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Agmatine ameliorates type 2 diabetes induced-Alzheimer's disease-like alterations in high-fat diet-fed mice via reactivation of blunted insulin signalling

Somang Kang [a, b], Chul-Hoon Kim [c], Hosung Jung [a, b], Eosu Kim [d], Ho-Taek Song [e], Jong Eun Lee [a, b, *]

[a] Department of Anatomy, Yonsei University College of Medicine, Seoul, 120-752, South Korea
[b] BK21 Plus Project for Medical Sciences, and Brain Research Institute, Yonsei University College of Medicine, Seoul, 120-752, South Korea
[c] Department of Pharmacology, Yonsei University College of Medicine, Seoul, 120-752, South Korea
[d] Department of Psychiatry, Yonsei University College of Medicine, Seoul, 120-752, South Korea
[e] Department of Diagnostic Radiology, Yonsei University College of Medicine, Seoul, 120-752, South Korea

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ABSTRACT

The risk of Alzheimer's disease (AD) is higher in patients with type 2 diabetes mellitus (T2DM). Previous studies in high-fat diet-induced AD animal models have shown that brain insulin resistance in these animals leads to the accumulation of amyloid beta (Aβ) and the reduction in GSK-3β phosphorylation, which promotes tau phosphorylation to cause AD. No therapeutic treatments that target AD in T2DM patients have yet been discovered. Agmatine, a primary amine derived from L-arginine, has exhibited anti-diabetic effects in diabetic animals. The aim of this study was to investigate the ability of agmatine to treat AD induced by brain insulin resistance. ICR mice were fed a 60% high-fat diet for 12 weeks and received one injection of streptozotocin (100 mg/kg/ip) 4 weeks into the diet. After the 12-week diet, the mice were treated with agmatine (100 mg/kg/ip) for 2 weeks. Behaviour tests were conducted prior to sacrifice. Brain expression levels of the insulin signal molecules p-IRS-1, *p*-Akt, and *p*-GSK-3β and the accumulation of Aβ and p-tau were evaluated. Agmatine administration rescued the reduction in insulin signalling, which in turn reduced the accumulation of Aβ and p-tau in the brain. Furthermore, agmatine treatment also reduced cognitive decline. Agmatine attenuated the occurrence of AD in T2DM mice via the activation of the blunted insulin signal.

1. Introduction

Clinical and epidemiological studies indicate a higher risk of Alzheimer's disease (AD) among patients with type 2 diabetes mellitus (T2DM) (Craft and Watson, 2004). Previous studies have found a high-fat diet to be common risk factor for T2DM and AD (Edwards et al., 2011; Valls-Pedret and Ros, 2013; Willette et al., 2015). Based on this work, rodents models with various diet-induced AD-like alterations have been established to examine the pathogenesis of AD in T2DM (Arnold et al., 2014; Luo et al., 1998; McNeilly et al., 2011; Stranahan et al., 2008). Using these models, several studies have demonstrated that brain insulin resistance is likely to be the main cause of AD-like alterations (Haan, 2006; Jayaraman and Pike, 2014; Kim and Feldman, 2012; Ma et al., 2015). Insulin signalling is important for various neuronal functions (Belfiore et al., 2009), and may be involved in the regulation of synaptic activities, cognitive processes (Zhao and Alkon, 2001), and learning and memory (Kim and Feldman, 2015). Furthermore, insulin stimulates Aβ extracellular secretion to inhibit its intracellular accumulation (de la Monte, 2012; Gasparini et al., 2001; Pratico et al., 2001; Watson et al., 2003) and blocks GSK-3β via phosphorylation to inhibit neuronal tau phosphorylation (Balaraman et al., 2006; Clodfelder-Miller et al., 2005; Schubert et al., 2004; Takashima, 2006). Therefore, AD may develop when insulin is unable to work in the brain due to brain insulin resistance (Kim and Feldman, 2012).

An adequate therapeutic treatment that targets AD in T2DM patients has not yet been established. Although metformin, which

* Corresponding author. Department of Anatomy, Yonsei University College of Medicine, Seoul, 120-752, South Korea.
E-mail address: jelee@yuhs.ac (J.E. Lee).

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is a popular treatment for T2DM, has been applied to AD, its effects remain controversial (Gupta et al., 2011; McNeilly et al., 2012; Moore et al., 2013; Picone et al., 2015). Meanwhile, we believe that agmatine could be a therapeutic option for treating AD in individuals with T2DM. Altered arginine metabolism is associated with diabetes (Lee et al., 2011) as well as the deterioration of memory functions, similar to those found in AD patients (Liu et al., 2014). Arginine is metabolized into several bioactive molecules, including agmatine. Agmatine, an endogenous aminoguanidine compound made from arginine by arginine decarboxylase, has had positive effects in animal models of several diseases, such as diabetes, stroke, spinal cord injury, and cognitive decline (Ahn et al., 2014; Cui et al., 2012; Park et al., 2013; Song et al., 2014; Su et al., 2009). For example, agmatine has exhibited anti-diabetic effects in type 1 and type 2 diabetic animals (Chang et al., 2010; Hwang et al., 2005; Ko et al., 2008; Su et al., 2009). Several studies have demonstrated the pharmacological potential of agmatine in treating cognitive decline and memory impairment in various animal models (Arteni et al., 2002; Liu and Bergin, 2009; McKay et al., 2002; Moosavi et al., 2014; Rastegar et al., 2011; Zarifkar et al., 2010). Recently, agmatine has been shown to improve memory function in type 1 diabetes-induced memory decline (Bhutada et al., 2012). Also, our previous report revealed that agmatine activates insulin signal transductions in the brain to prevent cognitive decline induced by an intracerebroventricular streptozotocin injection (Song et al., 2014).

Although the effects of agmatine on diabetes and memory impairment have been independently reported, the possible therapeutic effect of agmatine on AD-like alterations in T2DM mice characterized by brain insulin resistance has not yet been investigated. The aim of the present study was to show that the regulation of insulin signalling by agmatine attenuates AD-like alterations in T2DM mice characterized by brain insulin resistance.

2. Materials and methods

2.1. Materials

Agmatine, streptozotocin, and glucose were purchased from Sigma Aldrich (St. Louis, MO, USA). Antibodies against IRS-1, p-IRS-1 (Tyr 632), p-tau (Ser 202, Tyr 205), TNF-α, and IL-1β were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Antibodies for detecting Akt, amyloid beta, and horseradish peroxidase (HRP)-conjugated anti-mouse, anti-rabbit, and anti-goat IgG antibodies were purchased from Abcam (Cambridge, UK). Beta-actin, FITC, or rhodamine-conjugated donkey anti-rabbit or anti-mouse antibodies and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Millipore (Billerica, MA, USA). Other antibodies against *p*-Akt (Ser473), *p*-GSK-3β (Ser9), and GSK-3β were purchased from Cell Signalling Technology (Beverly, MA, USA). The polyvinylidene difluoride (PVDF) membrane for western blot assay was purchased from Millipore. The chemiluminescence reagents (ECL) for western blot assay were from Life Technologies (Carlsbad, CA, USA). The high-fat diet (60% kcal fat) was purchased from Research Diets (New Brunswick, NJ, USA) and normal diet was purchased from LabDiet (St. Louis, MO, USA). The portable glucometer (CareSensII Meter) was purchased from Pharmaco (NZ) Ltd. (Auckland, New Zealand). The serum insulin ELISA was purchased from ALPCO (Windham, NH, USA) and tissue insulin ELISA was purchased from Shibayagi (Gumma, Japan). The amyloid beta ELISA is purchased from Invitrogen (Carlsbad, CA, USA). All the other chemicals used in this experiment were purchased from Sigma Aldrich.

2.2. Establishment of the T2DM mice with AD-like alterations characterized by brain insulin resistance

Adult male ICR mice (7 weeks old, Central Lab Animal Inc., Seoul, Korea) were used in this study. The mice were raised in a standard laboratory animal facility under a 12 h light/dark cycle and had free access to food and water *ad libitum*. All procedures were conducted in accordance with the Yonsei University College of Medicine Animal Care and Use Committee and the National Institutes of Health guidelines for the Care and Use of Laboratory Animals. We modified previously established methods (Byrne et al., 2015; Jiang et al., 2012; Luo et al., 1998; Rahigude et al., 2012; Tahara et al., 2011)to develop a T2DM mouse model with AD-like alterations characterized by brain insulin resistance. After a week of acclimatization to the laboratory conditions, mice were randomly divided into two groups. Mice were administered either a normal chow diet (NC; 13.1% kcal fat) or a high-fat diet (HFD; 60% kcal fat) for 12 weeks (Table 1). The mice fed HFD were injected once at week 4 with a low dose of streptozotocin [STZ; 100 mg/kg/ip, dissolved in citrate buffer (pH 4.4)] to shorten the time taken for the animal model to be established by inducing partial insulin deficiency (Fig. 1).

Mice with fasting serum glucose level >200 mg/dl, body weight >55 g, and impaired glucose, insulin tolerance were classified as T2DM (Tabak et al., 2012). T2DM mice were divided into two groups: HFD mice treated with saline and HFD mice treated with agmatine (HFD + AGM; 100 mg/kg/ip, dissolved in saline). These groups were treated with agmatine daily for 2 weeks (Fig. 1). Twelve mice were included in each group (a total of 36 mice were used).

2.3. Determination of body weight and serum glucose levels

Body weights (BW) and fasting serum glucose levels (Piletz et al., 2013) of all animals were monitored weekly. To measure fasting glucose levels, mice were fasted for 4 h before the test. Blood glucose concentrations from blood samples taken from the tip of the tail were measured using a glucometer.

2.4. Intraperitoneal glucose tolerance test (IPGTT)

Glucose tolerance test is a widely used clinical test to diagnose glucose intolerance and T2DM (American Diabetes, 2007; Muniyappa et al., 2008). Food was removed a night before the test. The mice were injected with glucose (2 g/kg/ip, dissolved in saline). Blood glucose levels from blood samples taken from the tip of the tail were measured using a glucometer at 0, 30, 60, and 120 min after the bolus. The area under the concentration versus time curve (AUC glucose 0–120 min, mg/dl * minutes) was calculated.

2.5. Intraperitoneal insulin tolerance test (IPITT)

Mice were fasted for 4 h before the test. The mice were injected with insulin (0.75 U/kg/ip, dissolved in saline). Blood glucose levels from blood samples taken from the tip of the tail were measured using a glucometer at 0, 15, 30, 60, and 120 min after the bolus. The

Table 1
Diet composition.

	Normal diet	High fat diet
Protein (kcal %)	24.5	20
Carbohydrate (kcal %)	62.4	20
Fat (kcal %)	13.1	60