrophic factor; BrdU, 5′-bromo-2′-deoxyuridine; CREB, cyclic-AMP response element-binding protein; DCX, doublecortin; DG, dentate gyrus; ECA, external carotid artery; EDTA, ethylenediaminetetraacetic acid; EGTA, thylene glycol tetraacetic acid; GFAP, glial fibrillary acidic protein; GKI, glucose potassium insulin; IP, intraperitoneal injection; MCAO, middle cerebral artery occlusion; mNSS, Modified Neurological Severity Score; NSPCs, neural stem/progenitor cells; PBS, phosphate-buffered saline; p-CREB, phospho-CREB; PI3K, phosphatidylinositol 3-kinase; ROI, regions of interest; SGZ, subgranular zone; SVZ, subventricular zone; TTC, 2,3,5-Triphenyltetrazolium chloride.

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reduced in type 1 and type 2 diabetic models (Jackson-Guilford et al., 2000; Beauquis et al., 2006, 2008; Zhang et al., 2008; Lang et al., 2009; Ramos-Rodriguez et al., 2014), which suggested a potential role of blood glucose in self-renewal and proliferation of NSPCs.

Clinical studies indicated that 40–70% of acute stroke patients had blood glucose >6.1 mM at admission (Capes et al., 2001; Dave et al., 2010; Jia et al., 2012; Desilles et al., 2013) and post-stroke hyperglycemia independently correlated with poor clinical outcome (Kruyt et al., 2010; Arnold et al., 2014). We reported previously that high glucose could induce apoptosis and suppress proliferation of immortalized adult rat neural stem cells subjected to 6 h oxygen and glucose deprivation (Chen et al., 2013). However whether elevated blood glucose negatively influenced post-stroke neurogenesis *in vivo* and to what extent still remain unknown.

Cyclic-AMP response element-binding protein (CREB) is a nuclear-localized basic leucine zipper superfamily transcription factor and activated by its phosphorylation on Ser133. Phospho-CREB (p-CREB) is at the hub of multiple cell-signaling cascades and regulates the expression of several downstream target genes essential for NSPCs proliferation, survival and differentiation such as brain-derived neurotrophic factor (BDNF) (Dworkin et al., 2009; Grimm et al., 2009; Mantamadiotis et al., 2012). It has been well documented that cerebral ischemia could trigger robust phosphorylation of CREB which is considered to be implicated in stroke-induced neurogenesis (Kitagawa, 2007; Tanaka et al., 2010).

In the present study, we examined the effect of hyperglycemia on neurogenesis in SVZ using a rat model of transient focal cerebral ischemia and further explored whether CREB signaling pathway is involved in the mechanism by which hyperglycemia affects stroke-induced neurogenesis.

EXPERIMENTAL PROCEDURES

Animal model

All studies were performed on adult male Sprague–Dawley rats (220–250 g) obtained from Experimental Animal Center of Southern Medical University. The animals were housed at 24 °C and maintained on a 12-h light/dark cycle (lights on 8:00 a.m. to 8:00 p.m.) with free access to food and water. All experiments were approved by the Animal Care and Use Regulation of the Southern Medical University and conducted in accordance with the the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23). Cerebral ischemia was generated by a 90-min intraluminal occlusion of the right middle cerebral artery (MCAO) with reperfusion as described originally by Longa et al. (1989). Before surgical procedures, rats were fasted overnight to ensure uniform basal blood glucose levels. Anesthesia was induced by intraperitoneal injection (IP) of 3% pentobarbital sodium (0.2 ml/kg). Then, the right cervical carotid bifurcation was exposed via a ventral midline cervical incision. Two branches of the external carotid artery (ECA), the occipital artery and superior thyroid artery, were carefully isolated and cut off. A 4-cm long 3–0 monofilament nylon suture with a thin silicon coat was introduced into the right internal carotid artery (ICA) through a micro-incision made on the ECA and was advanced 21–22 mm from the carotid bifurcation. Ninety minutes after occlusion, the filament was withdrawn for reperfusion. Body temperature was maintained at 37 ± 0.5 °C with a heating pad. Sham-operated rats underwent the same surgical procedure without suture insertion.

Induction of acute hyperglycemia

Rats were rendered hyperglycemic with IP injection of dextrose (Sigma; 50%) at 5 min after MCAO and another two indicated time points with a 45-min interval (Fig. 1A). The initial injection volume was 2.5 or 8.0 ml/kg for the target blood glucose concentrations of 10 and 20 mM, respectively. Subsequent injection volumes were all 2.0 ml/kg. Normoglycemic and sham groups received 2.0 ml/kg saline vehicle at the three time points. Tail blood was obtained at four indicated time points to measure glucose levels (Optium Xceed Glucometer, Abbott). Since an increased mortality rate (36%) was observed in 20 mM glucose group in our preliminary experiment (data not shown), we had to raise the number of rats in that group to ensure at least 5 rats used to analyze at each time point.

2,3,5-Triphenyltetrazolium chloride (TTC) staining

Rats were decapitated 24 h after the onset of ischemia (n = 5 each group). Brains were removed rapidly and a total of six 2-mm-thick coronal sections for every rat were serially cut throughout the brain using a rodent brain matrix, then immersed in 2% TTC (Sigma) in saline for 12 min at 37 °C. The stained sections were fixed with 4% paraformaldehyde and photographed by a scanner. Infarct area, left hemisphere area, and total brain areas were measured by a blinded investigator using Image J software. As previously described (Bederson et al., 1986; Swanson et al., 1990), infarct volume was expressed as the percentage of the volume of the contralateral hemisphere.

Behavioral testing

Each rat was subjected to a battery of behavioral tests, which consisted of Modified Neurological Severity Score (mNSS) and rotarod motor test, to evaluate neurologic function both before and 24 h after MCAO (n = 6 each group).

mNSS. The score system is composite of motor (muscle status, abnormal movement), sensory (visual, tactile, and proprioceptive), and reflex tests (De Ryck et al., 1989; Li et al., 2000, 2001). Neurological function was graded on a scale of 0–14 (normal score, 0; mild injury, 1–4; moderate impairment, 5–9; severe deficit score, 10–14). In the severity scores of injury, one point is awarded for the inability to correctly perform the tasks or for the lack of a tested reflex.