Short Communication

# Neurogenesis and memory function via hippocampal insulin signaling independent of the hypoglycemic effect

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## Abstract

The aging systemic milieu leads to a decline in hippocampal neurogenesis and cognitive functions, which also occurs in diabetes. Despite growing concern regarding the potential role of diabetic drugs in neural abnormalities, their effects on progressive deterioration of neurogenesis and cognitive functions remain unknown. Metformin, a biguanide anti-diabetic medication is the firstline drug for type-2 diabetes and lowers blood glucose levels by decreasing basal hepatic glucose output and increasing glucose uptake by skeletal muscle through activation of the AMP-Activated Protein Kinase (AMPK). Here we show that prolonged treatment with metformin enhances hippocampal neurogenesis while countering the microglial activation in the context of the combination of aging and diabetes in mice. Although chronic therapy with metformin fails to achieve recovery from hyperglycemia, a key feature of diabetes, it improves hippocampal-dependent spatial memory functions accompanied by increased serine/threonine phosphorylation of AMPK, Atypical Protein Kinase C ζ (aPKC ζ) and Insulin Receptor Substrate 1 (IRS1), a major mediator of the insulin/IGF1R signaling, in the hippocampus. Our findings suggest that signaling networks acting through long-term metformin-stimulated phosphorylation of AMPK, aPKC ζ/λ and IRS1 serine sites contribute to neuro-protective effects on hippocampal neurogenesis and cognitive function independent of a hypoglycemic effect.

## Introduction

Adult neurogenesis occurs throughout life in specific brain regions of mammals, including the hippocampus and subventricular zone. Alterations in hippocampal neurogenesis are associated with neurological and psychiatric disorders, such as epilepsy, Alzheimer's disease (AD), Parkinson's disease, Huntington's disease (HD), and depression. In addition, the systemic milieu in the context of aging leads to a decline in hippocampal neurogenesis and cognitive functions in animals, which also occurs in model animals with type 1 or type 2 diabetes such as streptozotocin-induced type 1 diabetes model and high-fat-diet (HFD)-induced type 2 diabetes (diet-induced obesity: DIO) model animals. C57BL/6J male mice supplied by Japan SLC, Inc. (Shizuoka, Japan), were maintained at room temperature (25 ± 2 °C) under a standard 12-h/12-h light–dark cycle with free access to water and food. To determine the basic characteristics of DIO mice, C57BL/6J mice were fed an HFD (D12492, 60% kcal from fat; Research Diets, Inc., New Brunswick, NJ, USA) from 4 to 29 weeks of age while age-matched control wild-type (WT) mice were fed a normal diet (CE-2; CLEA Japan, Tokyo, Japan). In the studies using middle-aged DIO mice with metformin treatment (metformin-treated DIO mice), for immunohistochemical analysis and behavioral testing, C57BL/6J mice received the HFD from 4 to 22 weeks of age, followed by the HFD plus chronic treatment with metformin (250 mg•kg−1•day−1) up to 35 weeks of age. For western blotting analysis, C57BL/6J mice were given the HFD from 4 to 34 weeks of age, followed by the HFD plus chronic treatment with metformin up to 45 weeks of age. Compared to metformin-treated DIO mice, age-matched (middle-aged) DIO mice were fed the HFD only. Metformin treatments of WT mice were carried out from 8 or 32 weeks (8 months) of age for 3 weeks, with the metformin being administered in drinking water. To conduct the analysis between young and middle-aged WT mice, C57BL/6J mice were fed a normal diet from 4 weeks of age up to 29 weeks of age. Behavioral and metabolic analyses were performed at 8 weeks of age (young WT mice) and 22–29 weeks of age (middle-aged WT mice). Young WT mice with/without metformin treatment were sacrificed at 10 or 11 weeks of age for each analysis. Middle-aged WT mice, middle-aged WT mice with metformin treatment, middle-aged DIO mice, and middle-aged DIO mice with metformin treatment were sacrificed at 21–45 weeks of age. All mice were fasted for 6 h and anesthetized before sacrifice. All animal experiments were performed in compliance with the guidelines following approval by the Animal Care and Use Committee of Miyazaki University and National Center for Geriatrics and Gerontology (Obu, Japan). Body weight was recorded weekly throughout the study. The amount of food intake in a day was recorded after 24-h fasting. The level of blood glucose at 6-h fasting was measured using a portable glucose meter (ACCU-CHEK® Aviva; Roche DC Japan K.K., Tokyo, Japan). The level of plasma insulin at 6-h fasting was determined using an insulin enzyme-linked immunosorbent assay kit (Morinaga, Yokohama, Japan). The levels of plasma free fatty acid (FFA), triglycerol (TG), total cholesterol (T-CHO), low-density lipoprotein (LDL) cholesterol (LDL-C), and high-density lipoprotein (HDL)-cholesterol (HDL-C) at 6-h fasting were assayed using enzymatic methods (Oriental Yeast Co., Ltd., Tokyo, Japan. Result: Hippocampal neurogenesis is implicated in hippocampal-dependent learning and memory functions. Previous studies have shown that aging and diabetes decrease hippocampal neurogenesis, which involves cognitive impairment in the hippocampus. However, it is unclear to what degree the combination of aging and diabetes affects neurogenesis and memory functions in the hippocampus. Therefore, we investigated hippocampal neurogenesis in middle-aged DIO mice, a physiological type 2 diabetic mouse model. Consistent with the findings in previous studies, middle-aged DIO mice manifested obesity, hyperglycemia, and hyperinsulinemia under our experimental conditions.First, we evaluated cell proliferation in the hippocampal DG of middle-aged DIO mice. Immunostaining with antibodies against BrdU, which is incorporated into DNA during S phase of the cell cycle, or Ki67, a nuclear antigen expressed during the cell cycle, revealed that the number of BrdU-labeled or Ki67-positive cells was profoundly decreased in middle-aged DIO mice compared with that in age-matched WT mice. Next, we examined the impact of HFD-induced diabetes on cell differentiation in DG of the hippocampus in those mice. The number of BrdU-retaining and Dcx (immature neuron marker)-expressing new neurons was remarkably decreased in middle-aged DIO mice compared with that in age-matched WT mice, whereas no significant difference in the total number of Dcx-positive immature neurons was observed between these two groups. Meanwhile, the numbers of newly generated BrdU and S100β (astrocyte marker) double-positive astrocytes and S100β-positive astrocytes were comparable.