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Original Article

High expression of Notch 1 with cognitive impairment in a rat model of type 2 diabetes mellitus

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Abstract: Objective: To study the possible role of the Notch 1 signaling pathway in cognitive impairment with hippocampal dendritic structure damage in a rat model of type 2 diabetes mellitus (T2DM). Method: In total, 60 rats with normal learning and memory function were randomly divided into a control group (30 rats) and an experimental group (30 rats). The T2DM model was treated with streptozotocin (STZ) and hippocampal dendritic structure endophenotype damage was established. Results: A rat model of damaged hippocampal dendritic structures was successfully established by feeding animals a high-fat, high-protein feed for eight weeks and intraperitoneally injecting STZ at a dose of 27 mg/kg. Compared with the control group, the mean latency to the platform of rats in STZ-treated group was significantly greater ($P<0.01$). The cross-platform time, time in the platform quadrant, and the ratio of swimming distance in the platform quadrant to total swimming distance was significantly lower among the treated group animals ($P<0.01$). The activity of plasma acetylcholinesterase (AChE) was significantly increased ($P<0.01$), while that of hippocampal AChE was increased ($P<0.01$) in the experimental group. Using light microscopy, very few brown granule deposits expressing Notch 1 were observed in hippocampal neuronal nuclei in the experimental group relative to the control group. Notch 1 integral absorbance was also significantly greater ($P<0.01$) in the experimental group tissues. Conclusions: Cognitive impairments in an animal model of T2DM with hippocampal dendritic structure damage may be associated with increased center AChE activity and activation of the Notch 1 signaling pathway.

Keywords: Notch 1, cognitive impairment, damage of hippocampal dendritic structures

Introduction

Along with the development of society, an increasingly aged population has become a major contemporary problem. As a highly prevalent disease among aged patients, diabetes mellitus is second only to cardiovascular and tumor diseases in terms of the number of associated complications, fatalities, and disability rates [1]. Individuals' cognitive function also declines with age. Statistics in China demonstrate that more than 10.0% of the country's total population is over the age of 65, and the percentage of citizens over the age of 80 in China increases at a rate of 5.0% annually [2]. However, there is currently no ideal drug for the treatment of cognitive impairment in Alzheimer's disease. The current primary means for prevention of cognitive impairment is to ensure good living habits and strengthen their identification ability [3]. It has been reported that acetylcholinesterase (AchE) in neurotransmission pathways, degrades acetylcholine (ACH) and inhibits postsynaptic membrane excitation. Critically, research has shown that AchE has been linked to cognitive impairments in patients [4].

At present, there is no clear consensus on the cause of cognitive impairment, although some scholars have argued that it may be caused by damage to hippocampal dendritic structures [5]. The Notch 1 signaling pathway has been implicated in developmental processes in vertebrates and invertebrates. Research shows that it can precisely regulate developmental processes including differentiation and cellular apoptosis in multiple organs and cell types in the body [6]. Furthermore, Notch signaling has a role in the regulation of embryonic nervous

Table 1. Weight measurement of rats in each group (g)

Group	Control group (n = 30)	Experimental group (n = 30)	t value	*P* value
2 week	275.45±24.74	280.65±22.51	0.851	0.398
4 week	287.36±32.54	298.84±35.63	1.303	0.198
6 week	304.39±36.71	337.60±38.69	3.349	0.001
8 week	325.70±40.55	362.62±43.50	3.400	0.001

system development and is linked to the growth of axons and dendrites, as well as synaptic plasticity [7].

To build upon the existent literature and provide new insights into potential therapeutic targets, this study sought to investigate the effect of the Notch 1 signaling pathway on memory formation in a rat model of type 2 diabetes mellitus (T2DM).

Materials and methods

Animals

In total, 60 male Sprague-Dawley rats were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. Their body weights ranged from 200-240 g and they were housed at room temperature (26°C) under regular light conditions and environmental noise levels (<45 dB) for 1 week before any manipulations were made or experiments performed.

Reagents

A Notch immunohistochemical kit was purchased from Beijing Zhongshan Gold Bridge Biological Company, Beijing, China. Streptozotocin was purchased from Sigma Company, St Louis, MO, USA. Notch 1 polyclonal antibody and the secondary antibody were purchased from Cell signaling Technology, Boston, MA, USA. ELISA assay kit was purchased from Nanjing Jiancheng Biological Company, Nanjing, China.

Establishment of animal model and screening

Rats were placed in the pool quadrant farthest from the platform, facing the pool wall. Animal learning and memory ability was then assessed and based on their Morris water maze performance, animals with weaker learning and memory performance (> 120 s) and those with stronger learning and memory performance (<40 s) were excluded in the experiment. Sixty rats were also randomly divided between a normal feed control group (30 rats) and a high-fat, high-protein feed experimental group (30 rats).

The rats in the experimental group were fed a diet enriched for carbohydrates, fat, protein, and sodium cholate, representing 26.0%, 58.8%, 15.2% and 1% of total dietary calories, respectively, for 8 weeks. The rats were weighed every 2 weeks and their appetite, hair color, other regular activities, and any spontaneous deaths were monitored. Rats in the experimental group were injected intraperitoneally with streptozotocin (27 mg/kg) after being fed the high-fat, high-protein diet for 8 weeks. Rats in the control group were injected with an equivalent volume of saline. Animal blood glucose and insulin content was assayed 3 days after injection to verify establishment of the T2DM rat animal model. Rats were maintained for an additional four weeks after injection administration at the above-mentioned diet and weighed weekly. After 4 weeks, the Morris water maze test was used to assess the learning and memory ability of rats in the two groups.

Morris water maze test

The Morris water maze was used, as has been described previously [8], for the place navigation test and spatial probe test. Time spent crossing the platform location during a probe trial in which the platform was removed, total swim distance in the platform quadrant was measured. A ratio of swimming distance in the platform quadrant to total swimming course was then calculated.

AChE assay

After undergoing Morris water maze testing, rats were anesthetized via intraperitoneal 1% pentobarbital sodium (0.4 mL/kg). Abdominal aorta blood was then collected. Chemical colorimetry was used to test the activity of plasma AChE in rats from each of the two conditions. Rats were euthanized after blood was collected. Part of the hippocampus was dissected, centrifuged to produce a homogenate, and chemical colorimetry was used to test the activity of hippocampal AChE of rats from each of the two groups.

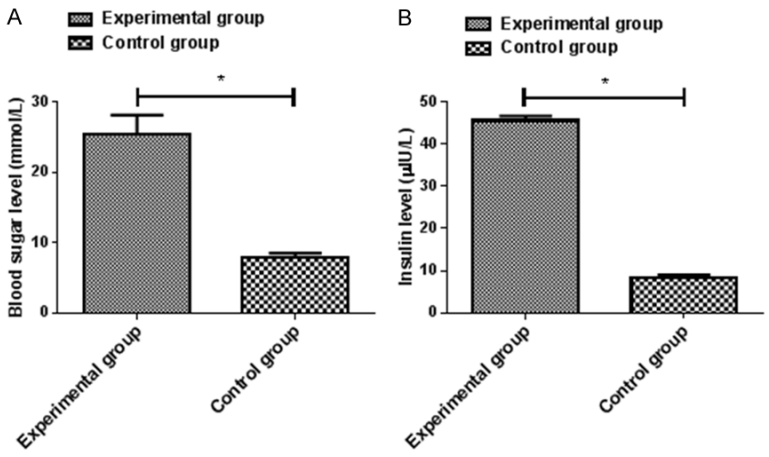

Figure 1. Blood-glucose and Insulin Levels. A. All blood-glucose levels in experimental group animals were > 16.7 mmol/L. Blood-glucose expression levels in the experimental group (25.46±2.68 mmol/L) were, on average, significantly higher (P<0.01) than those in the control group (7.84±0.65 mmol/L). B. Insulin levels in the experimental group (45.62±5.64 μIU/L) were significantly (P<0.01) higher than those in the control group (8.45±3.25 μIU/L).

Table 2. Body weight of rats in two groups 4 weeks after injection (g)

Group	Control group (n = 30)	Experimental group (n = 30)	t value	*P* value
1 week	342.30±20.84	358.52±35.20	2.172	0.034
2 week	358.65±25.60	345.88±23.21	2.024	0.048
3 week	365.44±26.28	340.86±24.68	3.734	0.001
4 week	384.51±28.69	336.58±20.21	7.481	0.001

Preparation of brain tissue sections and Notch 1 assay

After undergoing Morris water maze testing, the left auricle was then cut and sterile 0.9% NaCl followed by 4% paraformaldehyde (250 mL) was perfused into the animal. When the outflow liquid became transparent, rats were decapitated and brain tissues removed, transferred to 4% paraformaldehyde, and fixed for 8 hours. After fixation, tissues were sectioned at 3 μm. The hypersensitive two-step method for immunohistochemistry testing was conducted according to the instructions. Citrate buffer solution treatment (6 minutes each time for 4 times) was used to improve antigen retrieval. Tissues were then incubated in goat anti-mouse primary Notch 1 polyclonal antibody (1:70) overnight at 4°C, followed by 1-hour incubation in the secondary antibody. Sections were then developed with DAB solution. Hematoxylin counterstaining was monitored under a microscope using transparent xylene and sections were sealed with neutral resins.

Statistical analysis

The SPSS17.0 software package was used for data processing and all statistical analysis. All quantitative data are represented by mean ± standard deviation (x±s). A Student's t-test was used for comparisons of group-wise means between the experimental and control groups. All percentages were represented with % and tested using the Chi-square test. P< 0.05 was considered a statistically significant difference.

Results

Model establishment

Food and water consumption of rats in the experimental group after injection of strepozotocin increased when compared to control group animals. No spontaneous deaths occurred in either of the two groups. Animals did not differ in body weight between the two groups. (P > 0.05). However, the body weight of rats in the experimental group significantly increased from week 6 after the altered diet and was significantly higher than those in the control group (P<0.05). Rats in the experimental group got significant weight gain 6 weeks after model establishment by injection of streptozotocin (P<0.01) (**Table 1**).

Blood glucose and insulin assessments

Blood-glucose levels across all 30 rats in the experiment experimental groups were greater than 16.7 mmol/L after 8 weeks on a high-protein, high-fat diet. Seventy-two hours after injection of streptozotocin, blood-glucose levels were 25.46±2.68 mmol/L, significantly higher in the experimental than in the control group animals (7.84±0.65 mmol/L, P<0.01). Insulin levels in the experimental group (45.62±5.64 μIU/L) were significantly higher (P<0.01) than

Table 3. Cross-platform number of rats, time of stay in the original platform quadrant, and swimming distance in total swimming course of rats in two groups

Group	Control group (n = 30)	Experimental group (n = 30)	t value	*P* value
Number of platform crossings (times)	2.24±1.23	7.56±1.64	14.214	0.001
Time (s)	16.61±6.52	43.57±8.84	13.443	0.001
Percentage (%)	25.69±4.25	32.34±7.58	4.191	0.001
Escape latencies (s)	26.36±1.84	55.46±4.51	32.41	0.001

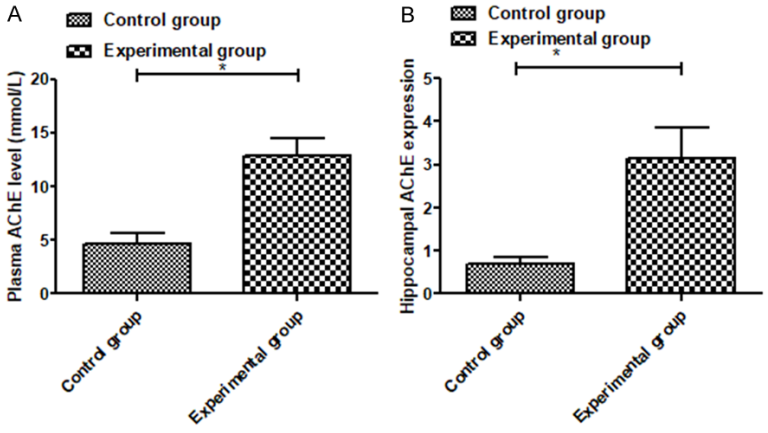

Figure 2. AChE Expression Levels. A. Expression of AChE in control animal plasma was significantly lower (P<0.01) than in experimental group animals. B. Expression of AChE in control group hippocampi was significantly higher (P<0.05) than in experimental group hippocampi.

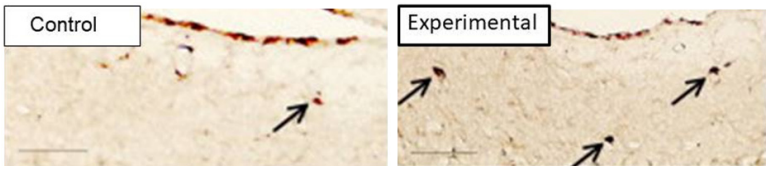

Figure 3. AC hE Expression Levels. Expression of Notch1 in the hippocampus using immunohistochemistry were observed in the experimental group and control group.

that in the control group (8.45±3.25 μIU/L), indicating that the T2DM animal model was successfully established with a high lipoprotein diet and streptozotocin treatment (**Figure 1A** and **1B**).

Memory assessment

Latency to the platform on the 1st and 2nd days of training did not significantly differ between the two groups (P > 0.05). Animals in the experimental group (40.47±4.32 s) had an increased latency compared to those in the control group (26.61±5.42) on the 3rd day of training (P<0.05). The number of platform crossings, time in the platform quadrant, and the percentage of swimming distance to total swimming distance of rats in the experiment group significantly decreased (**Table 2**).

Cognitive function of experimental group

On the 5th day of Morris water maze place navigation training, escape latencies were 55.46±4.51 s and 26.36±1.84 s in the experimental and control groups, respectively. The results show that the experimental group exhibited cognitive impairment (**Table 3**).

Plasma and hippocampal AChE activity

The activity of AChE in plasma and the hippocampus was determined using chemical colorimetry. As shown in **Figure 2A** and **2B**, the expression of AChE in control group plasma was significantly lower (P<0.01) than that in experimental group plasma. However, we found that the expression of AChE in control group hippocampal tissues was significantly higher than that in the experimental group (P<0.05).

Notch 1 expression

In examining expression of Notch in the hippocampus using immunohistochemistry, As shown in **Figure 3** trace amounts of representative of Notch 1 expression were observed in the experimental group while a greater density

of Notch 1 brown sediment particles were observed in hippocampal neurons of experimental group. Notch positive expression integral absorbance in the experimental group was significantly greater than that in the control group

Discussion

Diabetes mellitus (DM) is a metabolic disease caused by elevated blood glucose levels, usually due to insufficient or defective insulin secretion. It is one of the most common chronic metabolic diseases [9, 10]. Survey data shows that there are approximately 93 million individuals suffering from DM in China, making it the country with the most DM sufferers worldwide [11]. Individuals with Type 2 diabetes mellitus (T2DM) account for up to 90% of those with DM [12]. T2DM is a metabolic disease primarily characterized by insulin resistance causing hyperglycemia and hyperinsulinemia [13]. Blood-glucose dysregulation, complications from DM, and many other factors can cause cognitive impairments in DM sufferers. However, how T2DM causes these impairments is unknown. Compared with other pathways, the Notch signaling pathway is a relatively simple pathway, composed of one major receptor, ligand, and a DNA binding protein [14, 15]. Research shows that the Notch signaling pathway serves a diversity of functions and is mainly composed of Notch 1, Notch 2, Notch 3 and Notch 4 in mammals [16].

Here, we have established a T2DM model of cognitive impairment using SD rats. We determined successful establishment of T2DM by assessing blood glucose and insulin expression. Studies show that hyperglycemia and insulin resistance are primarily caused by insufficient or defective synthesis of acetylcholine in the brain [17]. The key enzyme for synthesis of acetylcholine is acetylase, which coexists with insulin. Insufficient levels or inhibited synthesis of insulin will result in decreased expression of acetylcholine, which is also attributed to the pathogenesis of Alzheimer's disease [18]. Our study demonstrates that hippocampal AChE expression in experimental group animals was significantly lower than in controls, but that serum levels were significantly increased.

By determining the hippocampal expression of Notch 1 using immunohistochemistry, we found that expression in the experimental group was significantly higher than that in control group. Synaptic plasticity is considered by a majority of researchers to be the primary memory storage and encoding mechanism in the central nervous system [19]. Research shows that the connection between the Notch signaling pathway and synaptic plasticity-Notch can regulate the maturity of dendrites and dendritic spines [20]. Therefore, we speculate that increased expression of Notch may cause cognitive impairments, such as those seen in our model, and may also be related to reduced synaptic plasticity. Lasky et al. [21] report that long-time memory formation deficits and cognitive impairments occur in fruit flies after mutation of the Notch gene. Mice with a mutated Notch gene also exhibit long-term spatial memory defects, as assessed by Morris water maze test performance. Further work demonstrates that mice with a CSL gene duplication, a key transcription gene in the Notch pathway, also exhibit defects after Morris water maze testing [22]. Another study shows that, by deleting the Notch gene, long-term potentiation is inhibited and hippocampal plasticity is affected, an effect which can be inhibited with the exogenous Notch ligand, Jag-1 [23].

Some limitations are present in the current study including an insufficient sample size. Whether this affects our study results is unknown. Furthermore, as a basic science study, whether these results may have particular clinical applications is yet to be determined. Further studies should examine Notch pathway gene transcription levels and link this to a causal role in diminished cognitive capacity in T2DM individuals.

In conclusion, the cognitive impairments in rats associated with T2DM may be related to increased serum AChE activity and activation of the Notch 1 signaling pathway.

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Disclosure of conflict of interest

None.

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