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Research report

Preventive effect of theanine intake on stress-induced impairments of hippocamapal long-term potentiation and recognition memory

Haruna Tamano[a], Kotaro Fukura[b], Miki Suzuki[a], Kazuhiro Sakamoto[b], Hidehiko Yokogoshi[b], Atsushi Takeda[a,*]

[a] Graduate School of Pharmaceutical Sciences, University of Shizuoka, Global COE, Japan
[b] Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, Global COE, Japan

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ABSTRACT

Theanine, γ-glutamylethylamide, is one of the major amino acid components in green tea. On the basis of the preventive effect of theanine intake after birth on mild stress-induced attenuation of hippocamapal CA1 long-term potentiation (LTP), the present study evaluated the effect of theanine intake after weaning on stress-induced impairments of LTP and recognition memory. Young rats were fed water containing 0.3% theanine for 3 weeks after weaning and subjected to water immersion stress for 30 min, which was more severe than tail suspension stress for 30 s used previously. Serum corticosterone levels were lower in theanine-administered rats than in the control rats even after exposure to stress. CA1 LTP induced by a 100-Hz tetanus for 1 s was inhibited in the presence of 2-amino-5-phosphonovalerate (APV), an N-methyl-D-aspartate (NMDA) receptor antagonist, in hippocampal slices from the control rats and was attenuated by water immersion stress. In contrast, CA1 LTP was not significantly inhibited in the presence of APV in hippocampal slices from theanine-administered rats and was not attenuated by the stress. Furthermore, object recognition memory was impaired in the control rats, but not in theanine-administered rats. The present study indicates the preventive effect of theanine intake after weaning on stress-induced impairments of hippocampal LTP and recognition memory. It is likely that the modification of corticosterone secretion after theanine intake is involved in the preventive effect.

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1. Introduction

Humans and animals are constantly exposed to environmental stress. Stressful life events are one of the causes of psychiatric disorders and are associated with suicidal behavior (Mann et al., 2005; Abreu et al., 2009). The hypothalamic–pituitary–adrenal (HPA) axis serves to respond to stress and controls glucocorticoid secretion from the adrenal gland (Keller-Wood and Dallman, 1984; Jacobson and Sapolsky, 1991; Linthorst and Reul, 2008). The HPA axis activation increases glucocorticoid secretion to maintain homeostasis in the living body through energy mobilization or to restore it (Chrousos and Gold, 1992; Chrousos, 2009). The hippocampus is enriched with glucocorticoid receptors, plays an important role in stress response in addition to cognitive function, and negatively modulates HPA axis activity (Kim and Yoon, 1998). However, the hippocampus is vulnerable to stress (McEwen, 1999; Garcia, 2001). Stress and glucocorticoids have diverse effects on cognitive behavior and synaptic plasticity such as long-term potentiation (LTP) that is thought to be a potential cellular mechanism of memory (Howland and Wang, 2008). Studies on how acute stress and the stress-related hormones affect learning and memory have shown inconsistent findings, which might be due to some variables such as the properties of stressors, the nature of memory, the protocols for behavioral tasks, and the characteristics of the subjects (Cazakoff et al., 2010).

Tea is one of the most widely consumed beverages worldwide (Graham, 1992). The interest in green tea has grown for human health. Green tea has the putative benefits to brain function (Bolling et al., 2009; Gonzalez de Mejia et al., 2009). Theanine, γ-glutamylethylamide, is one of the major amino acid components in green tea and is synthesized from ethylamine and glutamate in green tea leaves (Terashima et al., 1999). It has been reported that theanine has an impact on brain function (Haskell et al., 2008; Kelly et al., 2008; Einöther et al., 2010; Kakuda, 2011). It can counteract excitotoxicity and/or mitochondrial radical formation. Theanine intake might lead to neuroprotective effects (Egashira et al., 2004; Cho et al., 2008; Di et al., 2010). Furthermore, theanine may improve cognitive function; an experimental study indicates that 0.3% theanine administration facilitates hippocampal neurogenesis in the developing rats, followed by enhanced recognition memory (Takeda et al., 2011b). It is likely that theanine

* Corresponding author. Tel.: +81 54 264 5700; fax: +81 54 264 5705.
E-mail address: takedaa@u-shizuoka-ken.ac.jp (A. Takeda).

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intake has benefits to the postnatal development of hippocampal function.

Theanine has been used historically as a relaxing agent (McKay and Blumberg, 2002; Shimbo et al., 2005). Kobayashi et al. (1998) report that theanine (200 mg) increases alpha waves when administered to resting participants. Theanine potentially reduces stress in humans (Kimura et al., 2007). However, the effect of theanine intake on acute stress and its mechanism remains to be solved. On the basis of the preventive effect of theanine intake after birth on mild stress (tail suspension for 30 s)-induced attenuation of hippocamapal CA1 LTP (Takeda et al., 2012), the present study evaluated the effect of theanine intake after weaning on acute stress-induced impairments of LTP and recognition memory. It is unknown whether theanine intake prevents stress-induced impairment of memory. Neuronal circuits are shaped by experience during critical periods of early postnatal life (Hensch, 2004). Dietary environment in the critical periods is important for the shaping and can irreversibly influence brain functions. Therefore, it is important to know whether theanine intake after weaning rescues the impairment of learning and memory after acute stress.

2. Materials and methods

2.1. Animals and chemicals

Male Wistar rats (3 weeks old) were purchased from Japan SLC (Hamamatsu, Japan). They were housed under the standard laboratory conditions (23 ± 1 °C, 55 ± 5% humidity) and had access to tap water containing 0.3% theanine and food (CE-2, CLEA JAPAN, Tokyo, Japan) for 3 weeks ad libitum. Six-week-old rats were used for the experiments. In the preliminary experiment, the increase in body weight after weaning was significantly suppressed in rats fed water containing 1–2% theanine (70–80% of the control). Thus, we used water containing 0.3% theanine, which is estimated to be approximately 10 times higher than theanine concentration in green tea usually prepared in Japan. Because water intake of a rat was 22 ml, an averaged amount per day, theanine intake was 66 mg/rat/day. All experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka that refer to American Association for Laboratory Animals Science and the guidelines laid down by the NIH (NIH Guide for the Care and Use of Laboratory Animals) in the USA.

L-Theanine was obtained from Taiyo Kagaku Co., Ltd. (Yokkaichi, Japan).

2.2. Exposure to acute stress

Rats (3 weeks old) were fed water containing 0.3% theanine for 3 weeks and then subjected to water immersion stress. Rats were placed into a plastic tank (diameter, 19.2 cm; height, 45 cm) containing water (400 ml, 23–24 °C) for 30 min. They moved around to explore the tank and to avoid water. A few minutes later, they stopped moving and kept the same posture against water in the tank.

2.3. Theanine concentration in the hippocampus

The hippocampus was excised from the brain of ether-anesthetized rats after feeding water containing 0.3% theanine for 3 weeks. The hippocampi were homogenized with 3-fold volume (v/w) of 3% sulfosalicylic acid solution to the weight of the hippocampi by using an ultrasonic homogenizer and centrifuged at 12,000 × *g* for 10 min (4 °C) to obtain the supernatant. The supernatant was filtered with a 0.45 μm cellulose acetate membrane filter and analyzed for theanine concentration (detection limit, 100 pM) in an automatic amino acid analyzer (L-8500, Hitachi Co. Ltd., Tokyo, Japan).

2.4. Serum corticosterone concentration

Blood samples were collected from the common carotid arteries of the control and theanine-administered rats under diethyl ether anesthesia. Because the peak of serum corticosterone level usually reaches 30–60 min after the start of acute stress, blood samples were also collected in the same manner from the control and theanine-administered rats immediately after exposure to water immersion stress for 30 min. The collection from each group was performed in the morning (10–11 O'clock) and quickly finished within 2 min. Blood samples were kept on ice and centrifuged for 10 min (6000 rpm, 4 °C). Corticosterone concentration in the serum obtained was determined by a corticosterone EIA kit (detection limit (80% B/B_0), 30 pg/ml; Cayman Chemical Company, Ann Arbor, MI).

2.5. Hippocampal slice preparation

The control and theanine-administered rats were anesthetized with ether and decapitated. In another experiment, the control and theanine-administered rats were anesthetized with ether 1 h after exposure to water immersion stress for 30 min and decapitated. The brain was quickly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) containing 119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 26.2 mM $NaHCO_3$, and 11 mM D-glucose (pH 7.3). Transverse hippocampal slices (400 μm) were prepared in an ice-cold ACSF using a vibratome ZERO-1 (Dosaka Kyoto, Japan). Slices were then maintained in a holding chamber at room temperature for at least 1 h. All solutions used in the experiments were continuously bubbled with 95% O_2 and 5% CO_2.

2.6. CA1 LTP induction

The hippocampal slices were transferred to a recording chamber and submerged beneath continuously superfusing ACSF, which was maintained at 26–27 °C. The Schaffer collateral/commissural-CA1 pyramidal neuron responses were induced by stimulation of the Schaffer collateral/commissural pathway with a bipolar tungsten electrode. Extracellular recording was obtained by using a glass micropipette filled with 3 M NaCl (2–10 MΩ). The recording electrode was placed along the trajectory of Schaffer collateral/commissural pathway. An Axopatch-200B amplifier was used. The signal was filtered at 300 Hz, digitized with a 12 bit analog-to-digital converter (Digidata 1322 A; Axon instruments, Foster City, CA) and acquired at 50 kHz by using pClamp10.2 software (Axon Instruments). Before each experiment, we determined the stimulus intensities that elicited threshold and maximum field excitatory postsynaptic potentials (fEPSPs) by gradually increasing stimulus intensity until the fEPSP amplitude reached a saturated level. The stimulus intensity was then set to produce approximately 40% (around 100 μA, 200 μs/pulse) of the maximum fEPSPs and responses were elicited every 30 s (test stimulation, 0.033 Hz). The stimulus intensity is varied even in slices prepared from the control rats. However, the averaged stimulus intensity was almost the same (around 100 μA) in slices prepared from four groups; the unstressed and stressed control rats, and unstressed and stressed theanine-administered rats. CA1 LTP was induced by tetanic stimuli at 100 Hz for 1 s. At the beginning of the experiments, the physiological state of the slices was tested by verifying the existence of paired-pulse facilitation, which was induced by application of paired pulses separated by 40 ms.

Field EPSP amplitudes were averaged over 180 s intervals. Each point and line (the mean ± SEM) in the figures shows the mean of 180 s (6 points) and expressed as percentages of the mean fEPSP amplitude measured during the 30 min baseline period perfused with ACSF prior to LTP induction. Grouped data are expressed as the mean ± SEM. An averaged fEPSP amplitude 45–60 min after tetanic stimulation was taken as a LTP magnitude; in the result section, the values in parentheses represent the mean ± SEM.

2.7. Open field

Behavior and locomotor activity of the control and theanine-administered rats were assessed in the open-field test for 10 min. In another experiment, the control and theanine-administered rats were subjected to water immersion stress for 30 min. One hour later, behavior and locomotor activity of rats were assessed in the open-field test for 10 min. Each rat was placed in an arena (70 cm × 70 cm × 40 cm) made of a black-colored polyvinyl chloride box where it has never been placed. Behavior of each rat in the arena was recorded with a camera and analyzed using software made from the NIH Image.

2.8. Object recognition memory

The control and theanine-administered rats were placed for 10 min into an open field, which was a 70 cm × 60 cm arena surrounded by 70 cm high walls, made of a black-colored plastic, once a day for 2 days. Twenty-four hours later, the rats were subjected to water immersion stress for 30 min. One hour later, the rats were trained and tested in a novel object recognition task. Training in the object recognition task took place in the same area used for the open field exploration. The open field exploration was thus used as a context habituation trial for the recognition memory task. The object recognition test requires that rats recall which of two earthenware objects they had been previously familiarized with. Training was conducted by placing individual rats into the field, in which two identical objects (objects A1 and A2; sake bottle) were positioned in two adjacent corners, 15 cm from the walls. Rats were left to explore the objects for 5 min. Rats were not used for the test when the total of the object exploration time was less than 20 s. Twenty-four hours after training, the rats explored the open field for 3 min in the presence of one familiar (A) and one novel object (B; cup). The familiar and novel objects were counterbalanced between rats in the object recognition task and the position of the novel object was also counterbalanced. All objects presented similar textures, colors and sizes, but distinctive shapes. Behavior of rats was recorded with a video camera during the training and the test. Two persons independently measured exploratory time and the averaged time was used. A recognition index calculated for each rat was expressed by the ratio (%) $T_B/(T_A + T_B) \times 100$ [T_A = time spent to explore the familiar object A; T_B = time spent to explore the novel object B]. In the training trial, T_B is the

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