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Rapid Communication

High-salt diet enhances hippocampal oxidative stress and cognitive impairment in mice

Yun-Zi Liu [a,b,1], Ji-Kuai Chen [c,1], Zhang-Peng Li [c], Ting Zhao [c], Min Ni [c], Dong-Jie Li [a], Chun-Lei Jiang [b], Fu-Ming Shen [a,c,*]

[a] Department of Pharmacy, Shanghai Tenth People's Hospital, Tongji University, Shanghai 200072, China
[b] Laboratory of Stress Medicine, Faculty of Psychology and Mental Health, Second Military Medical University, Shanghai 200433, China
[c] Department of Pharmacology, School of Pharmacy, Second Military Medical University, Shanghai 200433, China

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ABSTRACT

Previous evidence suggests that a high-salt (HS) diet may increase oxidative stress and contribute to the development of hypertension that is already present. Oxidative stress is thought to play a critical role in the development of neurodegenerative diseases. Lower dietary sodium intake putatively contributes to a lower rate of cognitive impairment; however, the specific effects of HS diet on cognitive function remain poorly understood. In this work, C57BL/6J mice were administered a normal-salt (NS) diet (0.4% NaCl) or a HS diet (7.0% NaCl) for 12 weeks, and cognitive ability and oxidative stress in the brain were measured. It was found that the HS diet significantly impaired retention of spatial memory. Additionally, superoxide anion production in the hippocampus was significantly increased in the HS diet mice compared with that in the NS mice. Interestingly, the antioxidant defense capacities for HS diet mice were markedly reduced in the hippocampus, but not in the cerebral cortex, compared with the NS mice. Taken together, these data demonstrate that HS diet directly impairs retention of spatial memory, which may be related to the increased oxidative stress observed in the hippocampus.

1. Introduction

Sodium intake is of great interest due to being inversely associated with all-cause and cardiovascular disease mortality (Alderman, Cohen, & Madhavan, 1998). A high-salt (HS) diet is strongly associated with increased blood pressure and cardiovascular disease (Strazzullo, D'Elia, Kandala, & Cappuccio, 2009). Hypertension is thought to contribute to the impairment of cognitive function and development of dementia. One recent study found that lower dietary sodium intake over three years was associated with decreased rates of cognitive impairment, especially in elderly subjects with low levels of physical activity (Fiocco et al., 2012). However, the mechanism behind impaired cognitive function due to a HS diet is poorly understood.

Oxidative stress refers to the imbalance between excessive production of reactive oxygen species (ROS) and limited antioxidant defenses (Halliwell, 1992). The brain is particularly susceptible to oxidative stress because it consumes large amounts of oxygen; however, it has a relative paucity of antioxidant defenses compared with other organs (Halliwell, 1992). The activities of antioxidant enzymes, such as catalase (CAT) and superoxide dismutase (SOD), as well as non-enzymatic antioxidants, such as reduced glutathione (GSH), have been found to be lower in animal models of cognitive dysfunction (Murali & Panneerselvam, 2008). Additionally, oxidative stress is believed to be associated with learning and memory deficits due to aging and metabolic disorders (Head, 2009).

Studies have shown that a HS diet increases oxidative stress both in the brains of stroke-prone spontaneously hypertensive rats and in mesenteric vessels of mice (Hao et al., 2011; Kim-Mitsuyama et al., 2005). The hippocampus is one of the brain regions critical to learning and memory (Morris et al., 2003). Furthermore, when memory loss occurs in the early stages in Alzheimer's disease, the hippocampus is one of the first regions to suffer damage (Small, 2001). Therefore, we hypothesized that a HS diet leads to enhanced oxidative stress in the hippocampus of mice, which causes cognitive impairment.

* Corresponding author. Address: Department of Pharmacology, School of Pharmacy, Second Military Medical University, 325 Guo-He Road, Shanghai 200433, China.
E-mail address: fumingshen@hotmail.com (F.-M. Shen).
[1] The first two authors contribute equally to this work.

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2. Methods

2.1. Animals and tissue preparation

Male C57BL/6J mice (6–8 weeks old, weighing 18–22 g) were purchased from the Sino-British SIPPR/BK Laboratory Animal Ltd. (Shanghai, China). Animals were given free access to normal drinking water and were fed chow with 0.4% NaCl (normal salt, NS) or 7.0% NaCl (high salt, HS) (SLAC Laboratory Animal Co. Ltd., Shanghai, China). After 12 weeks, mice were tested in the Morris water maze (MWM), and then were killed by decapitation under anesthesia. The brain was immediately removed and washed with ice-cold normal saline, and the hippocampus and cerebral cortex were dissected (Xu et al., 2009).

2.2. Morris water maze

Cognitive spatial ability was evaluated using the MWM, as described previously (Blokland, Geraerts, & Been, 2004; Small, 2001). To measure reference learning and memory retention, mice were placed in a 100-cm pool that was divided into four equal quadrants. A 9-cm transparent platform was placed 1.5-cm below the surface of the water in the center of quadrant 3. An assortment of 2-D visual cues surrounded the pool. For each of four successive 1-min trials per day, mice were started in a different quadrant; the same quadrant start pattern was used across five days of acquisition. Latency to find the platform (maximum of 60 s) was recorded for each trial, and the four daily trials were averaged for statistical analysis. Once a mouse found the platform, it was allowed to stay on it for 20 s; if a mouse did not find the platform, it was gently guided to the platform, where it was allowed to stay for 20 s. Animals that did not find the platform were given a latency of 60 s. On the day following acquisition testing (day 6), a probe trial was performed. For this 30 s trial, the platform was removed, and the mouse was started in the quadrant opposite to the platform-containing quadrant. The percentage of time spent in each quadrant, platform crossings, and average swimming speeds were determined from videotape recordings of the probe trial.

2.3. Measurement of blood pressure, heart rate and blood glucose

Mice fed either the NS or HS diet for 12 weeks were anesthetized with a combination of ketamine (100 mg/kg, *i.p.*) and midazolam (2 mg/kg, *i.p.*). Heart rate and systolic and diastolic blood pressure were measured by cannulation of the right internal carotid artery under anesthesia and analyzed using the Powerlab Data Acquisition System and Lab Chart 7 software (AD Instruments Ltd., Australia). Whole blood was obtained from the mouse tail vein, and glucose levels were measured at random time points using the blood glucose monitoring system (Maochang, Taibei, China).

2.4. Determination of superoxide anion

Superoxide anion was detected according to the manufacturer's instructions (Probes Inc., Eugene, OR, USA). In brief, frozen, enzymatically intact, 5-μm thick sections were prepared from mouse brain and immediately incubated with dihydroethidium (DHE) (10 μmol/L; Probes Inc., Eugene, OR, USA) in phosphate-buffered saline for 30 min at 37 °C in a humidified chamber. DHE is oxidized upon reaction with superoxide to ethidium, which binds to DNA in the nucleus and fluoresces red. Nuclei were counterstained with Hoechst 33258 (10 μmol/L; Sigma–Aldrich, St. Louis, MO, USA). Images were observed using a fluorescence microscope (DMI 3000B, Leica, Wetzlar, Germany). The intensity of the fluorescence was analyzed and quantified using Image J. Pixel intensities of the DHE fluorescent signal were quantified and normalized to Hoechst 33258 intensity. A minimum of three sections were quantified per animal.

2.5. Measurement of SOD, CAT, GSH, and T-AOC

The activities of SOD and CAT and the levels of GSH and total antioxidant capacity (T-AOC) were assayed with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

SOD activity was measured following the reduction of nitrite by a xanthine–xanthine oxidase system, which is a superoxide anion generator. The absorbance at 550 nm was recorded for the calculation of SOD activity. One unit of SOD is defined as the amount that shows 50% inhibition. The activity was expressed as U/mg-protein.

CAT activity was assayed by measuring absorbance at 240 nm with an ultraviolet light spectrophotometer and was expressed as U/g-protein. The definition of its activity was based on the hydrogen peroxide decomposition rate at 240 nm in the reactive mixture, of which the absorbance was between 0.50 and 0.55.

The level of GSH was assayed by measuring absorbance at 412 nm with an ultraviolet light spectrophotometer and was expressed as μmol/g-protein. Fresh tissue (200 mg) was ground with 2 ml of 2% metaphosphoric acid, and centrifuged at 17,000 rpm for 10 min. The supernatant was neutralized by adding 0.6 ml of 10% sodium citrate. One milliliter of assay mixture was prepared by combining 100 μl of extract, 100 μl of distilled water, 100 μl of 5,5-dithio-bis-(2-nitrobenzoic acid) and 700 μl of NADPH. The mixture was stabilized at 25 °C for 3–4 min. Then, 10 μl of glutathione reductase was added, and the absorbance was measured.

The level of T-AOC was based on the reduction of Fe^{3+} to Fe^{2+} by antioxidants, following which, Fe^{2+} was reacted with phenanthroline to form a complex with an absorbance of 520 nm. Increases of 0.01 units at A_{520} in 1 min/mg protein was considered to be one unit of antioxidant capacity (U), and T-AOC levels were expressed as U/mg protein.

2.6. Statistics

Values are presented as the mean ± SEM (standard error of the mean). The data from the MWM were analyzed with a repeated measures and multivariate analysis of variance (ANOVA) test with

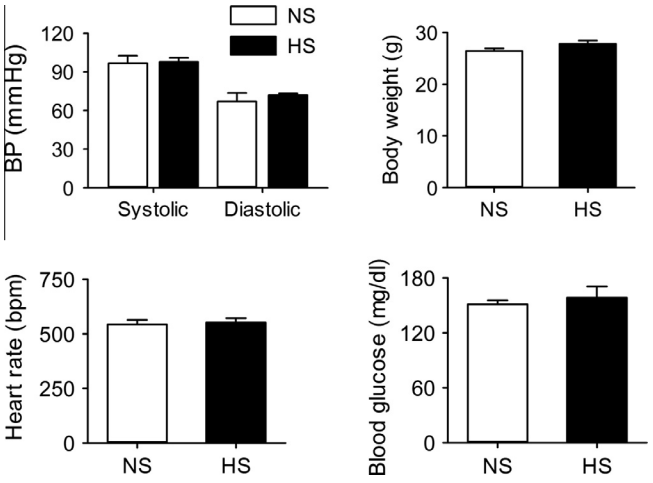

Fig. 1. Blood pressure (BP), body weight, heart rate and blood glucose in normal-salt (NS) and high-salt (HS, 12 weeks) diet treated mice. bpm: beats per minute. Values are means ± SEM; $n = 10$.