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BRAIN RESEARCH

Research Report

Diabetes impairs learning performance and affects the mitochondrial function of hippocampal pyramidal neurons

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ABSTRACT

Previous research has demonstrated that diabetes induces learning and memory deficits. However, the mechanism of memory impairment induced by diabetes is poorly understood. The present study investigated the effect of streptozotocin (STZ)-induced diabetes on spatial learning and memory using the Morris Water Maze. The effects of diabetes on CA1 pyramidal neurons in hippocampus were also examined. Diabetes impaired spatial learning and memory of rats. Diabetes induced the apoptosis of neurons and translocation of Bax from cytoplasm to mitochondria. On the contrary, diabetes induced cytochrome c release into the cytoplasm from mitochondria. Release of Cyt-c from mitochondria into cytoplasm may play a role in apoptosis of the CA1 pyramidal neurons, which resulted in a decrease of the number of neurons in hippocampus and impaired the performance function. These results partially explain the mechanism of the effect of diabetes on learning and memory. To protect mitochondrial function is possible candidate for preventing the impairments of diabetes on hippocampal function.

1. Introduction

Diabetes mellitus (DM) is a heterogeneous metabolic disorder characterized by hyperglycemia resulting from defective insulin secretion, resistance to insulin action or both (Gaven et al., 1997). Poor academic performance in diabetic children and memory impairment in adults with diabetes are viewed as increasing public health concerns. There is accumulating evidence that diabetes adversely affects the central nervous system independently from atherosclerotic disease. In humans, diabetes mellitus is associated with moderate impairments in cognitive function and patients present a high risk of affective disorders, dementia and Alzheimer disease (Northam et al. 2006; Ott et al., 1999; Ristow, 2004; Brismar et al., 2007; Stewart and Liolitsa, 1999). Learning and memory deficits also occur in streptozotocin (STZ)-induced diabetic rats (Baydas et al., 2003; Kucukatay et al., 2007; Lupien et al., 2003; Stranahan et al., 2008; Tiwari et al., 2009; Biessels et al., 1996), which have been partly associated with the structural and functional deficits in certain brain regions such as the hippocampus and cerebral cortex (Baydas et al., 2003; Hasanein and Shahidi, 2010; Biessels et al., 1996). The multifactorial pathogenesis of learning and memory impairments in diabetes has not been fully elucidated. Several factors such as vascular complications, metabolic disturbances, and the release of free radicals are implicated (Hasanein and Shahidi, 2010). However, less attention has been given to the effects of diabetes on the intrinsic properties

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Abbreviations: STZ, streptozotocin; Cyt-c, cytochrome c; MTP, mitochondria permeability transition pore

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of neurons, which are thought to be critical for the formation of memories at a cellular level (Bliss and Collingridge, 1993).

Here we began by assessing the impact of diabetes on hippocampus-dependent spatial learning and memory; we then followed this with an examination of the effects of diabetes on the morphological character of CA1 pyramidal neurons of the hippocampus, an area crucial to the formation and encoding of memories (Henninger et al., 2007; Eichenbaum, 2000). Also, the effect of diabetes on the mitochondria of the CA1 neurons was studied.

Using the Morris Water Maze spatial-learning set protocol, we found that diabetic rats are slower to learn the location of the platform than control groups, and are deficient in memory maintenance. The data from the behavior tests indicated that diabetes impairs the ability of spatial learning and memory of rats. We further found that diabetes induces translocation of Bax to mitochondria and cytochrome c (Cyt-c) release into cytoplasm, and induces the apoptosis of CA1 pyramidal neurons, which may contribute to the deficits in performing the spatial learning task.

2. Results

2.1. Blood glucose levels and body weight

Streptozotocin injection resulted in a diabetic syndrome verified by the presence of polydypsia, polyuria, hyperglycemia, and weight loss in the diabetic animals. Mean blood glucose level in the diabetic group was significantly higher than the control group ($p<0.01$) after STZ injection, and blood glucose levels in the diabetic group remained significantly elevated (Fig. 1A). Significant weight loss was observed in STZ-induced diabetic rats compared with the control group (Fig. 1B, $p<0.01$).

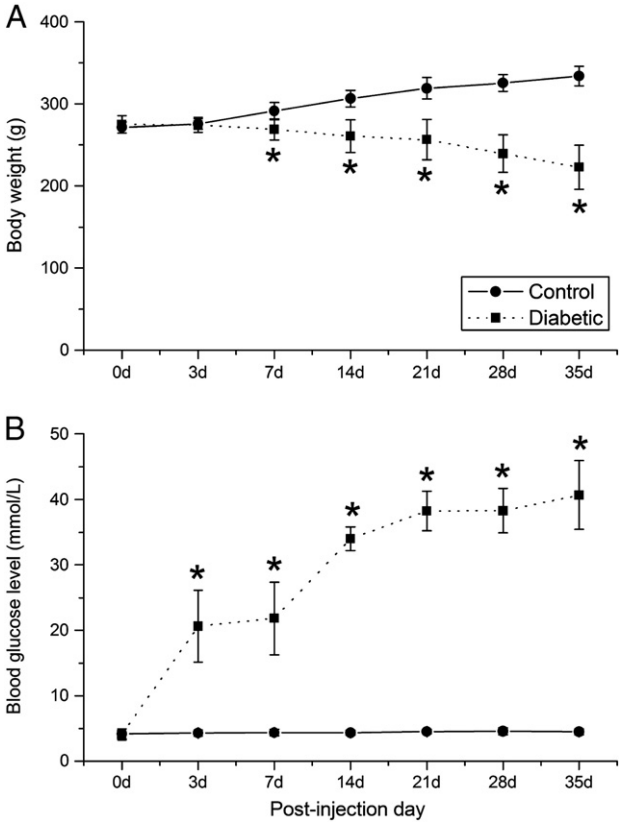

Fig. 1 – Body weight and blood glucose after STZ-injection. (A) Body weight significantly decreased in STZ-injected rats. (B) STZ injection induced rapid hyperglycemia on day 3 and remained at a high level up to 35 days. * $p<0.01$, Diabetic vs Control; # $p<0.05$, after STZ-injection vs baseline (0 day).

2.2. Diabetes impairs spatial learning and memory

After 5 weeks of STZ-injection, the control group and the rats that were diagnosed as diabetic were trained in the Morris Water Maze. Daily, the rats were tested between 0900 h and 1200 h, in a place-learning set paradigm as described in Methods.

The latency and distance traveled to find the platform in either Trial 1 or Trial 2 were shown in Fig. 2. Both the diabetic and control group exhibited significant, substantial reductions in their times ($F=52.097$, $p<0.01$) and distance ($F=26.735$, $p<0.01$) to find the platform over the training sessions. There were significant difference between the two groups ($F=12.805$, $p<0.01$) in both trials. However, there is not significant interaction ($F=1.604$, $p=0.172$). Compared to the control group, diabetic rats took significantly longer to find the platform, implying a significant impairment of learning and memory, and this impairment occurred from training day 2 and persisted through day 5 ($p<0.01$). These results are shown in Figs. 2A–D. Swimming speed was calculated from distance and latency. The swimming speeds of diabetic rats in Trial 2 showed a small decrease which did not quite reach significance ($p=0.337$; Figs. 2E and F).

In the probe trial, the platform was removed and the animal was placed into quadrant 2, which is opposite the target quadrant (quadrant 4). The time that an animal spent in the target quadrant and the number of times that the same animal crossed the former platform area were recorded. During the 120 s of the probe trial, animals in control group spent significantly more time in the target quadrant ($p<0.001$), however, the diabetic rats spent a similar percentage of time in the target quadrant to the other three quadrants, which was lower than that of the control group ($p<0.001$) (Fig. 3). The crossing times of diabetic rats was significantly less than of the control group (2.6 ± 0.5 and 4.9 ± 0.6, respectively, $p<0.05$).

2.3. Ultrastructural changes of CA1 pyramidal neurons

To investigate the possible mechanism of the deficit of learning performance, we used transmission electron microscopy to examine the effects of diabetes on the ultrastructural features of CA1 pyramidal neurons. Observation in the hippocampal CA1 region of the control group revealed the appearance of healthy CA1 pyramidal neurons, displaying a large nucleus, dispersed chromatin, and a well-defined nucleolus (Fig. 4A). Mitochondria were elongated or round and had numerous transversae cristas with a regular pattern and an electron-dense intra-mitochondrial matrix (Fig. 4C). In diabetic rats, CA1 pyramidal neurons showed chromatin aggregate, chromatin clumps appeared in the nucleus (Fig. 4B). The mitochondria were swollen like empty bubbles, and the cristas broken and reduced, and the internal membranes fragmented (Fig. 4D). The axon swelled obviously, and a large gap, increased periaxonal space, is present between axolemma and the surrounding myelin sheath. The synapses in