Physiology & Behavior 140 (2015) 15–22

Contents lists available at ScienceDirect

Physiology & Behavior

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

Genistein improves spatial learning and memory in male rats with elevated glucose level during memory consolidation

Yumi Kohara [a], Shinichiro Kawaguchi [b], Rika Kuwahara [c], Yutaro Uchida [a], Yushi Oku [a], Kimihiro Yamashita [c,*]

[a] Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, Nagasaki 852-8521, Japan
[b] Graduate School of Science and Technology, Nagasaki University, Nagasaki 852-8521, Japan
[c] Division of Environmental Chemistry and Ecotoxicology, Institute of Environmental Studies, Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, Nagasaki 852-8521, Japan

HIGHLIGHTS

- Genistein improved spatial learning and memory with limited effect in normal rats.
- Genistein improved spatial learning and memory at elevated blood glucose.
- Genistein had no effects on emotionality or aversive memory.

ARTICLE INFO

Article history:
Received 1 May 2014
Received in revised form 25 November 2014
Accepted 2 December 2014
Available online 4 December 2014

Keywords:
Genistein
Phytoestrogen
Glucose load
Oral administration
Rat
Spatial learning

ABSTRACT

Cognitive dysfunction due to higher blood glucose level has been reported previously. Genistein (GEN) is a phytoestrogen that we hypothesized might lead to improved memory, despite elevated blood glucose levels at the time of memory consolidation. To investigate this hypothesis, we compared the effects of orally administered GEN on the central nervous system in normal versus glucose-loaded adult male rats. A battery of behavioral assessments was carried out. In the MAZE test, which measured spatial learning and memory, the time of normal rats was shortened by GEN treatment compared to the vehicle group, but only in the early stages of testing. In the glucose-loaded group, GEN treatment improved performance as mazes were advanced. In the open-field test, GEN treatment delayed habituation to the new environment in normal rats, and increased the exploratory behaviors of glucose-loaded rats. There were no significant differences observed for emotionality or fear-motivated learning and memory. Together, these results indicate that GEN treatment improved spatial learning and memory only in the early stages of testing in the normal state, but improved spatial learning and memory when glucose levels increased during memory consolidation.

1. Introduction

Genistein (GEN) is a naturally occurring phytoestrogen present in soy, with a higher binding affinity to estrogen receptors (ER) compared with other phytoestrogens [1–3]. It has been reported that GEN can be detected in the brain soon after intraperitoneal (i.p.) administration [4], suggesting that GEN can pass through the blood–brain barrier and affect the central nervous system (CNS).

A number of studies have shown that GEN has a neuroprotective or memory-improving effect in animal models of Alzheimer's disease [5] and global cerebral ischemia [6] and in ovariectomised (OVX) rats [7–9]. However, few studies have discussed the effects of GEN on learning and memory in the normal state. To understand the effects of GEN on neuronal functioning, it is important to study its effects in normal animals.

Diabetes is the most common serious metabolic disorder in humans [10], and is associated with long-term complications that affect the eyes, kidneys, heart, blood vessels and nerves [10]. Cognitive dysfunction due to diabetes has been reported previously [11,12]. A recent study revealed that the increased oxidative stress in diabetes produces oxidative damage in many regions of rat brain including the hippocampus [13].

It has been previously reported that GEN decreases plasma glucose levels in streptozotocin (STZ)-induced diabetic rats [14]. Furthermore, GEN also ameliorates hyperglycemia in a mouse model of type 2 diabetes [15]. This suggests that GEN might be an effective antidiabetic agent [16].

However, it has not been reported the effects of GEN on learning under the state that the blood glucose level is elevated. We thought that the investigation about this point will be important on thinking about the effects of GEN on the prevention of the cognitive decline by

* Corresponding author.
E-mail address: kimihiro@nagasaki-u.ac.jp (K. Yamashita).

http://dx.doi.org/10.1016/j.physbeh.2014.12.005
0031-9384/

the elevation of blood glucose level. Therefore, we performed simultaneous glucose load and GEN treatment in adult male rats. Specifically, this study employed a battery of behavioral tests to investigate the effects of GEN on the CNS in normal versus elevated blood glucose states, at the time of memory consolidation in male rats.

2. Materials and methods

2.1. Animals

We used male Sprague–Dawley (SD) rats, which were obtained at 5 weeks of age from Kyudo Co. Ltd. (Kumamoto, Japan). All animals were maintained in a 12:12-h light–dark cycle (lights on from 0700 to 1900) at 22 ± 2 °C and 55 ± 10% humidity.

The animals were food restricted (12 g/day food and 33.3 mL/day water per rat) from 6 weeks of age, to increase the motivation for reward in MAZE test which was an appetite-motivated MAZE test. Once a week, food restrictions were lifted to avoid an excessive reduction in body weight. Experimentations were conducted as follows (Fig. 1).

Animal care and experimental procedures were performed in accordance with the Guidelines for Animal Experimentation of Nagasaki University, with the approval of the Institutional Animal Care and Use Committee.

2.2. Drug administration

GEN was purchased from LKT Laboratories Inc. (Minnesota, USA). Rats were divided into six groups: three groups each of normal and glucose-loaded rats, comprising a vehicle, a 1 mg/kg/day of GEN (1 mg/kg GEN), and a 10 mg/kg/day of GEN (10 mg/kg GEN) group. Vehicle groups received 0.5% Carboxymethyl Cellulose Sodium Salt (CMC-Na; Wako Pure Chemical Industries, LTD., Osaka, Japan), while other groups were administered GEN dissolved in this solution. For the glucose-loaded groups, rats were administered 20% glucose solution to elevate blood glucose level (D (+)-glucose; Wako Pure Chemical Industries, LTD., Osaka, Japan) at a rate of 1 g/kg body weight. Glucose was administered by intraperitoneal injection to avoid gastric physical stimulation because the MAZE test, used subsequently, was an appetitive-motivated task. Oral administrations (1 mL/kg/day) of vehicle or GEN were conducted by feeding needles. After this administration, the glucose solution was administered immediately. All administrations were performed under light anesthesia using halothane (Fluothane, Takeda Pharmaceutical Co. Ltd., Tokyo, Japan).

For the MAZE test, drugs were administered within 30 min following training or each testing. For the open-field and elevated plus maze test, drugs were administered the day before the tests. In the step-through passive avoidance test, drugs were administered within 30 min following the training session.

2.3. The open-field test

We assessed locomotor activity, emotionality, and exploratory behavior in rats using the open-field test, following the method previously described by Hall [17]. The open-field apparatus had a circular bottom 60 cm in diameter, and an enclosing wall 50 cm high. The floor of the apparatus was illuminated by a light (100 W) placed 80 cm above the floor, and was divided by black lines into 19 equal regions (Fig. 2). The form of divisions was slightly different, but its area was mostly same. The open-field consisted of an inner circle (placed 12–30 cm from the wall) and an outer ring (placed 0–12 cm from the wall). Rats were placed on the center of the floor, and then ambulation (total number of times the black lines were crossed), inner-cross (number of times the black lines were crossed in the inner circle), and rearing (number of times the rat stood up on its hind legs) were counted for 3 min. These events were measured three times, with a two-hour interval. Ambulation, inner-cross, and rearing were used as indices of locomotor activity, emotionality, and exploratory behavior, respectively. The test was conducted when rats were 8 weeks old.

2.4. The elevated plus maze test

Anxiety was measured using the elevated plus maze test [18]. This maze consisted of apparatus in the form of a plus sign, with two closed arms surrounded by walls 60 cm in height and two open arms (no wall), and placed 60 cm above the floor. Each arm was 50 cm × 10 cm, painted black, and connected to a central neutral zone (14 cm × 14 cm). In each trial, rats were placed in the neutral zone facing an open arm, and the total number and total time spent in each arm were measured for a

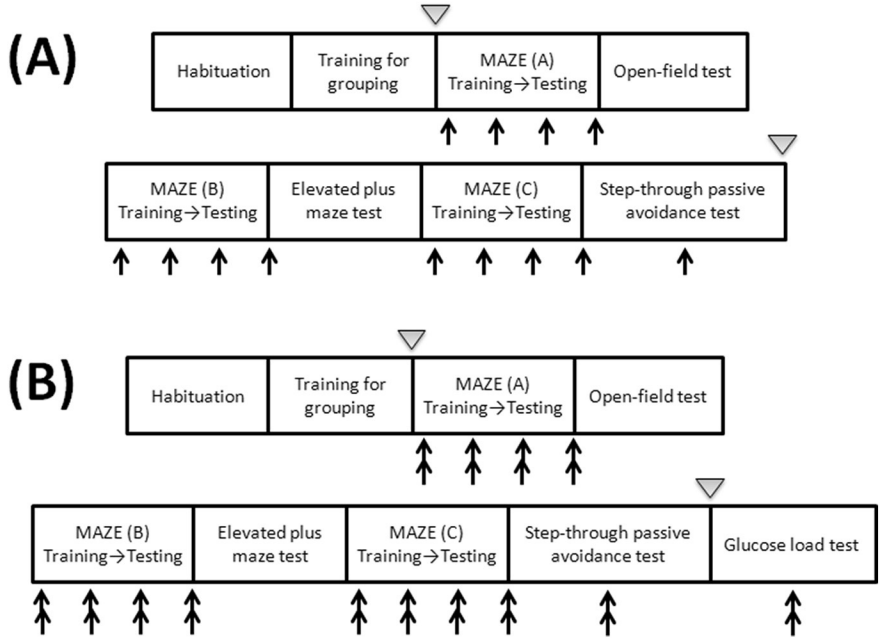

Fig. 1. Experimental procedures. Rats received either oral administration only (A) or oral administration + intraperitoneal (i.p.) injection of 20% glucose solution (B) under the schedule of normal (A) or glucose-loaded group (B). The arrow and the inverted-triangle indicate the points of administration and measuring the fasting blood glucose level, respectively.