Anti-Stress Effect of Theanine in Mice and Humans

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Summary

Theanine is the most abundant amino acid in tea. Theanine ingestion prevented and relieved psychosocial stress through the modulation of hypothalamic–pituitary–adrenal (HPA) axis activity in mice that were stressed under confrontational housing. In addition, in aged mice, the intake of theanine suppressed stress-derived malfunctions such as shortened lifespan, cognitive dysfunction and brain atrophy. In addition, the anti-stress effect of theanine on humans was evaluated in 5th-year university students during pharmacy practice. The participants (n=20) were randomly assigned to theanine or placebo groups. Theanine or placebo (lactose) tablets (200 mg, twice a day) were taken from 1 week prior to the pharmacy practice and continued for 10 days into the practice period. Salivary α-amylase (sAA) activity was measured as a marker of sympathetic nervous system activity. The activity of sAA in the morning was significantly higher in the placebo group than in the theanine group. Theanine intake significantly suppressed the stress response of students assigned to a long-term commitment.

Introduction

It is widely accepted that chronic psychosocial stress is associated with the development of depression, mood disorders, cardiovascular and other age-related diseases. Since suppression or prevention of stress-induced alterations is a potential therapeutic strategy for a healthy life, experimental animal models under psychosocial stress are beneficial for finding new components acting against psychosocial stress. A conflict between two male members can be a source of severe social psychological stress in mammals. We have developed a new model of psychosocial stress, confrontational housing, by introducing a partition in a cage (Unno et al. 2011). To study how mice are stressed under confrontational housing, we investigated the time-course of adrenal hypertrophy, which is a marker of the stress-responsive organ, and the levels of corticosterone and ACTH in blood. Then, we tried to find anti-stress components using mice under confrontational housing. Theanine (γ-glutamylethylamide), an amino acid in green and black tea, has recently been reported to have neuroprotective effects, to modulate neurotransmitters and to reduce psychological stress. The effects of theanine ingestion on lifespan, cognitive dysfunction and brain atrophy were investigated in aged mice that were chronically stressed under confrontational housing.

Next, we aimed to investigate the effect of theanine supplementation on stress responses in 5th-year college students of the school of pharmaceutical sciences. They were assigned to practice outside the university such as in a hospital or a drug store, for 11 weeks. Such a long-term commitment in new environments provides a stressful condition for young students. Salivary α-amylase activity (sAA), an oral cavity enzyme, was measured as a stress marker. Measurement of sAA has been demonstrated as a useful tool for monitoring the autonomic nervous system (ANS) reactivity to stress. The measurement of sAA is an efficient and non-invasive assessment to study the effect of psychosocial stress. In the present study, considering possible individual variability in responding to the same stressful condition, the state-trait anxiety inventory (STAI), physical condition and subjective stress were scored and integrated with the changes in sAA in each participant during pharmacy practice.

Materials and methods

Theanine and other tea components: L-theanine (Suntheanine, Taiyo Kagaku Co. Ltd., Yokkaichi, Japan)

was used at 5-100 μg/ml of water. The concentrations of green tea catechin (Polyphenon 70S, Mitsui Norin Co. Ltd., Tokyo, Japan) and caffeine (Wako Pure Chemical Industries, Ltd., Osaka, Japan) used in this study were 200 and 30 μg/ml, respectively. Mice consumed water containing theanine and/or other tea components ad libitum.

Animals and housing conditions for stress experiments: All experimental protocols were performed in accordance with the guidelines for the care and use of laboratory animals of the University of Shizuoka. Male SAMP10/TaSlc (SAMP10) and ddY mice were purchased from Japan SLC Co., Ltd. (Shizuoka, Japan). All mice transported at 4 weeks of age were housed in groups of six in a cage for 5 days for habituation to novel conditions. Then, mice were divided in two groups of confrontational and group housings. These mice were individually housed to allow for the establishment of an individual territory. Then, the partition was removed to expose the mice to confrontational stress, and subsequently the two mice coexisted in a cage (confrontational housing). On the other hand, mice for group housing were used as control in which six mice were housed per cage.

Participants: Twenty healthy 5th-year students of the University of Shizuoka, who participated in the experiment, were randomly divided into two groups with matching sex: theanine (n=10, 7 men and 3 women; average age 22.5 ± 0.2 yr) and placebo (n=10, 7 men and 3 women; average age 22.2 ± 0.1 yr) via sealed envelopes to receive theanine or placebo tablets. The students were assigned to practice outside the university, in a hospital or a drug store for 11 weeks. The first 10 days of the practice program were analyzed. The study was conducted in accordance with the Helsinki Declaration. The study protocol was approved by the Ethics Committee of the University of Shizuoka. All the participants received verbal and written information about the study and signed an informed consent form before entering the study. This study was registered at clinicaltrials.gov (registration ID no. NCT01361204).

Measurement of sAA: To assess the physiological stress response, sAA was measured using a colorimetric system (Nipro Co., Osaka, Japan). Saliva was collected twice a day, in the morning after waking up and in the evening after practice, for 10 days during the practice. Prior to sampling, participants washed their mouths with water. After saliva was collected for 30 sec using a sampling tip, each participant measured their own sAA immediately every morning and evening for 10 days. To establish a no-stress and no-medication baseline of sAA, the participants measured sAA every morning and evening for 10 days during routine daily life at the university. The measurement was carried out before the pharmacy practice.

Results and discussion

Altered HPA axis in mice under confrontational housing: The body weight on the last day of the experiment was not different between group and confrontational housings. However, the adrenal weight of male mice housed confrontationally was significantly higher than that of group housing male mice. The level of corticosterone in serum was investigated because corticosterone was secreted from the adrenal gland. The level of corticosterone is generally higher in the evening and lower in the morning in mice. To confirm the effect of housing condition on the circadian rhythm of corticosterone, the level was measured each 6 h on the 7th day. The level of corticosterone was highest at 18:00 and lowest at 6:00 in the mice under group housing. However, serum corticosterone levels became low and the diurnal rhythm flattened in mice under confrontational housing. The flattened level was similarly observed in mice under confrontational housing for 2 days. However, when mice ingested theanine under confrontational housing, the levels exhibited a clear diurnal rhythm with a peak at 18:00 and a trough at 6:00. The level of ACTH in plasma was investigated because ACTH mainly regulates the adrenal gland. The plasma ACTH levels became low and there was a flattened diurnal rhythm in mice under confrontational housing. A diurnal rhythm was observed in mice that ingested theanine under confrontational housing, although the levels were lower than those in mice under group housing.

Effect of theanine consumption in mice: When the mice continued to consume theanine at 5-100 μg/ml for 2 weeks, that is, 1 week of single housing and 1 subsequent week of confrontational housing, the adrenal weight was significantly lower than in confrontational housing mice that drank water. The suppressive effect

of theanine could be observed from the lowest concentration (5 μg/ml). The average volume of drinking water was 8.3 ml/day/mouse. The consumption of theanine at 5 μg/ml was 1.1 mg/kg/day. Next, the time-dependent alterations of the adrenal gland were measured for 10 days. After the confrontation, the weight of the adrenal gland increased significantly and apparent hypertrophy continued for at least 1 week. When mice continued to consume theanine in drinking water at 20 μg/ml, no adrenal hypertrophy was observed in mice under confrontational housing. When mice consumed theanine at 20 μg/ml after the confrontation, the adrenal weight increased slightly but not significantly. However, in mice that consumed 40 μg/ml theanine after the confrontation, no adrenal hypertrophy was observed. Prior intake of theanine prevented psychosocial stress at a low dose (>5 μg/ml). Even after psychosocial stress, a higher dose of theanine (40 but not 20 μg/ml) was effective.

The effect of the coexistence of catechin and caffeine with theanine was examined. The suppressive effect of theanine on adrenal hypertrophy at 20 μg/ml (4.5 mg theanine/kg/day) was abolished by the addition of catechin (200 μg/ml) or caffeine (30 μg/ml). However, the consumption of 40 μg/ml theanine suppressed adrenal hypertrophy even when catechin and caffeine were added. The volume of drinking water was not affected by the concentration of theanine and other tea components.

Effect of psychosocial stress on lifespan in mice: Mice under confrontational housing began to die from about 4 months of age. The mean lifespan of mice under confrontational housing was 13.6 ± 1.5 months. On the other hand, the mean lifespan of group housing mice was 17.6 ± 1.2 months. The lifespan of mice under confrontational housing was significantly shorter than that under group housing. Next, the lifespan of mice drinking theanine in water under confrontational housing was investigated. The lifespan was significantly longer in mice that ingested theanine (17.9 ± 1.4 months) than that of mice that ingested water under confrontational housing. Theanine consumption did not affect mice under group housing.

Learning ability and cerebral atrophy in mice: The time for learning not to enter a dark room was measured in SAMP10 mice at 8 months using a step-through passive avoidance task. A longer learning time implies lower learning ability. The learning time was significantly longer in mice under confrontational housing than mice under group housing. On the other hand, mice that ingested theanine showed a significantly shorter learning time, even though the mice were under confrontational housing. Cerebral weight was significantly lower in 9-month-old mice under confrontational housing than that of mice of the same age under group housing. The progression of cerebral atrophy was accelerated about 3 months by confrontational housing. On the other hand, atrophy was suppressed in mice that consumed theanine under confrontational housing.

Changes of sAA in students: There was no significant difference between morning and evening levels of sAA during routine daily life at the university. During the practice, the level of pre-practice sAA (i.e. in the morning) (40 U/ml) was considered to be a baseline of sAA in the participants. The pre-practice sAA was significantly higher in the placebo group than in the theanine group (Fig. 1). The theanine group maintained the baseline observed during routine activity at the university. There was no significant difference in post-practice sAA (i.e., in the evening) although the placebo group tended to have higher levels than the theanine group. In the theanine group, post-practice sAA tended to be higher than pre-practice sAA. Considering individual variability, the mean values of pre- and post-practice sAA of each participant were analyzed. Participants of higher pre-practice sAA exhibited higher post-practice sAA. The correlation between pre- and post-practice sAA tended to be higher in the theanine-group than in the placebo-group.

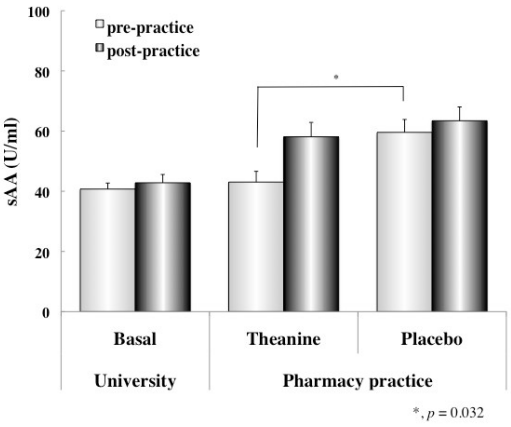

Fig. 1 Effect of theanine ingestion on sAA

STAI value and subjective stress in students: The average STAI values were examined to assess anxiety based on an appraisal standard. There was no significant difference between both groups. However, a positive correlation was observed between STAI values and pre-practice sAA in the placebo group. Participants with a high STAI value exhibited a high level of pre-practice sAA. In the theanine group, a positive correlation between STAI and pre-practice sAA was also observed. The correlation coefficients between STAI value and post-practice sAA were low in both the placebo and theanine groups. Psychosocial stress was evaluated by each participant at the end of daily practice using visual analog scales (0-10). The average score was significantly lower in the theanine group than in the placebo group, a notable trait from the first day of pharmacy practice. A positive correlation between subjective stress and post-practice sAA was observed in both groups. No participants of the theanine group showed high subjective stress. A close relationship between subjective stress and STAI was observed in the theanine group but not in the placebo group.

Effect of theanine: Orally consumed theanine is easily absorbed from the intestinal tract and partially transported into the brain competitively via the L-system at the blood brain barrier (Yokogoshi et al. 1998). The level of theanine, when administered intragastrically, reached the highest level in the brain of rats after 5 h, and completely disappeared within 24 h (Terashima et al. 1999). In the theanine group, the level of theanine in the brain might have been high from noon to evening and gradually decreased toward the next morning. Theanine incorporation into the brain is reported to reduce the release of glutamate from presynapse to the synaptic cleft by strongly acting as a glutamine transporter (Kakuda 2011). It then inhibits the incorporation of extracellular glutamine into neurons, which suppresses the conversion of glutamine to glutamate, a potent excitatory amino acid, by glutaminase. We have shown that theanine intake completely suppressed HPA-axis alteration and behavioral depression in mice (Unno et al. 2013). These results suggest that theanine has an anti-stress effect by suppressing adverse alteration of the HPA axis under stressful conditions. Therefore, theanine intake may suppress ANS and HPA axis excitability by reducing the release of glutamate, and lead to low subjective stress.

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