Behavioural Brain Research 280 (2015) 78–83

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

The dendrites of granule cell layer neurons are the primary injury sites in the "Brain Diabetes" rat

Akiko Sheala Shingo[a,*], Ronald F. Mervis[b], Tomomichi Kanabayashi[c], Shozo Kito[d], Toshio Murase[a]

[a] Okinaka Memorial Institute for Medical Research, Tokyo, Japan
[b] University of South Florida College of Medicine, FL, USA
[c] Biopathology Institute, Ohita, Japan
[d] Chigasaki Tokushu-kai Clinic, Kanagawa, Japan

ARTICLE INFO

Article history:
Received 21 August 2014
Received in revised form
20 November 2014
Accepted 25 November 2014
Available online 2 December 2014

Keywords:
Dendritic spine
Insulin
Streptozotocin
Dentate gyrus

ABSTRACT

We previously demonstrated that rats that receive dorsal third ventricle (3V) streptozotocin (STZ) injections (STZ-3V-rats) exhibit cognitive decline as measured by the Morris Water Maze (MWM) and can be used as an animal model of Alzheimer's disease (AD). Immunohistochemical studies of the hippocampal formations of these animals have revealed significant changes in cerebral insulin signalling pathways, as well as marked increases of amyloid beta (Ab) deposition. Here, we performed Sholl analyses of granule cell layer dendrites and measured dendrite spine densities to assess the effect of STZ on hippocampal morphology. In STZ-3V rats as the results, more branching, complex dendrite arborisation, and increased soma size of the granule cells were observed, while spine densities were decreased in all three spine types. An intraventricular injection of a long-acting insulin analogue improved STZ-induced behavioural and immunohistochemical changes. Nevertheless, dendrite spine densities remained diminished, presumably due to overall null changes since new spine formation due to insulin stimulation has been compensated by loss of old spines. It is concluded that cognitive decline in the "Brain Diabetes" rats is primarily due to impaired intracerebral insulin signalling and the ultimate results were injured excitatory inputs through the perforant pathway.

1. Introduction

Recent epidemiological studies have linked type 2 diabetes mellitus with an increased risk of developing Alzheimer's disease (AD). A significant decrease in glucose utilization has been reported in the brains of AD patients, and this observation has led to the hypothesis that cognitive dysfunction in AD is associated with decreased central glucose metabolism [1], in addition to cholinergic deficit and amyloid accumulation in the brain [2].

We previously demonstrated the effects of streptozotocin (STZ) injection into the dorsal third ventricle (3V) on cognitive performance in rats tested with the Morris water maze (MWM) task and described insulin-related neurochemical changes observed by immunohistochemical analyses of the hippocampus [3,4]. The STZ-3V-treated rats showed significant declines in all the parameters of the MWM task (escape latency, path efficiency, and swim path) compared to control rats except average swimming speed. Immunohistochemical analyses of the hippocampal formations revealed significant decreases in phospho-cyclic AMP response element-binding protein (pCREB), Akt, somatostatin (SST), insulin receptor (IR), and insulin-degrading enzyme (IDE) immunoreactivities, and a significant increase in amyloid beta (Ab) immunoreactivity that was especially marked in the crest of the dentate gyrus. Our experiments confirmed that intraventricular administration of STZ led to cognitive impairment and changes in hippocampal immunohistochemical markers. We concluded that cognitive decline in diabetes was primarily due to impaired intracerebral insulin signalling.

These experiments were followed by an intracerebral injection of a long-acting insulin analogue that successfully rescued

Abbreviations: 3V, dorsal third ventricle; Ab, amyloid beta; AD, Alzheimer's disease; IDE, insulin-degrading enzyme; IR, insulin receptor; MWM, Morris water maze; pCREB, phospho-cyclic AMP response element-binding protein; STZ, streptozotocin; SST, somatostatin.

* Corresponding author at: 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan. Tel.: +81 03 3588 1111x7893; fax: +81 03 3583 3496.
E-mail address: akisheashin@toranomon.gr.jp (A.S. Shingo).

http://dx.doi.org/10.1016/j.bbr.2014.11.041
0166-4328/

the STZ-induced decline in learning ability. Insulin treatment also normalized hippocampal levels of IDE, IR, Akt, SST, and Ab. The STZ-induced decrease of granule cell layer neurons was also recovered by insulin administration as confirmed with Golgi–Cox staining. Such changes following intracerebral STZ injection are considered to be the result of serious impairment in signalling in the granule cell layer which receives inputs from the perforant pathway. Because it is technically challenging to assess the perforant pathway itself, we examined the state of granule cell dendrites by means of Sholl analysis and spine density counting.

The molecular layer of the dentate gyrus is the main entrance for information into the hippocampus, where perforant path axons from the entorhinal cortex synapse onto the spines and dendrites of granule cells [5]. The entorhinal cortex mediates the flow of information through the hippocampal formation and sends back the information processed in the hippocampal formation to the neocortex. Notably, the entorhinal cortex is also the first site affected in the earliest stage of Alzheimer's disease [6].

The present study was undertaken to elucidate the detailed mechanisms of granule cell layer networks in STZ-3V-treated rats before and after intracerebral insulin injection.

2. Materials and methods

2.1. Animals

Four-week-old male Wistar rats (80–100 g) were purchased from Japan SLC, Inc. (Hamamatsu, Japan) for use in the study. The animals had free access to water and standardized food pellets (LabDiet 5002, PMI Nutrition International, St. Louis, MO, USA). They were maintained under a 12/12-h light/dark cycle (lights on 0700–1900). These experiments were approved by the institutional review board and adhered to the National Institutes of Health (NIH) guidelines (Guide for the Care and Use of Laboratory Animals, Bethesda, MD, USA).

2.2. Materials

STZ was purchased from Sigma–Aldrich (St. Louis, MO, USA). Antibodies raised against IDE, Ab (1–42), insulin receptor, SST, and Akt were obtained from Abcam (Cambridge, UK). Nichirei-Histofine® Simple Stain Rat MAX-PO (MULTI) kits were from Nichirei Biosciences Inc. (Tokyo, Japan). The insulin analogue, detemir, was a kind gift from Novo Nordisk Pharma (Bagsværd, Denmark).

2.3. Surgery

For behavioural checks, rats received a single STZ injection (4 mg dissolved in phosphate-buffered saline [PBS]) into the 3V for 2-week treatment (STZ-3V rats) prior to receiving an injection of detemir (0.5 units = 12 nmol) into the same zone (STZ-ins rats). The next day after the behavioural tests were performed to confirm the effects of insulin for 9-day successive trials, brains were extracted and used for morphological studies by Golgi–Cox staining.

The stereotaxic coordinates used for injection were −1.8 mm anterior, 0.0 mm lateral, and −4.0 mm ventral from the bregma. STZ was administered into the 3V to avoid injuring the cerebral cortex (Fig. 1). Intracerebrally STZ-treated rats do not exhibit increased blood sugar or alterations in blood insulin levels. Control animals underwent the same surgery but received PBS instead of STZ. The numbers of STZ-3V, STZ-ins, and control rats used for analysis were 5, 5, and 4, respectively.

2.4. Golgi–Cox staining

Golgi–Cox staining was performed using the different solutions in the FD Rapid GolgiStainTM® kit (FD NeuroTechnologies, Columbia, MD, USA) according to the manufacturer's instructions. After behavioural checks were performed, the brains were extracted and immersed in impregnation solution comprised of equal volumes of Solutions A and B at room temperature for 2 weeks in the dark. The impregnation solution was refreshed after the first 24 h. The tissue was transferred into Solution C and stored at 4 °C for 4 days in the dark. Again, the solution was replenished after the first 24 h of immersion. Sagittal sections were prepared at 80-μm thickness on a cryostat. Each section was stained with Solution D/E mixture and counterstained with cresyl violet before being dehydrated and cleared in xylene prior to mounting.

2.5. Sholl analyses and dendritic branching analyses

Camera lucida drawings of randomly selected granule cells were made on Zeiss bright-field research microscopes equipped with drawing tubes using a 40× oil immersion lens and a 1.25 Optivar (intermediate magnification) setting and a 10× eyepiece for a final magnification of 500×.

Selected neurons had to meet the following criteria: (1) well-stained and (2) branches were not obscured by other neuronal branches, glia, or blood vessels.

Sholl analyses were used to evaluate the amounts and distributions of the dendritic arbours. In this analysis, a template of circles is superimposed on a camera lucida drawing, and the number of intersections of the dendritic branches with each shell is quantified.

Dendritic Branch Point analyses measure dendritic branching complexity. A branch point is defined as when a dendrite branch bifurcates into two sub-branches. A branch emanating from the soma is a primary or first-order branch. When this branch splits into two second-order branches, this occurs at a first-order branch point. A larger number of branch points indicates a more complex dendritic arbour.

Using these two methods, the number of intersections of the dendrites with each shell was quantified, and a profile was generated depicting the amount of dendritic branch material at increasing distances from the soma.

2.6. Dendritic spine analysis

On each neuron, dendritic spines were counted along three internal branch segments. For the quantitative assessment of spines, only visible (flanking) spines emanating from the dendritic branches were counted. Segment lengths were measured using the Zeiss drawing tube and a digitizing tablet with Scios imaging software.

In addition to total spine density (spine number/dendritic segment length), we also evaluated spines in terms of spine configurations, based primarily on the appearance of the spine head and neck. We divided spines into three types: (1) thin spines = thin spine necks and definitive spine heads, (2) mushroom spines = large definitive spine heads and thickened spine necks, and (3) nubby spines = poorly defined spine heads, and thickened spine necks. Spines were counted using a 100× oil-immersion objective and a 2.0 Optivar setting for a final magnification of 2000×.

2.7. Statistical analyses

For both spine counts and dendritic branching, the individual data for each animal were averaged, and the average from each animal was considered as one data point. The average and standard deviation of the mean (SD) were calculated for each group.