



L-Theanine reduces psychological and physiological stress responses

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Abstract

L-Theanine is an amino acid contained in green tea leaves which is known to block the binding of L-glutamic acid to glutamate receptors in the brain. Because the characteristics of L-Theanine suggest that it may influence psychological and physiological states under stress, the present study examined these possible effects in a laboratory setting using a mental arithmetic task as an acute stressor. Twelve participants underwent four separate trials: one in which they took L-Theanine at the start of an experimental procedure, one in which they took L-Theanine midway, and two control trials in which they either took a placebo or nothing. The experimental sessions were performed by double-blind, and the order of them was counterbalanced. The results showed that L-Theanine intake resulted in a reduction in the heart rate (HR) and salivary immunoglobulin A (s-IgA) responses to an acute stress task relative to the placebo control condition. Moreover, analyses of heart rate variability indicated that the reductions in HR and s-IgA were likely attributable to an attenuation of sympathetic nervous activation. Thus, it was suggested that the oral intake of L-Theanine could cause anti-stress effects via the inhibition of cortical neuron excitation.

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1. Introduction

In recent years, various healthful effects of the ingredients contained in green tea have been scientifically verified (Yamane et al., 1991; Cooper et al., 2005). L-Theanine, one of the major amino acids contained in green tea, has been a focus of attention due to its physical characteristics. As a biochemical characteristic of this substance, Yokogoshi et al. (1998a,b) reported that L-Theanine could pass through the blood–brain barrier, and that it increased by 1 h at the latest in serum, the liver, and the brain after administration, thereafter decreasing sharply in the serum and liver but only beginning to decrease in the brain 5 h after administration. Furthermore, another study reported that L-Theanine could influence the secretion and function of neurotransmitters in the central nervous system even at 30 min after oral administration (Terashima et al., 1999). L-Theanine binds to the glutamate receptor subtypes (AMPA, kainate, and NMDA receptors) and blocks the binding of L-glutamic acid to the glutamate receptors in cortical neurons (Kakuda et al., 2002). Despite the lower affinity of L-Theanine with glutamate

receptor subtypes than with L-glutamic acid (about 80-fold lower with the AMPA and kainate receptors, and about 30,000-fold lower with the NMDA receptor), several reports demonstrating the neuroprotective effect of L-Theanine in cortical neurons via the antagonistic role (Kakuda et al., 2000; Nagasawa et al., 2004) suggest the functional role of L-Theanine in brain dynamics. These chemical and functional characteristics of L-Theanine in the brain suggest that it might down-regulate cerebral functions, at least to a moderate degree.

In fact, previous animal studies have reported that the administration of L-Theanine reduced blood pressure (Yokogoshi et al., 1995) and inhibited the excitatory effects of caffeine (Kakuda et al., 2000). Since emotional or physiological states in humans are modulated by the chemical behaviors of neurotransmitters, psychological and physiological states could also be influenced by L-Theanine. Although empirical findings concerning the effects of L-Theanine on human participants have been limited, Kobayashi et al. (1998) reported that the oral administration of 200 mg of L-Theanine resulted in an increase of the α -band component of electroencephalograms (EEG) in the occipital and parietal scalp regions when participants rested in a relaxing state. Furthermore, the observed result that the α -band component of EEG increased more remarkably at 30 min after oral administration was

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consistent with the result in the animal study showing that neurotransmitters in the brain were affected by L-Theanine 20 min after its administration (Yokogoshi et al., 1998a,b), and might indicate that there was a time-lag between when L-Theanine was administered and when it took effect. Unfortunately, however, the researchers examined the influence of L-Theanine on EEG only during a resting situation. It is well known that the extracellular level of glutamate in the brain is increased by acute stressors (Lowy et al., 1993; Moghaddam, 1993), and it seems likely that such a glutamate increase would also result in facilitation of the activity of the sympathetic nervous system. Considering the competitive role of L-Theanine against excitation of the glutamatergic phenotype, we hypothesized that L-Theanine might be able to reduce stress-induced excitation of the peripheral sympathetic activity.

In laboratory acute stress studies, changes in several autonomic parameters, such as heart rate and blood pressure (BP), have been evaluated when participants were engaged in acute stress tasks such as the mental arithmetic, the public speaking, a stroop task, and a cold pressor task (Bachen et al., 1992; Herbert et al., 1994; Breznitz et al., 1998; Willemsen et al., 1998, 2000, 2002; Winzer et al., 1999; Atanackovic et al., 2002; Bosch et al., 2003). We previously developed a mental arithmetic task which elicits a continuous increase in the levels of HR, BP, and salivary secretory immunoglobulin A (s-IgA) (Isowa et al., 2004; Kimura et al., 2005). In addition, we attempted to estimate activity in the autonomic nervous system during the task by analyzing heart rate variability (HRV). HRV describes the variations between consecutive heartbeats, and has been widely used as a quantitative marker of the autonomic nervous system (Task Force of the European Society of Cardiology, The North American Society of Pacing and Electrophysiology, 1996). The frequency domain of HRV was divided into two major frequency bands, a high-frequency band (HF component: 0.15–0.5 Hz) and a low-frequency one (LF component: 0.05–0.15 Hz). The former is related to respiratory sinus arrhythmia and is exclusively attributable to parasympathetic influence reflecting vagal activity, while the latter mirrors the baroreceptor feedback loop that controls blood pressure and appears to reflect both sympathetic and parasympathetic activity. Furthermore, the relative contribution of LF and HF power (LF/HF) was thought to reflect the sympathovagal balance. Our observation of the significant decrease in the HF component and a remarkably increased LF/HF ratio during the task indicated that the sympathetic nervous system was prominently activated by this task. Moreover, the stress task successfully induced not only a physiological stress state, but also subjective stress feelings and state anxiety. Thus, this robust experimental paradigm allowed us to examine the possibility that L-Theanine could buffer autonomic activity and subjective stress feelings during an acute stress challenge in human participants.

In the present study, therefore, we measured the subjective stress intensity, HR, HRV, and the concentration of s-IgA as indices of acute stress responses, and examined the buffering effects of L-Theanine on such responses by asking participants to drink a cup of water containing L-Theanine before the acute stressor started. Additionally, to examine the buffering effects

more precisely, we administered L-Theanine at two different time points, since previous studies pointed out the possibility that there was a time-lag effect after oral intake. If the time-lag effect existed, the observed stress responses would be buffered differentially between the time points. However, it is known that the effects of drug treatment are, in general, dependent on participants' expectations. Because we had to take this expectation effect, called the placebo effect, into consideration, in addition to above two L-Theanine administration conditions, we set a placebo condition in which participants were required to drink a cup of water which did not contain L-Theanine. Furthermore, a control condition was added to the experimental protocol to confirm the effect of the acute psychological stressor and the oral administration of the solution itself. Together, all participants engaged in the four conditions in a double blind and counterbalanced order. In our hypotheses, as previous studies indicated, the acute stress task induces remarkable stress feeling and sympathetic nervous activation, which is reflected by the elevated HR, s-IgA level, relative decrement in HF component of HRV, and increment of LF/HF ratio. The oral administration of L-Theanine should reduce these variations via inhibiting sympathetic nervous activation based on the antagonistic role in glutamate receptors. That is, it is thought that an elevation in the HR, s-IgA, and LF/HF ratio should be reduced while the HF component reflecting vagal activity shows a lower decrement.

2. Methods

2.1. Participants

Twelve male undergraduate students (age range, 20–25 years; mean = 21.50, S.D. = 1.38) who were not suffering from any chronic or oral illnesses and not taking any medication known to influence immunity participated in the experiment. All participants received a detailed explanation of the study and gave their informed written consent to participate.

2.2. Treatment

200 mg of L-Theanine (Suntheanine, Taiyo Kagaku Inc., Japan) dissolved in 100 ml of water was used as a treatment. The safety of the oral administration of L-Theanine has been confirmed by toxicological research (Fujita et al., 1994). We decided to use this particular dose because one study reported that it could elicit a significant anti-anxiety effect in humans (Kobayashi et al., 1998).

2.3. Stress task

The participants performed a mental arithmetic task for 20 min. They were told to add a currently displayed number (from 2 to 9) to the next number displayed on a PC monitor, and to answer only one digit of the current answer by pressing a key (from 0 to 9). Each number was displayed for 500 ms and followed by a 1500 ms interval during which no number was displayed. During the task, once the participants responded by pressing a key on the keyboard after each set, an X or O was displayed on the monitor to indicate whether or not the response was correct.

2.4. Psychological measures

The participants were asked to evaluate the subjective perception of stress on visual-analog scales (0–100%). In addition, they completed the Japanese version (Nakazato and Mizoguchi, 1982) of the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970). The STAI was designed to measure state anxiety and consists of 20 items.

2.5. Cardiovascular measures

Cardiodynamic activity was recorded using an electrocardiogram (ECG) at 500 Hz by an MP 100 system (Biopac Systems Inc.) and by placing Ag/AgCL electrodes on the extremities. The following analysis of the ECG waveforms was performed using AcqKnowledge software for MP 100. After rejection of the artifacts in the ECG waveforms which were obtained, HR and inter-beat-interval data were derived from each data. In addition, the mean values of HR were calculated for the last 2 min of the baseline period, at four periods during the stress tasks (0–5, 5–10, 10–15, and 15–20 min), and for the last 2 min of each rest period. The inter-beat-interval data were subsequently analyzed to yield HRV. To equalize the duration of the tachogram epochs used for HRV spectral analyses in each condition and period, we analyzed 10 min inter-beat-interval data in each period as the focused epoch. Therefore, we calculated the spectral data from the epochs derived for the last 10 min of the baseline period, for the first 10 min of the task period, and for all the time in each rest period. The obtained 10 min inter-beat-interval data were resampled at 4 Hz to obtain equidistant time series values. A power spectrum density was then obtained through a fast Fourier transformation of the tachogram. In connection with the fast Fourier transformation, the data were detrended linearly and filtered through a rectangular window. The integral of the power spectrum was studied in two major frequency bands, namely the HF component (0.15–0.5 Hz) and the LF component (0.05–0.15 Hz).

2.6. s-IgA

To determine the volume of secreted saliva and concentration of s-IgA, we collected samples of unstimulated saliva using cotton swabs (Salivettes, Sarsted Ltd.). A cotton swab was placed underneath the tongue of each participant for 3 min. The swab was then removed and the saliva extracted from the cotton by centrifugation at 3.5×10^3 rpm for 10 min. The saliva was stored frozen in capped test tubes at -20°C until assay. We determined the s-IgA concentration in the saliva (in micrograms per milliliter) by an enzyme-linked immunosorbent assay (using an IgA test; MBL Inc.). The thawed saliva aliquots (10 μl) were diluted 40 times. Saliva samples were reacted with polystyrene beads that labeled the antihuman secretory component. After incubation at 37°C for 1 h, the beads were washed twice and reacted with peroxidase standard antihuman IgA (rabbit IgG/Fab') (secondary reaction). After incubation at room temperature for 1 h, the beads were washed three times, and then enzyme metrical fluid (orthophenylenediamine + 4 mM H_2O_2) (3rd reaction) was added. After incubation at room temperature for 30 min, the reaction was stopped by the addition of H_2SO_4 . The reaction product was quantified spectrophotometrically at 492 nm with a microplate reader (Model 550; Bio-Rad Inc., Hercules, CA).

2.7. Procedure

The participants were instructed to eat a light breakfast on the morning of the experiment; caffeine-containing beverages were not allowed. After each participant entered the experimental chamber, electrodes for the electrocardiographic measurements were attached. The experimental session is shown in Fig. 1. As seen in Fig. 1, it began with a 20 min rest period, followed by a mental arithmetic task for 20 min and two rest periods for 10 min. The saliva samples and psychological measures were obtained at the end of each period. Cardiodynamic activity was

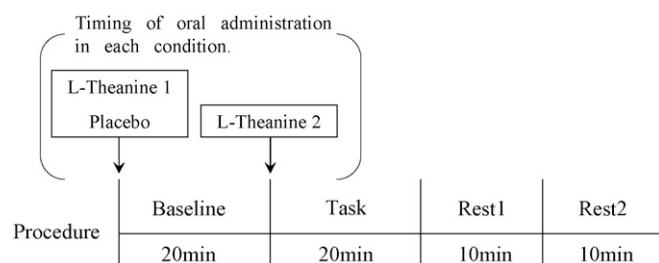


Fig. 1. Experimental protocol of the present study. Arrowed line indicates the time point at which participants took the water with or without L-Theanine in each condition.

Table 1

The order of experimental conditions in each participants

Participants' number	Control	Placebo	L-Theanine 1	L-Theanine 2
1–3	1	2	3	4
4–6	2	3	4	1
7–9	3	4	1	2
10–12	4	1	2	3

Twelve participants were divided into four groups and the order was formed according to a Latin square design.

monitored continuously throughout the experimental session. There were four conditions in the experiment: (1) oral administration of L-Theanine immediately after initiation of the session (L-Theanine 1 condition); (2) oral administration of L-Theanine immediately after the first rest period (L-Theanine 2 condition); (3) oral administration of a placebo at the same time as the L-Theanine in the L-Theanine 1 condition (placebo condition); (4) no administration of any treatment, and rest periods in place of the task periods (control condition). Thus, the participants experienced the experimental sessions four times. However, we considered the fact that the order of the experimental conditions, which the participants performed sequentially, could affect the data obtained on the psychological and physiological parameters because of the effect of habituation. To exclude this order effect reflecting experience of the experiment, the order was counter-balanced by dividing the participants into four groups randomly (with three participants in each group), and conducting the experimental conditions differentially among the groups according to a Latin square design described in Table 1. Moreover, to prevent contamination of the placebo effect, the administration of the water solution was conducted by a double-blind method. These procedures allowed us to conduct the study without any contamination.

2.8. Statistical analyses

Because in our research attention was paid to the stress responses in the task period, change scores (the mean value at each period – the mean value at the baseline) of all parameters were computed and used in statistical analyses. The HR data were analyzed using repeated-measures analyses of variance (ANOVAs) with two within-participant factors: the condition (control, placebo, L-Theanine 1, and L-Theanine 2) and the period (baseline, stress 5 min, stress 10 min, stress 15 min, stress 20 min, rest 1, and rest 2). In addition, the other data were analyzed using repeated-measures ANOVAs with a within-participant factor: the condition (control, placebo, L-Theanine 1, and L-Theanine 2) and the period (baseline, stress, rest 1, and rest 2). The Greenhouse–Geisser epsilon correction factor, ϵ (Jennings and Wood, 1976), was used where appropriate. In cases where significant interactions were found in the ANOVAs, post hoc analyses using LSD tests ($p < 0.05$) were conducted to examine which combinations of data points differed significantly. For each condition, Pearson correlation coefficients were computed among change scores (scores at the task period – scores at the baseline) of each parameter to examine the relationship between the psychological and physiological parameters.

3. Results

3.1. Psychological measures

Psychological data during the stress task and rest periods are presented in Fig. 2 and Table 2. Two-way ANOVAs revealed significant interactions between the condition and period in perception of stress ($F(6, 83) = 3.91, p < 0.01$) and state of anxiety ($F(6, 80) = 4.29, p < 0.01$). Post hoc comparisons using LSD tests clarified that the perception of stress in the task period showed a higher value under the placebo condition than under the other conditions. Furthermore, the change scores of the subjective stress in the L-Theanine 1 and 2 conditions were

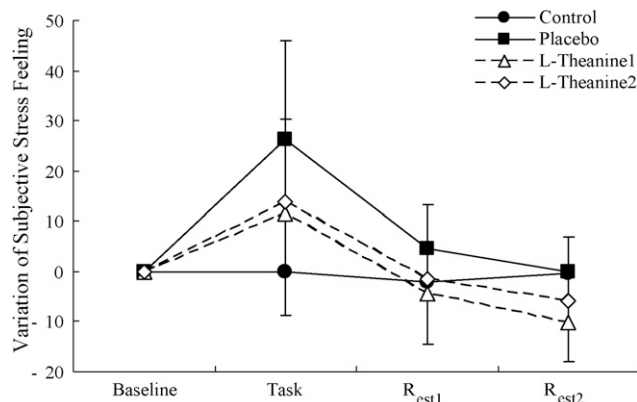


Fig. 2. Change scores from baseline scores at each time point in the subjective stress feeling. Error bars indicate standard deviations.

Table 2
Means (S.D.s) of change scores from baseline scores in state anxiety score

Conditions	Task	Rest 1	Rest 2
Control	1.83 (4.15)	0.83 (4.13)	0.08 (4.98)
Placebo	11.58 (9.15)	0.00 (3.93)	-1.67 (4.54)
L-Theanine 1	1.58 (7.32)	-1.00 (3.98)	-1.92 (5.66)
L-Theanine 2	3.17 (10.06)	0.33 (5.14)	-1.08 (4.10)

significantly lower than those under the two control conditions. In addition, the STAI state anxiety score was remarkably higher during the task period than during the rest periods, and the level of state anxiety was significantly higher under the placebo condition than under the other three conditions during the task period. In these two measures, any differences between the two L-Theanine conditions were not observed.

3.2. Heart rate and heart rate variability

The changes in HR and parameters in HRV are illustrated in Fig. 3 and Tables 3 and 4. ANOVAs yielded a significant interaction between the condition and period for HR ($F(8, 118) = 2.26, p < 0.05$). This meant that the HR was at higher

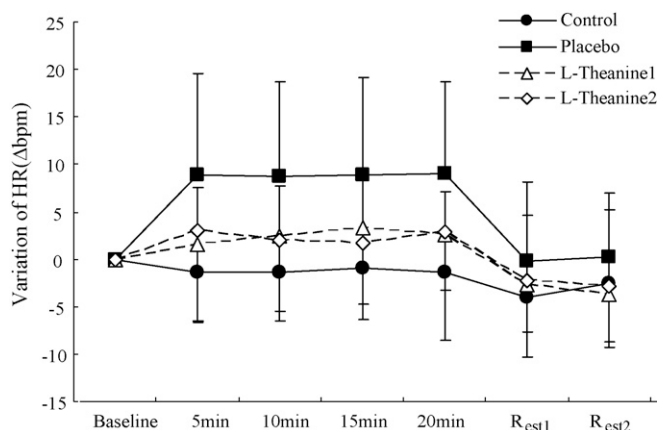


Fig. 3. Changes scores from the baseline scores at seven time points in HR. Error bars indicate standard deviations.

Table 3
Means (S.D.s) of change scores from baseline scores in LF/HF ratio

Conditions	Task	Rest 1	Rest 2
Control	-1.97 (4.65)	-1.15 (5.34)	-1.34 (4.64)
Placebo	4.09 (6.17)	1.93 (4.59)	1.12 (3.30)
L-Theanine 1	3.77 (7.54)	2.15 (4.31)	1.48 (4.47)
L-Theanine 2	2.78 (4.99)	2.49 (4.36)	0.83 (2.67)

Table 4
Means (S.D.s) of change scores from baseline scores in HF component of HRV

Conditions	Task	Rest 1	Rest 2
Control	-7 (19)	-1 (15)	-4 (17)
Placebo	15 (25)	5 (29)	9 (26)
L-Theanine 1	14 (23)	12 (21)	10 (27)
L-Theanine 2	10 (21)	5 (23)	1 (18)

levels during execution of the stress task than during the rest periods. Moreover, post hoc analyses revealed that the mental arithmetic task significantly increased the HR under the placebo condition while the HR in the L-Theanine 1 and 2 conditions showed lower increments than under the placebo condition. However, in contrast to our hypothesis, we did not find any significant differences between the two L-Theanine conditions in HR. On the other hand, as seen in Table 3, the mean values in the LF/HF ratio—which is thought to reflect sympathetic activity—during the task period showed a pattern similar to that of the HR. However, the interactions between condition and period were not statistically significant for the LF/HF ratio ($F(6, 92) = 26.82, p = 0.157$) or the HF component of HRV ($F(7, 102) = 1.23, p = 0.292$). A main effect of period was statistically significant for the LF/HF ratio ($F(2, 92) = 3.48, p < 0.05$) and statistically marginal for the HF component ($F(2, 102) = 2.49, p < 0.1$). Despite the lack of significant interactions, we reanalyzed the HRV data by using LSD tests to examine the observed pattern in more detail. Consequently, the post hoc analyses indicated that the LF/HF ratio was significantly higher under the placebo condition than under the other conditions in the task period, suggesting that the participants showed more activation of the sympathetic nervous system during an acute stress period under the placebo condition than under the other three conditions. Furthermore, no significant differences among the other three conditions were revealed.

3.3. Salivary s-IgA

Fig. 4 indicates the temporal variation of s-IgA under each condition. Repeated-measures ANOVA indicated a significant interaction between condition and period ($F(7, 102) = 3.61, p < 0.01$). LSD tests revealed that the elevation of s-IgA during the task period was more remarkable under the placebo condition than under the other three conditions. However, no significant differences were observed among the other three conditions.

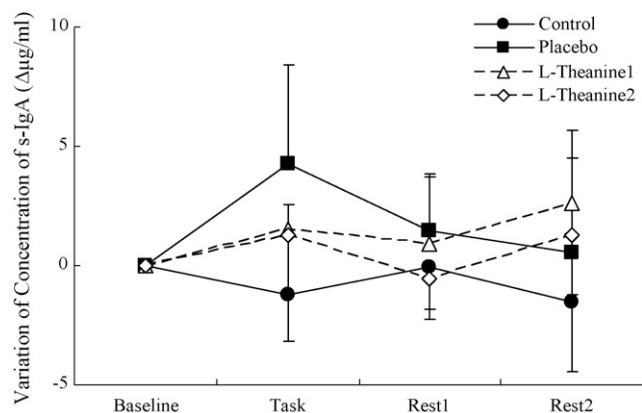


Fig. 4. Changes in scores from baseline scores at each time point in s-IgA. Error bars indicate standard deviations.

4. Discussion

The main findings in this study were that the acute stress responses elicited by the mental arithmetic task were reduced by the oral administration of L-Theanine. Moreover, this effect of L-Theanine was consistently observed not only in the subjective perception of stress but also in physiological stress responses such as HR and s-IgA. Although there is a possibility that the buffering effect was induced by a placebo effect, as is frequently seen in clinical trials of medicine, we prevented the placebo effect by employing a double-blind method. And in retrospect, after termination of the experimental sessions, no participants could identify whether they drank water or water containing L-Theanine. In addition, any effect from the repeated experience of the experimental conditions by the participants was removed by conducting the present experimental conditions differentially among groups according to a Latin square design. These valid controls suggested the buffering effect of L-Theanine in psychological and physiological stress responses. However, this parallel change between the psychological and physiological stress responses represented in the mean values should not indicate that the treatment used in this study affected the same participants in both kinds of parameters. Thus, to consider a pattern of the change, we tried to calculate the correlations coefficients in the psychological and physiological parameters. From the results, although the results showed a positive correlation between the changes, the correlations did not reach significant levels (for example, $r = 0.37$, $p = 0.24$ between s-IgA and the perception of stress in the placebo condition and $r = 0.43$, $p = 0.16$ between them in the L-Theanine 2 condition). It was thought that the result was probably due to the low number of participants in the present study. Therefore, we should not conclude the detailed buffering effects of L-Theanine. Further study using a larger number of participants is needed.

The change pattern of physiological indices under the control condition compared to that in the placebo condition clearly certified that the task used in this study elicited temporal elevations of physiological responses, and suggested that the task adequately functioned as an acute stressor. Consistent with

previous findings on acute stress responses (Willemsen et al., 1998, 2000, 2002; Isowa et al., 2004; Kimura et al., 2005), the mental arithmetic task used in the present study produced temporal increments of HR and s-IgA. On the one hand, as our previous studies using the same mental arithmetic task showed, present study also reported increments of the LF/HF ratio reflecting the degree of sympathetic nervous activation. On the other hand, there have been several reports that s-IgA was affected by acute psychological stressors, but that the direction of the change was dependent on the type of stressors used (Bosch et al., 2001, 2003; Isowa et al., 2004). For example, previous studies suggested that a memory test and mental arithmetic task elevated the concentration and secreted rate of s-IgA, while a cold pressor task and the presentation of unpleasant stimuli such as a surgical procedure video did not affect concentration and the rate of s-IgA secreted, at least during stressor exposure (Bosch et al., 2001; Isowa et al., 2004). These results indicated that the elevation of s-IgA was modulated by the distinct pattern of autonomic activation. More directly, Carpenter et al. (2000) examined this issue in an animal study and reported that stimulation of the sympathetic nervous fibers released s-IgA from plasma cells placed on submandibular glands (Carpenter et al., 2000). After considering these previous suggestions and the elevation of HR and the LF/HF ratio in HRV observed in this study, the elevation in s-IgA should be attributed to sympathetic nervous activation. In fact, it was thought that the variations in HR and s-IgA showed a similar tendency of variation in the present study. Taken together, these results indicate that the physiological changes induced by the stress task used in the present study should be interpreted as having been mediated by sympathetic activation.

The present study suggested the possibility that L-Theanine can buffer such sympathetic activity during an acute stressor. Kakuda et al. (2002) reported that L-Theanine could block the binding of L-glutamic acid to the glutamate receptors in cortical neurons. Although the affinity of L-Theanine with the glutamate receptors was remarkably lower than the L-glutamic one (about 80–30,000 fold less), there have been several reports indicating the functional role of L-Theanine, such as a neuroprotective effect in cortical neurons via the antagonistic role with glutamate receptors (Kakuda et al., 2002; Nagasawa et al., 2004). Furthermore, it has been suggested that another mechanism, such as an effect on the glutamate transporter, might play a role in the modulation of L-glutamic acid in the brain (Kakuda et al., 2002). Since there are so many neurons activated by glutamic acid in the limbic system of the brain, and because these neurons could modulate the activation of the autonomic nervous system, it is possible that the results in the present study were induced by the antagonistic role of L-Theanine to excitation of the glutamatergic phenotype. However, considering that several studies have reported that the oral administration of L-Theanine modified the secretion of neurotransmitters, such as serotonin or dopamine (Kimura and Murata, 1986; Yokogoshi et al., 1998a,b), there is also a possibility that the observed reduction of acute stress responses is attributable not only to the antagonistic role to glutamatergic

receptors but also to other neurotransmitter systems. For example, it is known that the intracerebral microinjection of L-Theanine increased dopamine release from the corpus striatum dose-dependently and a concentration of serotonin in several brain regions such as the hippocampus and hypothalamus (Yokogoshi et al., 1998a,b). Although, to our knowledge, there are no studies which reveal the relative affinity of L-Theanine with receptors of these neurotransmitters and the underlying mechanisms systemically, it should be taken into account as another possible mechanism because changes in these neurotransmitters generally modulates physiological and psychological states. In any case, the present results could be interpreted as indicating that the change in central nervous system elicited the reduction of acute stress responses. Furthermore, such reduction of physiological stress responses can be subjectively monitored, at least partly, and might induce lower evaluation of subjective stress feelings and state anxiety. Consequently, it was suggested that the L-Theanine which was absorbed had an anti-stress function both physiologically and psychologically via modulation of the activity of the central nervous system.

Despite previous studies reporting that it took about 30 min to affect several parameters such as the α -band component of EEGs in humans (Kobayashi et al., 1998) and dopamine release (Yokogoshi et al., 1998a,b) in rats, we failed to find any significant differences between the two L-Theanine administration conditions. This should lead us to reconsider the time-lagged effect of this substance. However, previous studies tended to focus on a modification or function of L-Theanine during resting situations, while the present study examined the effect during the acute stress situation. This point of difference should be recognized. Because not only the neural activities in the central nervous system, but also the peripheral nervous system activities such as ingestion, insorption, and hemodynamics would be greatly different in a stress situation from a resting situation, it is possible that the functional aspect of L-Theanine is also different between these two types of situations. Since this is just a speculation, more detail studies to examine this issue are needed.

Although this study strongly indicated that L-Theanine has anti-stress effects, several limitations must be acknowledged. First, we examined only male participants, whereas the previous study (Willemsen et al., 2002) reported sex differences in some immune responses to acute stressor. Thus, the generalizability of the present findings must be further tested using a larger sample composed of both sexes. Second, relatively few parameters were measured in the present study. Because we did not measure blood pressure, vascular resistance, noradrenaline, and adrenaline, we can only speculate about activation and reduction of the sympathetic nervous system on the basis of the HR, HRV, and s-IgA data. Third, we concluded that the reduction of acute stress responses was mediated by changes in the amount of neurotransmitters or an antagonistic role of L-Theanine against glutamic acid receptors. However, we did not directly evaluate any parameters reflecting such functions. Further studies investigating such neural mechanisms are needed.

In conclusion, despite the limitations mentioned, our results suggested that L-Theanine was effective for reducing the stress responses elicited by the mental arithmetic task. These results suggest that L-Theanine exerts anti-stress effects during an acute stress challenge.

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