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Research report

Disturbance of endogenous hydrogen sulfide generation and endoplasmic reticulum stress in hippocampus are involved in homocysteine-induced defect in learning and memory of rats

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HIGHLIGHTS

- Exposure of rats to homocysteine (Hcy) leads to learning and memory dysfunctions.
- Hcy decreases the generation of endogenous H_2S in the hippocampus of rats.
- Hcy up-regulates the endoplasmic reticulum (ER) stress in the hippocampus of rats.
- Disturbed H_2S formation and ER stress involve in Hcy-caused memory disorder.

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ABSTRACT

Homocysteine (Hcy) is a risk factor for Alzheimer's disease (AD). Hydrogen sulfide (H_2S) acts as an endogenous neuromodulator and neuroprotectant. It has been shown that endoplasmic reticulum (ER) stress is involved in the pathological mechanisms of the learning and memory dysfunctions and that H_2S exerts its neuroprotective role *via* suppressing ER stress. In the present work, we explored the effects of intracerebroventricular injection of Hcy on the formation of learning and memory, the generation of endogenous H_2S, and the expression of ER stress in the hippocampus of rats. We found that intracerebroventricular injection of Hcy in rats leads to learning and memory dysfunctions in the Morris water maze and novel of object recognition test and decreases in the expression of cystathionine-β-synthase, the major enzyme responsible for endogenous H_2S generation, and the generation of endogenous H_2S in the hippocampus of rats. We also showed that exposure of Hcy could up-regulate the expressions of glucose-regulated protein 78 (GRP78), CHOP, and cleaved caspase-12, which are the major mark proteins of ER stress, in the hippocampus of rats. Taken together, these results suggest that the disturbance of hippocampal endogenous H_2S generation and the increase in ER stress in the hippocampus are related to Hcy-induced defect in learning and memory.

1. Introduction

Homocysteine (Hcy) is a thiol-containing amino acid formed by demethylation of methionine [1,2]. Elevated plasma homocysteine levels were found to be associated with increased risk of neuronal cells [2–9]. More than 40% Alzheimer's disease (AD) patients were found to have high Hcy levels in the plasma and the patients with high plasma Hcy levels displayed more rapid neural atrophy than those with normal levels of Hcy by epidemiological studies, which has accumulated that elevated plasma Hcy is a strong, independent risk factor of AD [10–14]. It has reported that treatment of rats with Hcy for 2 weeks could induce the deficit in spatial memory [15]. Therefore, preventing the neurotoxicity of Hcy may be a novel

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therapeutic strategy for AD. However, the underlying mechanisms that Hcy-induced AD-like lesions were not investigated clearly.

Hydrogen sulfide (H_2S) is the third endogenous signaling gasotransmitter, besides nitric oxide and carbon monoxide [16,17]. H_2S plays an important role in brain functions, probably acting as a neuromodulator as well as a neuroprotectant [18–20]. In the mammalian brain, H_2S is formed from the amino acid cysteine by the action of cystathionine beta-synthase (CBS) [16,17,21], the key enzyme in the transsulfuration pathway that processes Hcy. It has been observed that H_2S was decreased, but that Hcy was increased in AD brains [22]. Our previous studies have shown that Hcy-associated neurotoxicity is due to reduced endogenous generation of H_2S [23] and that H_2S directly antagonizes the toxicity of Hcy to neuronal cells [24]. Therefore, we want to know whether Hcy-impaired learning and memory involves in the disturbance of H_2S generation.

The endoplasmic reticulum (ER) lumen is a unique oxidative environment, critical for protein folding and formation of disulfide bonds [25]. Excessive and prolonged ER stress can trigger cell death [26]. Glucose-regulated protein 78 (GRP78), C/EBP homologous protein (CHOP), and cleaved caspase 12 are molecular markers of ER stress. ER stress is considered to be an important mechanism involved in neurodegenerative diseases, including AD [27]. Recently, ER stress was proposed to explain the pathogenic effects of Hcy [28–31], which may be a common pathway of injury of tissues and cells induced by Hcy. Interestingly, Wei et al. has reported that H_2S could antagonize cardiomyocytic ER stress in Hcy-induced cardiomyocytic injury [31]. Given the importance of Hcy in the pathogenesis of AD, altered endogenous production of H_2S in Hcy exposure, and the antagonistic role of H_2S in Hcy-induced ER stress, it is conceivable that Hcy-impaired learning and memory involves the disturbance of H_2S generation, which in turn causes ER stress in the hippocampus of rats.

In this study, we demonstrated that intracerebroventricular exposure of brain to Hcy impairs the learning and memory in Morris water maze and novel object recognition test, inhibits the generation of hippocampal H_2S, and up-regulates the expressions of GRP78, CHOP, and cleaved caspase12 in the hippocampus. Our study implied that the disturbance of H_2S generation is involved Hcy-induced deficit in learning and memory and that the ER stress in the hippocampus may be a potential contributing mechanism.

2. Materials and methods

2.1. Reagents

Hcy [dissolved in phosphate-buffered saline (PBS)] was purchased from Sigma Chemical Co (St. Louis, MO, USA). Specific monoclonal anti-CBS was purchased from Sant Cruz Biotechnology, Inc (Sant Cruz, CA, USA). Specific monoclonal anti-GRP78 and anti-CHOP antibodies were purchased from Epitomic Inc (Burlingame, UK). Specific monoclonal anti-Caspase-12 antibody was obtained from Sigma Chemical (St. Louis, MO, USA).

2.2. Animals

The male Sprague-Dawley rats (250–280 g), supplied by the SJA Lab Animal Center of Changsha (Changsha, China), were individually housed with free access to food and water under a 12:12 h reversed light–dark cycle. After being housed, the rats were handled for 1 week to habituate them to the experimenter. All animal experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Use and Protection Committee of University of South China.

2.3. Intracerebroventricular injection

PBS or Hcy (0.2, 0.6, or 2 μmol) in 2.5 μl was injected into the bilateral ventricle at the following coordinates: anterior/posterior −1.4 mm; medial/lateral 1.8 mm; dorsal/ventral −3.0 mm, from Bregma, with an injection rate of 0.5 μl per min under the control of micropump, respectively [32]. In order to make sure that the entire injection had been delivered, the injection cannula was allowed to remain in place for an additional minute before being removed.

2.4. Spatial learning and memory tests

The water maze was a circular pool (diameter 180 cm, height 60 cm). The water temperature (23 ± 2 °C), light intensity, external cues in the room, and water opacity were rigorously reproduced. A transparent Plexiglas non-slippery platform (diameter 12.5 cm) was immersed under the water surface (2 cm) during acquisition trials. Swimming was recorded using a camera capture, and analyzed using video track software. The software divided the pool into four quadrants. Several landmarks were fixed to the walls of the water maze room as the distal spatial extra-maze cues for the rats to find the platform.

2.4.1. Acquisition trail

The place navigation training consisted of four swims per day for 7 days, with about a 20-min intertrial time. Four start positions were pseudo-randomly selected across the four trials each day, and each animal was allowed a 120-s swim to find the platform. Once the rat reached the platform, it was left 20 s on the platform; if an animal did not reach the platform within 120 s, it was guided to the platform to remain for 20 s. The path and the escape latencies were recorded by an MT-200 Morris image motion system (Chengdu Technology and Market Corp, Chengdu, China).

2.4.2. Probe trail

The probe test was performed 24 h after the last swim on day 7. The platform was removed and each animal was allowed a free 120-s swim. The start position for each mouse corresponded to one of two positions remote from the platform location in counterbalanced order. The platform quadrant was termed the target quadrant. The percentage of time spent in the target quadrant and the number of times that the same animal crossed the former platform area were determined.

2.4.3. Visible platform test

After the probe test, visual, motor, and motivation skills were also tested with a visible platform to rule out the possible deficits in sensorimotor processes. The platform was raised 2 cm above the water surface. The platform was moved to a novel quadrant in the pool at a fixed location for the four consisted trials, and the latency to reach the platform and the average speed were recorded.

2.5. Novel Objects Recognition (NOR) test

The NOR test consisted of two sessions: a training session followed by a retention trial 24 h later. Two days prior to training, rats were habituated to the arena (50.0 × 50.0 × 60) for 5 min once a day in the absence of objects. During the training session, two different objects (A and B) were placed in the testing arena. Each animal was allowed to explore the objects for 5 min. The rat was considered to be exploring the object when the head of the animal was facing the object, or the animal was touching or sniffing the object. The total time spent exploring each object was recorded by a trained observer blind to treatments, and expressed as percentage of total exploration time. In the retention session, one identical and one novel object (A and C) were used. A rat was allowed to explore