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Research Report

Enhanced autophagy signaling in diabetic rats with ischemia-induced seizures

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ABSTRACT

Seizures are among the most common neurological sequelae of stroke, and ischemic insult in diabetes notably increases the incidence of seizures. Recent studies indicated that autophagy influences the outcome of stroke and involved in epileptogenesis. However, the association of autophagy and post-ischemic seizures in diabetes remains unclear. The present study aimed to reveal the involvement of autophagy in the seizures following cerebral ischemia in diabetes. Diabetes was induced in adult male Wistar rats by intraperitoneal injection of streptozotocin (STZ). The diabetic rats were subjected to transient forebrain ischemia. The neuronal damage was assessed using hematoxylin-eosin staining. Western blotting and immunohistochemistry were performed to investigate the alteration of autophagy marker microtubule-associated protein light chain 1B (LC3B). The results showed that all diabetic animals developed seizures after ischemia. However, no apparent cell death was observed in the hippocampus of seizure rats 12 h after the insult. The expression of LC3B was significantly enhanced in naïve animals after ischemia and was further increased in diabetic animals after ischemia. Immunofluorescence double-labeling study indicated that LC3B was mainly increased in neurons. Our study demonstrated, for the first time, that autophagy activity is significantly increased in diabetic animals with ischemia-induced seizures. Further studies are needed to explore the role of autophagy in seizure generation after ischemia in diabetic conditions.

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1. Introduction

Stroke is the leading cause of death and long-term disability worldwide (Feigin et al., 2014; Lozano et al., 2012). Seizures are common neurological sequelae of stroke (Ferro and Pinto, 2004). Diabetes is one of the major risk factors of stroke. Hyperglycemia in diabetes mellitus has a damaging effect on the central nervous system (CNS), which might trigger seizures following stroke (Ottman et al., 2011). It has been shown that diabetic animals frequently develop post-ischemic seizures and the incidence of seizures was related to the blood glucose levels (Li et al., 1998). Based on the temporal relation with stroke onset, seizures are classified as early- and late-onset seizures. Early-onset seizures are defined as seizures that occur within the first 14 days after stroke, late-onset seizures are defined as seizures occur after this time (Bladin et al., 2000; Procaccianti et al., 2012). It has been reported that the incidence of early-onset seizures in acute stroke ranges 3–33%, with 50–78% of the seizures appearing within the first 24 h (Chung, 2014). Patients with early-onset seizures are typically present acute symptomatic seizures, not qualified as having epilepsy (Loscher et al., 2015). The definition of epilepsy requires the development of at least one epileptic seizure (Fisher et al., 2005). Up to 86% patients with posttraumatic seizure develop recurrent seizures (epilepsy) within 2 years (Haltiner et al., 1997). Therefore, it is important to prevent the development of epilepsy by effective treatment of early-onset seizures.

Autophagy is a critical and highly conserved degradation pathway for the turnover of dysfunctional organelles or damaged proteins to lysosome to maintain cellular metabolic homeostasis

Abbreviations: 4-VO, four-vessel occlusion; GFAP, glial fibrillary acidic protein; H&E, hematoxylin and eosin; STZ, streptozotocin; LC3B, microtubule associated protein light chain 3B; CNS, central nervous system.

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(Uchiyama et al., 2008). It plays a central role in many physiological and pathological processes (Klionsky, 2007). Autophagy is characterized by the formation of autophagosomes, a double membrane organelle that engulf cytosolic components and organelles (Esteban-Martinez and Boya, 2015). Autophagosomes are induced in numerous neurological disorders including stroke, traumatic brain injury and other neurodegenerative diseases (Chu et al., 2009). In pathological conditions, autophagy abnormalities always exhibit marked accumulations of autophagy-related compartments in affected neurons (Yu et al., 2004), and over-activated autophagy would lead to autophagic cell death (Shi et al., 2012). Autophagy has been found to be compromised in electroconvulsive seizures (Otabe et al., 2014) and Lafora disease, which is strongly associated with epilepsy (Knecht et al., 2010). It has also been reported that autophagy activity markedly increased in the immature rodent brain after status epilepticus (Benz et al., 2014). Accumulated evidence indicates that autophagy plays an important role in epileptogenesis and epilepsy-induced brain damage (Giorgi et al., 2015).

To reveal the involvement of autophagy in post-ischemic seizures under diabetic conditions, the present study examined the autophagy activity in diabetic animals following transient cerebral ischemia. Our results demonstrated that autophagy activity significantly increased in diabetic rats with ischemia-induced seizures.

2. Results

2.1. The incidence of post-ischemic seizures significantly increases in diabetic animals

The animal model of diabetes was induced in adult male Wistar rat via intraperitoneal injection of streptozotocin (STZ). Six days after induction, blood glucose levels in these animals were markedly elevated (Control: 117 ± 5 mg/dl, n=10; STZ: 471 ± 15 mg/dl, n=18, $p < 0.01$, Fig. 1A). In comparison with the age-matching groups, the diabetic animals exhibited higher water intake (36 ± 1 ml/day vs. 107 ± 10 ml/day, n=4/5, $p < 0.01$, Fig. 1B) and lower body weight at 6th days after STZ injection (169 ± 1 g vs. 142 ± 2 g, n=4/5, $p < 0.01$, Fig. 1C). These results indicate that the animals showed typical diabetic symptoms after STZ injection. 15 min cerebral ischemia was induced using the 4-Vessle Occlusion (4-VO) method. No seizures were observed in control animals after ischemia whereas all diabetic rats developed seizures after ischemia (Fig. 1D). The seizures occurred at different time points ranging from 1.5 h to 24.5 h following ischemia (14.4 ± 1.5 h, n=18). The post ischemic seizures were status epilepticus, starting with forelimbs and hind limbs rigidly pushed away from the body, followed by violent shaking, vibrating, and jumping (Fig. 1E). The rats usually died within 2 h after the onset of seizures. As a result, seizure rats were sacrificed immediately after the observation of seizure activities.

2.2. No apparent cell death is observed in diabetic animals after ischemia

The morphology of hippocampal neurons following ischemia was examined on brain slides containing hippocampus with H&E staining (Fig. 2). Ischemic neuronal injury is evident by the eosinophilic cytoplasm, nuclear pyknosis, and shrinkage of the cell body in H&E sections. Neuronal damage in CA1 zone of hippocampus was quantified using the 3-scale method developed by Pulsinelli et al. (1982). In this method, no neuronal damage is scale 0 and majority of neuronal damage is scale 3. In the present study, no neuronal damage was observed in the control and diabetic rats (scale 0, n=3). Within 24 h after ischemia, the neuronal damage was scale 0.75 ± 0.75 (n=4) in normoglycemic rats and scale 0.40 ± 0.40 (n=4) in diabetic rats. These results suggest that brain damage is dispensable for the development of post-ischemic seizures in diabetic rats.

2.3. Autophagy activity increases in diabetic animals with ischemia-induced seizures

LC3-II is a widely used marker for autophagosome, which lipoxidized from a cytosolic form of microtubule-associated protein light-chain I (LC3-I) during autophagy (Kabeya et al., 2000; Mizushima et al., 2010). LC3 represents a mammalian homologue of the yeast autophagy related gene ATG8. It was originally characterized as light chain 3 of the microtubule associated protein 1 (MAP1LC3). The protein family consists of LC3 A, B, and C and the GABARAP subfamilies. But only LC3B-II correlates with increased levels of autophagic vesicles, and therefore anti-LC3B antibodies were used in the present studies (Barth et al., 2010). To investigate the changes of autophagy activities after ischemia in diabetic animals, the expression of LC3B-II was compared in different experimental groups. Immunostaining using antibodies against LC3B showed that the LC3B positivity significantly increased in normoglycemic animals after ischemia, especially in CA3 and dentate gyrus (DG) (Fig. 3 and 4). Quantitative studies using optical density measurement showed that the LC3B immunostaining was further enhanced in diabetic animals following ischemia (0.84 ± 0.11, n=3, $p < 0.05$) compared with control animals (0.41 ± 0.11, n=3, Fig. 5A). To further quantify the alteration of LC3B, Western blotting was used to compare the changes of LC3B-II after ischemia.

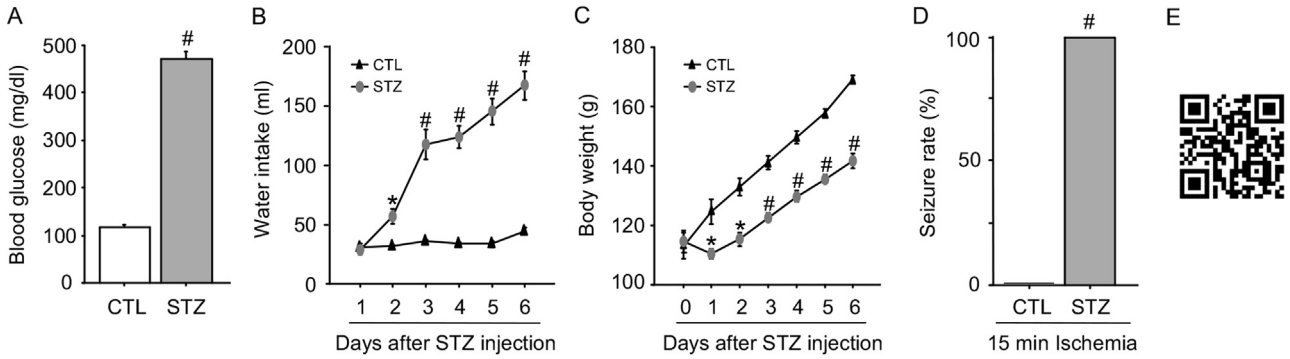

Fig. 1. Diabetic animal model and post-ischemic seizures. A: Blood glucose levels of control rats (n=10) and rats that received STZ injection (n=18). B: The volume of water intake increased significantly after injecting STZ (n=5). There was no change in control rats during the same time period (n=4). C: The body weight of STZ injected rats (n=5) significantly decreased as compared to the control ones (n=4). D: All animals with hyperglycemia developed seizures following 15 min ischemia, whereas none of the rats with normoglycemia had seizures after ischemia. E: Video clips showing the seizures in diabetic rats after ischemia. *$P < 0.05$, # $P < 0.01$ vs. control groups.