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Research report

Diabetes impairs spatial learning and memory and hippocampal neurogenesis via BDNF in rats with transient global ischemia

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ABSTRACT

Diabetic conditions worsen the prognosis of stroke. The molecular mechanism underlying the impairment of post-stroke recovery is not very clear. Here, we establish a rat model resembling human cerebral infarction with or without diabetes to determine how diabetes impairs cognitive recovery. Our data show that diabetes inhibits hippocampal BDNF expression and impairs the survival and differentiation of the newborn neural cells in rats with ischemia. Consequently, the rats of diabetic ischemia have a significantly lower score in spatial learning and memory in the Morris water maze test than the non-diabetic ischemia model rats. On the other hand, treatment with BDNF effectively improves hippocampal neurogenesis and the spatial learning and memory in rat with diabetic ischemia. All together, our data suggest that diabetes impaired spatial learning and memory and hippocampal neurogenesis in rats with ischemia by inhibition of the BDNF expression in the hippocampus.

1. Introduction

Diabetes, which affected one-fourth of the ischemic stroke patients, was found to be associated with a high risk of death and disability after onset of stroke(Huang et al., 2013; Jia et al., 2011). Experiments in rats also showed that diabetes or acute hyperglycemia worsens ischemic stroke-induced brain damage and tried to figure out the mechanism involved (Huang et al., 2013; Kim et al., 2014; Li et al., 2013; Rehni et al., 2014). However, the molecular mechanisms underlying impairment of post-stroke recovery induced by diabetes are still not very clear.

Previous studies showed that pathological conditions such as ischemia may induce neuron regeneration (Burns et al., 2009; Liu et al., 1998). Recent studies suggest that cognitive recovery after ischemia may at least partly depends on the neural regeneration process (Abe et al., 2012). On the other hand, diabetes inhibits the survival of regenerated neural cells in hippocampus (Lang et al., 2009). It has been found that diabetes impairs cortical plasticity and functional recovery following ischemic stroke (Sweetnam et al., 2012). It is not clear yet if diabetes affects cerebral ischemia-induced neurogenesis.

Brain-derived neurotrophic factor (BDNF), which is a key neuroprotective protein in the brain, has been shown to play a role in the survival and differentiation of neurons (Scharfman et al., 2005). BDNF expression is down-regulated by diabetes. A clinical research showed that plasma levels of BDNF were decreased in humans with type 2 diabetes (Krabbe et al., 2007). Another research in rat showed that streptozotocin-induced diabetes reduced the expression of BDNF in retinas, which may be involved in early retinal neuropathy (Seki et al., 2004). Recent research showed that streptozotocin-induced diabetes decreased BDNF expression and BDNF-mediated neuroprotection in the brain (Navaratna et al., 2011). We wondered if diabetes affects post-ischemia recovery and neurogenesis through impaired BDNF signaling.

To address this issue, we established diabetic cerebral ischemia and non-diabetic cerebral ischemia model rats and investigated spatial learning and memory. We observed neurogenesis and BDNF expression in dentate gyrus (DG) of hippocampus since neurogenesis in the DG plays a crucial role in cognitive recovery in rats after ischemia (Yan et al., 2007). To verify the role of BDNF, recombinant BDNF was administrated intracerebroventricularly in

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the diabetic cerebral ischemic rat. Our findings indicated diabetes impairs cognitive recovery and hippocampal neurogenesis in rats with ischemia by inhibition of the BDNF expression in hippocampus.

2. Materials and methods

2.1. Animals

Adult Sprague-Dawley rats (250 ± 10 g) were acquired from Anhui Experimental Animal Center (Anhui, China). They were maintained at a constant temperature (21–23 °C) with a cycle of 12 h light and 12 h dark with access to food and water ad libitum. Animal housing, care, and application of experimental procedure were in accordance with all relevant local guidelines and legislation to minimize pain and suffering of the animals. All animal experiments were performed in consistency with the policy of Animal Care and Use Committee, First Affiliated Hospital, Anhui University of Traditional Chinese Medicine, The approval number is 2010090208.

The experimental rats were randomly assigned to five experimental groups: sham, Diabetes, ischemia, ischemia with diabetes, as well as ischemia with diabetes administered with BDNF.

2.2. Induction of cerebral ischemia and diabetic cerebral ischemia rat models

The diabetes rat model was established by given a single intra-peritoneal injection of by 60 mg/kg streptozocin (STZ, S0130, Sigma-Aldrich, St. Loui, MO, U.S.A) prepared in 100 mM sodium citrate buffer at pH 4.5 to rats after they have been fasted for 12 h. Age-matched normal rats received citrate buffer only. 3 days after STZ treatments, Diabetes was confirmed by assaying the fasting glucose concentration with a blood glucose monitoring sensor (Accu-Chek, Roche) using a blood sample from the snipped tail. Only the rats with glucose levels > 16.7 mmol/l were considered to be a successful diabetic model (Ren et al., 2012). The transient global cerebral ischemia model and sham rats were established following a previously published procedure of our group (Yan et al., 2007) on day 30 after STZ injection. Briefly, rats were anesthetized by chloral hydrate (350 mg/kg, intraperitoneal). The vertebral arteries were irreversibly electro cauterized. Common carotid arteries were exposed and a small-diameter silk thread looped around each artery to facilitate subsequent occlusion. Rats were allowed to recover for 24 h. Ischemia was induced by clamping the common carotid arteries with microvascular clamps in awake animals. Rats that lost their righting reflex within 1 min and those whose pupils were dilated and unresponsive to light were selected for the experiments. Carotid artery blood flow was restored by releasing the clamps. During 15 min ischemia and 2 h reperfusion, rectal temperature was maintained at about 37 °C. Sham received the same dorsal and ventral surgical procedures except that the vertebral arteries were not electro cauterized and the common carotid arteries were not occluded. During and post surgery, antibiotics(penicillin, 1000 U/ml)was locally administered to the rats to prevent infections.

2.3. Placement of a chronic intracerebroventricular (ICV) catheter

23 days after STZ treatments, the rats were anesthetized with intra-peritoneal injections of 350 mg/kg chloral hydrate. After anesthesia, rats were fixed with stereotaxic instruments, an incision was made in the flesh along the midline, and a hole was made 1.3 mm posterior, 1.9 mm lateral, and 3 mm deep from the bregma. A cannula made by 21-gauge syringe needle was inserted into the lateral ventricle and the orifice was occupied with a wire sized to fit inside the cannula. The brain cannula was secured to the surface of the skull using a jewler's screw and acrylic dental cement.

2.4. Treatments

In order to allow ICV administration of recombinant human BDNF or solvent arificial cerebrospinal fluid (aCSF, pH 7.4), the wire placed in the cannula was removed. A syringe needle that matched the length of the cannula was inserted, and an injection was given over a period of 5 min. The syringe was removed 5 min after the injection, and then the orifice of cannula was occupied with a wire again. 1 μg BDNF (2 μl per rat) (Scharfman et al., 2005) was administrated every second day from postischemic days 5–21. 5-bromo- 20-deoxyuridine (BrdU, 50 mg/kg) was intraperitoneally administrated twice daily on postischemic day 6 to determine cell generation, survival, and the phenotype of the Brdu-positive cells. On postischemic days 7 and 28, Rats were killed by decapitation at a specified time under anesthesia. Brain was collected for immunohistochemistry and western blotting.

2.5. Morris water maze test

To detect the capacities of spatial learning and memory, a recent version of the Morris water maze (MWM) test (Choi et al., 2006) was used and all animals were tested 5 days (from 23 to 27 days after ischemia). The water maze was located in a large room, where several visual signs (120–150 cm high) were put around the tank to show the orientation. The visual signs were visible from the pool and presumably used by the rats for spatial orientation. The position of the cues remained unchanged throughout the experiment. The water maze is a black round water tank (r = 107 cm, h = 80 cm) divided into four quadrants (north, south, east, and west). Water was filled to a height of 50 cm. The water temperature was maintained at 21–24 °C. A black circular platform (r = 4.5 cm) was placed in the center of one of the four quadrants and was 2 cm under the water surface, hidden from the rat's view. In the test, the rat was first trained to find the hidden platform.

The rat was placed in the water facing the wall at a start position in any of the four areas. Each rat was allowed to swim for 90 s or until it found the platform. If the animal found the platform, it was allowed to remain on it for 10 s. If the rat failed to find the hidden platform within 90s, it was placed on the platform for extra 10s. The procedure was repeated for all the four start locations. The latency, representing the average of the four trials, to reach the platform and swimming speed were recorded. Two sessions of four trials were conducted on the first day, and the interval was 4 h. The first session was considered as training procedure. One session of four trials was conducted daily for the next 4 days. On postischemic day 28, a probe trial was given within 90 s in which the platform was removed from the tank. The rat was placed in the water at the same random start location, and time spent in the target quadrant of the pool which previously contained the platform was recorded. This was to test how well the rats remembered the location of the platform or whether the rats had a learned bias to navigate toward the goal quadrant. Thigmotaxis and speed of the animals were analyzed to rule out motor and other non-cognitive deficits that may interfere with the performance of the task. Thigmotaxis, as a test for anxiolytic activity in rats, was detected by the percentage of time that an animal spent within 15 cm of the wall of the tank during the experiment (Harris et al., 2009).

2.6. Bromodeoxyuridine incorporation for neural cells proliferation and regeneration

The BrdU-positive neural cells provide measurement of the newly regenerated neural stem cells. The cell proliferation rate