Behavioural Brain Research 292 (2015) 36–43

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Betaine prevents homocysteine-induced memory impairment via matrix metalloproteinase-9 in the frontal cortex

Kunisawa K.[a], Nakashima N.[a], Nagao M.[a], Nomura T.[a], Kinoshita S.[a], Hiramatsu M.[a,b,*]

[a] Department of Chemical Pharmacology, Faculty of Pharmacy, Graduate School of Pharmacy, Meijo University, Nagoya, Japan
[b] Laboratory of Neuropsychopharmacology, Graduate School of Environmental and Human Sciences, Meijo University, Nagoya, Japan

HIGHLIGHTS

- Betaine has beneficial effects on acute stress and lipopolysaccharide-induced memory impairment. In the present study, we investigated whether betaine ameliorates Hcy-induced memory impairment and the underlying mechanisms of this putative effect.
- Betaine suppressed the memory impairment induced by repeated Hcy injections.
- Treatment with betaine significantly inhibited Hcy-induced MMP-9 activity in the frontal cortex but not in the hippocampus after acute Hcy injection.
- These results suggest that the changes in MMP-9 activity after betaine treatment might have been partially responsible for the amelioration of the memory deficits and that MMP-9 might be a candidate therapeutic target for HHcy.

ARTICLE INFO

Article history:
Received 18 February 2015
Received in revised form 29 May 2015
Accepted 3 June 2015
Available online 6 June 2015

Keywords:
Betaine
Homocysteine
Hcy
Memory
Matrix metalloproteinase-9
MMP-9
Frontal cortex

ABSTRACT

Betaine plays important roles that include acting as a methyl donor and converting homocysteine (Hcy) to methionine. Elevated plasma Hcy levels are known as hyperhomocysteinemia (HHcy) and contribute to impairments of learning and memory. Although it is commonly known that betaine plays an important role in Hcy metabolism, the effects of betaine on Hcy-induced memory impairment have not been investigated. Previously, we demonstrated the beneficial effects of betaine on acute stress and lipopolysaccharide-induced memory impairment. In the present study, we investigated whether betaine ameliorates Hcy-induced memory impairment and the underlying mechanisms of this putative effect. Mice were treated with Hcy (0.162 mg/kg, s.c.) twice a day for nine days, and betaine (25 mg/kg, s.c.) was administered 30 min before the Hcy injections. The memory functions were evaluated using a spontaneous alternation performance test (Y-maze) at seven days and a step-down type passive avoidance test (SD) at nine and ten days after Hcy injection. We found that betaine suppressed the memory impairment induced by repeated Hcy injections. However, the blood concentrations of Hcy were significantly increased in the Hcy-treated mice immediately after the passive avoidance test, and betaine did not prevent this increase. Furthermore, Hcy induces redox stress in part by activating matrix metalloproteinase-9 (MMP-9), which leads to BBB dysfunction. Therefore, we tested whether betaine affected MMP-9 activity. Interestingly, treatment with betaine significantly inhibited Hcy-induced MMP-9 activity in the frontal cortex but not in the hippocampus after acute Hcy injection. These results suggest that the changes in MMP-9 activity after betaine treatment might have been partially responsible for the amelioration of the memory deficits and that MMP-9 might be a candidate therapeutic target for HHcy.

1. Introduction

Betaine is widely distributed in plants, microbes and various dietary sources [1,2]. Betaine is utilized as a methyl donor in a reaction that converts homocysteine (Hcy) into methionine via betaine-homocysteine methyltransferase (BHMT; EC 2.1.1.5) [3]. Indeed, betaine supplementation can effectively facilitate the metabolism of Hcy and decrease plasma Hcy levels in healthy subjects [4–6].

Abbreviations: AD, Alzheimer disease; BBB, blood–brain barrier; BGT-1, betaine/GABA transporter-1; BHMT, betaine-homocysteine methyltransferase; COX-2, cyclooxygenase-2; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; GAT, GABA transporter; Hcy, homocysteine; HHcy, hyperhomocysteinemia; iNOS, inducible nitric oxide synthase; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; SD, step-down type passive avoidance test; TNF-α, tumor necrosis factor-α; Y-maze, spontaneous alternation performance test.

* Corresponding author. Correspondence Department of Chemical Pharmacology, Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tenpaku-ku, Nagoya 468-8503, Japan. Fax: +81 52 834 8780.

http://dx.doi.org/10.1016/j.bbr.2015.06.004
0166-4328/

Hcy is a thiol-containing excitatory amino acid that enhances the susceptibility of neurons to excitotoxic and oxidative injuries [7]. Hcy also alters hippocampal plasticity and synaptic transmission resulting in learning and memory deficits [8,9]. Because elevated plasma Hcy levels known as hyperhomocysteinemia (HHcy) contribute to neurodegenerative diseases [10,11], homocysteine-decreasing therapies are potentially beneficial. However, the role of betaine treatment in Hcy-induced memory impairment has not yet been studied.

It is important to note that the increase in oxidative stress that follows Hcy treatment plays an essential role in the dysfunction of the blood–brain barrier (BBB) due to the activation of matrix metalloproteinases (MMPs) [12,13]. MMPs have a key role in the degradation of extracellular matrix (ECM) components within the basement membranes around cerebral blood vessels and neurons. MMPs are synthesized as pre-enzymes, secreted from cells as pro-enzymes, and activated by other proteases and free radicals in the extracellular compartment [14]. Specifically, several authors have suggested that pathological concentrations of Hcy exclusively increase MMP-9 production [15–17] and macrophages [18] in brain injury. Thus, reducing MMP-9 is an attractive strategy for combating Hcy-induced neurological diseases. Additionally, these reports suggest that MMP-9 might be involved in the memory impairments caused by Hcy injection.

Dietary betaine suppresses the activation of nuclear factor-κB (NF-κB) that is induced by oxidative stress and also suppresses protein expressions of proinflammatory molecules, such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and tumor necrosis factor (TNF)-α, in aged rat kidneys [19,20]. However, the effects of betaine on central nervous system function in animals are poorly understood. Previously, we found that treatment with betaine improves the memory impairment induced by acute stress [21,22] and increases the expression of the mRNAs of GABA transporter 1 (GAT1), GAT2, and GAT3 in the hippocampus [21]. The mouse transporter homologue of GAT2 is known as the betaine/GABA transporter-1 (BGT-1), is this transporter in an integral membrane transporter that is capable of utilizing both betaine and GABA as substrates [23,24]. Interestingly, because GAT2 is co-localized with P-glycoprotein, which is BBB-specific marker, in brain capillaries [25], it might also be involved in betaine transport across the BBB and the regulation of MMP-9 activation in HHcy-level animals. In the present study, we explored the role of betaine treatment in Hcy-induced memory impairment and asking whether MMP-9 is involved in the neuroinflammatory response and whether betaine supplementation is a promising novel strategy for the prevention of Hcy-induced neurotoxicity.

2. Materials and methods

2.1. Animals

Male ddY strain mice (7–9 weeks old, 28–40 g; Japan SLC, Shizuoka, Japan) were used. The mice were housed in groups of four or five per cage (28 × 44 × 20.5 cm) and were maintained in a regulated environment (24 ± 1 °C, 55 ± 5% humidity) on a 12-h light/dark cycle (lights on 7:00 a.m.). The animals received food and tap water ad libitum. The experimental protocols concerning the use of the laboratory animals were approved by the Animal Ethics Board of Meijo University and followed the guidelines of the Japanese Pharmacological Society (Folia Pharmacol. Japan, 1992, 99: 35A), the Interministerial Decree of May 25th, 1987 (Ministry of Education, Japan), and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). All efforts were made to minimize animal suffering and reduce the number of animals used.

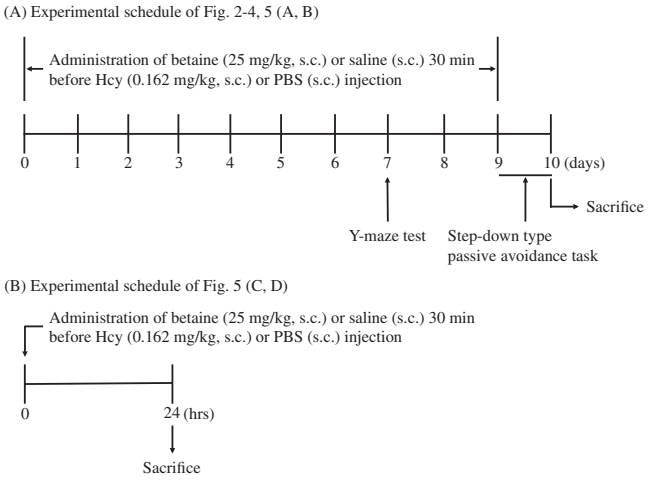

Fig. 1. Experimental protocols.

2.2. Drug challenges

Betaine hydrochloride (betaine; 25 mg/kg, Sigma, St. Louis, USA) was dissolved in 0.9% saline. DL-homocysteine (Hcy; 0.162 mg/kg, Nacalai tesque, Kyoto, Japan) was dissolved in phosphate-buffered saline (PBS). Betaine was administered 30 min before Hcy injection, and both drugs were injected subcutaneously (s.c.). Drug or vehicle was administered to the mice in volumes of 0.1 ml/10 g body weight. In the repeated administration schedule, the mice were treated with betaine and/or Hcy twice per day from days 1 to 9, with the exceptions of days 7 and 9. The performances in the spontaneous alternation and passive avoidance tests were examined on day 7 and/or days 9 and 10 after Hcy injection, respectively. On the experimental days, the mice were treated with drugs once after the behavioral experiments. The animals were sacrificed immediately after the passive avoidance tests to evaluate the Hcy and MMP-9 levels. On the acute administration schedule, the mice were administered a single dose of betaine 30 min before Hcy and sacrificed 24 h after the drug treatment. The sham control animals were injected with vehicle (s.c.) the same number of times as the drug-injected animals.

The experimental schedules are shown in Fig. 1. The behavioral and biochemical experiments were conducted as described below and based on our previous studies.

2.3. Behavioral testing

2.3.1. Spontaneous alternation performance

Immediate working memory was assessed by recording spontaneous alternation behavior during a single session in a Y-maze [26] made of black painted wood. Each arm was 40 cm long, 12 cm high, 3 cm wide at the bottom, 10 cm wide at the top, and converged in an equilateral triangular central area. The procedure was similar to that described previously [27]; each mouse, none of which had any prior experience with the maze, was placed at the end of one arm and allowed to move freely through the maze during an 8-min session, and the arm entries were counted. Each series of arm entries was recorded visually, and an arm entry was defined as the hind paws of the mouse being completely within the arm. Alternation was defined by successive entries into the three arms in overlapping triplet sets. The percentage alternation was calculated with the following formula:

$$\frac{\text{number of alternations}}{\text{total number of arm entries} - 2} \times 100\%$$