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The acute effects of L-theanine in comparison with alprazolam on anticipatory anxiety in humans

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L-Theanine (δ-glutamylethylamide) is one of the predominant amino acids ordinarily found in green tea, and historically has been used as a relaxing agent. The current study examined the acute effects of L-theanine in comparison with a standard benzodiazepine anxiolytic, alprazolam and placebo on behavioural measures of anxiety in healthy human subjects using the model of anticipatory anxiety (AA). Sixteen healthy volunteers received alprazolam (1 mg), L-theanine (200 mg) or placebo in a double-blind placebo-controlled repeated measures design. The acute effects of alprazolam and L-theanine were assessed under a relaxed and experimentally induced anxiety condition. Subjective self-reports of anxiety including BAI, VAMS, STAI state anxiety, were obtained during both task conditions at pre- and post-drug administrations. The results showed some evidence for relaxing effects of L-theanine during the baseline condition on the tranquil–troubled subscale of the VAMS. Alprazolam did not exert any anxiolytic effects in comparison with the placebo on any of the measures during the relaxed state. Neither L-theanine nor alprazalam had any significant anxiolytic effects during the experimentally induced anxiety state. The findings suggest that while L-theanine may have some relaxing effects under resting conditions, neither L-theanine not alprazolam demonstrate any acute anxiolytic effects under conditions of increased anxiety in the AA model.

KEY WORDS — L-theanine; alprazolam; anticipatory anxiety; anxiety; GABA; anxiolytic; anxiety models; benzodiazepine

INTRODUCTION

L-Theanine (δ-glutamylethylamide) is one of the predominant amino acids found in green tea and historically it has been used as a relaxing agent. It was first isolated and identified in green tea leaves (*Camella sinensis*) in 1949 by Sakato (1949) and in mushrooms (*Xerocomus badius*) in the early 1950s (Casimir *et al.*, 1960).

The pharmacology of L-theanine is relatively unknown. Animal studies have shown evidence for multiple pharmacological effects on various neurochemical systems. These pharmacological effects include: (1) inhibition of glutamate reuptake by inhibition of the glutamate transporter (Sadzuka *et al.*, 2001); (2) increases in γ-aminobutyric acid (GABA) concentrations (Kimura and Murata, 1971); (3) increases in dopamine release in the striatum in rats (Yokogashi *et al.*, 1998a); (4) increases in serotonin levels in specific brain regions including the striatum, hippocampus and hypothalamus in rats (Yokogashi *et al.*, 1998b); and (5) neuroprotective effects in the hippocampus through blockade of multiple glutamate receptor subtypes, NMDA (N-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors (Kakuda, 2002; Kakuda *et al.*, 2000; Lu *et al.*, 2004).

While historically L-theanine has been shown to have relaxing properties (Juneja *et al.*, 1999; Lu *et al.*, 2004), the anxiolytic effects of L-theanine have not been established scientifically in animal or human studies. However, the pharmacological effects of L-theanine reported in animals suggest that it may have

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some anxiolytic properties given that both serotonin and GABA play a fundamental role in the neurobiology of anxiety and are molecular targets in the treatment of various anxiety disorders (Kent *et al.*, 2002; Charney, 2003; Millan, 2003). Supporting the preclinical pharmacological effects of L-theanine, one electrophysiological study in healthy human subjects reported possible relaxing effects of L-theanine (200 mg) as indicated by increased alpha activity in the occipital and parietal cortex (Ito *et al.*, 1998). While useful, the latter finding does not provide strong evidence for an anxiolytic effect of L-theanine, as alpha activity is regarded as an indirect and crude measure anxiety and behavioural measures of anxiety or relaxation were not evaluated and reported.

Both pharmacological and psychological methods have been used to examine anxiety in humans. These include pharmacological methods such as cholecystokinin tetrapeptide (CCK-4), lactate, carbon dioxide and pentagastrin induced anxiety (Benkelfat *et al.*, 1995; Bellodi *et al.*, 1998; Ponto *et al.*, 2002; Bradwejn *et al.*, 1995; Javanmard *et al.*, 1999; Boshuisen *et al.*, 2002; Zedkova *et al.*, 2003; Zwanzger *et al.*, 2003a, 2003b), and psychological (experimental) methods such as extemporaneous public speaking, aversive conditioning, fear-potentiated startle response, Stroop colour word task performance and anticipation of electric shock (Baas *et al.*, 2002; Chua *et al.*, 1999; Graeff, 2002, 2003; Grillon and Ameli, 2001; Grillon *et al.*, 1991, 1993a,b; Palma *et al.*, 1994; Reiman *et al.*, 1989; Riba *et al.*, 2001; Silva *et al.*, 2001; Simpson *et al.*, 2001; Tillfors *et al.*, 2002). Recently, it has been shown that some of these models of experimental anxiety, particularly the fear-potentiated startle response are also sensitive to benzodiazepine anxiolytic agents (Zuardi *et al.*, 1993; Patrick *et al.*, 1996; Hellewell *et al.*, 1999; Leite *et al.*, 1999; Bitsios *et al.*, 1999; Riba *et al.*, 2001; Graeff, 2003). Furthermore, the 5-HT agonist (d-fenfluramine), and 5-HT antagonist (nefazodone) have also been shown to attenuate the anxiety induced by both simulated public speaking and aversive conditioning to tones, as indicated by the anxiety dimension of the VAMS and the bodily symptoms scale (BSS) (Hetem *et al.*, 1996; Silva *et al.*, 2001; Graeff 2002, 2003). These findings suggest that experimental models of anxiety may be useful in detecting acute anxiolytic effects of potential anxiolytic drugs.

Anticipatory anxiety (AA) is one of the basic forms of anxiety, and commonly occurs in response to an immediate negative event or stressor (Reiman *et al.*, 1989). It is commonly experienced in normal individuals and in patients suffering from anxiety disorders, especially panic disorder (Barlow *et al.*, 1996). Previously AA has been used as a model of anxiety and is induced within healthy human subjects via the expectation of mild electric shocks (Reiman *et al.*, 1989; Chua *et al.*, 1999; Simpson *et al.*, 2001; Gray *et al.*, 2003). Induced AA is associated with increased subjective anxiety, phasic skin conductance and heart rates, and produces changes in blood flow and electrical activity in cortical areas associated with anxiety (Reiman *et al.*, 1989; Chua *et al.*, 1999; Simpson *et al.*, 2001; Gray *et al.*, 2002, 2003). Recently, in an electrophysiological brain imaging study it was shown that this model of anxiety is also sensitive to the three main classes of anxiolytics, namely selective serotonin-reuptake inhibitors (SSRI) (citalopram), 5-HT_{1A} partial agonists and benzodiazepines (alprazolam) (Gray *et al.*, 2002).

Given the sensitivity of the AA model to anxiolytics, the acute effects of L-theanine were examined in comparison with a standard benzodiazepine anxiolytic, alprazolam, on behavioural measures of anxiety in healthy human subjects using the AA model. It was hypothesized both alprazolam and L-theanine would reduce the subjective experience of anxiety in the AA model, and that the effects of L-theanine would be comparable to that of alprazolam.

METHODS

Participants

Sixteen healthy participants (12 males aged between 18 and 34 years (mean $\pm$ SD $= 24.8 \pm 5.4$) and four females aged between 28 to 31 years (mean $\pm$ SD $= 29.0 \pm 1.4$)) were recruited through University advertisements. All participants were considered for selection if they were healthy, non-smokers, non-medicated (no contraceptive medication for females), and had no known personal or family history of physical or psychiatric disorders as determined by semi-structured clinical interview by a physician. All participants gave written informed consent to the take part in the study, which was approved by the Swinburne Research Ethics Committee.

Study design

The study was a double-blind, placebo-controlled, repeated measures design, in which all subjects were tested under three treatment conditions. The treatment conditions were: placebo, L-theanine (200 mg; Suntheanine®, Taiyo Kagaku, Japan) and alprazolam

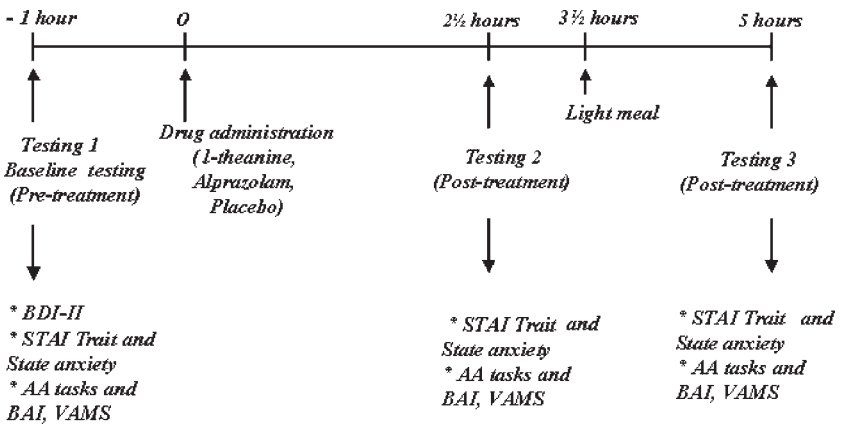

Figure 1. Subject testing schedule and behavioural measures of anxiety in each treatment day

(1 mg; Xanax®, Pharmacia and UpJohn Ltd). Alprazolam was used as a positive control in order to compare its effects with L-theanine. Individual assignment to the order of treatment condition was randomized using a Latin square design. All participants were required to attend 3 full-day repeated testing sessions with a minimum 7 days between testing days to allow for a sufficient drug washout period. On each treatment session, testing was conducted at baseline (pre-treatment) and 2½ h and 5 h post-treatment (see Figure 1). Testing times were selected to coincide with peak pharmacokinetic and pharmacodynamic effects of alprazolam and L-theanine (Fawcett and Kravitz, 1982; Terashima *et al.*, 1999). A total of nine testing sessions was completed in the 3 day testing period over 3 weeks.

Procedure

The study was conducted at the Neuropsychopharmacology Laboratory, Brain Sciences Institute, Swinburne University of Technology. Subjects were initially interviewed by telephone to screen for health and fitness suitability. All subjects were screened further for psychiatric and physical illnesses by a medical physician using the Prime-MD (based on the DSM-IV criteria for psychiatric disorders) (APA, 1994) and a semi-structured clinical interview. At the time of the initial telephone contact, subjects were given a verbal explanation of the study. Upon their arrival in the laboratory for the medical examination, written instructions and information about the study was provided and they were asked to sign the consent form if they agreed to take part in the study.

In an attempt to control for metabolic differences, subjects were instructed to consume a light breakfast with a low protein content such as toast or cereal prior to each testing day. They were instructed not to consume alcoholic or caffeinated beverages, including coffee or tea, particularly green tea, in the previous 24 h. A standard meal (one apple and 300 ml of orange juice) was provided 3½ h after drug administration. Female participants were tested during the follicular phase (days 1–13) of their menstrual cycle in order to control for the possible influence of phase-dependent variation in mood.

Figure 1 shows the subjects' testing schedule on each treatment day. At baseline (pre-drug testing), the subjects were asked to complete pre-anxiety self-rating measures including the Beck depression inventory-II (BDI-II) and trait and state anxiety (see Behavioural Measures Section). Subjects were then asked to complete the AA task conditions (see Testing Methodology section). Behavioural measures of anxiety including Beck anxiety inventory (BAI) and VAMS were used to measure subjective anxiety during the AA task. Subjects received either L-theanine (200 mg), alprazolam (1 mg) or placebo immediately after the baseline testing was completed. The AA task and behavioural measures of anxiety were re-administered at 2½ and 5 h post-drug administration. A side-effect checklist was also administered to monitor subjective physiological symptoms. The checklist took the form of a 1-5 Likert scale, that contained the following items: headache, feeling cold, feeling hot, dizziness, blurred vision, nausea, heart palpitations, dry mouth and gastric complaints, to give a mean subjective physiological symptoms score for each item.

Testing methodology (anticipatory anxiety)

The subjects completed two task conditions, an AA condition and a relaxed condition. In both conditions, the subjects were instructed to focus their gaze on a computer monitor and focus their attention on their current feelings. During the AA condition, a red border framed the computer screen. Subjects were informed that they would randomly receive electrical shocks during the red border presentation. Subjects completed two blocks of AA tasks in this condition, which had a total of 180 s duration, 135 s on the first block and 45 s on the second block. The subjects also stopped to complete the VAMS and Beck anxiety inventory (BAI) measures in between the two blocks (see Behavioural Measures). The relaxed (baseline) condition was identical to the AA condition, except in this condition, a blue border framed the computer screen and subjects were informed that no shocks would be delivered during the blue border presentation. The current experimental methodology is similar to that used in previous neuroimaging studies and has been shown to activate areas associated with anxiety (Gray *et al.*, 2002, 2003).

Stimuli and apparatus. The electrical stimuli were delivered through two electrodes with gel and adhesive attached to the back of the participant's right hand. The stimulus was delivered by an Isolated Stimulator, Dogwood Scientific Equipment, model CMS 1-200. The shocks had an intensity of 30 mA, a voltage of 110 V (maximum) and duration of 0.1 ms. This level of electrical stimulation has previously been shown to reliably induce anticipatory anxiety in healthy human subjects without causing pain (Gray *et al.*, 2002, 2003).

Behavioural measures

The following self-rating scales were used to assess behavioural (subjective) states of anxiety.

The visual analogue mood scale (VAMS: Bond and Lader, 1974) requires subjects to place a single mark with a pen along a 100 cm horizontal line separated by two adjectives in the current study: (1) calm–excited, (2) relaxed–tense and (3) tranquil–troubled.

The state-trait anxiety inventory (STAI: Spielberger *et al.*, 1970) is a 20-item scale assessing two types of anxiety: *state anxiety* measures the intensity of anxiety at a particular moment from 1 'not at all' to 4 'very much so', and *trait anxiety* measures anxiety as a relatively stable personality trait from 1 'almost never' to 4 'almost always'.

The Beck depression inventory-II (BDI-II: Beck *et al.*, 1996) is a widely used 21-item inventory for assessing the severity of depression. Each item is rated on a scale ranging from 0 'normal' to 3 'most severe' with summary scores ranging from 0 to 63.

The Beck anxiety inventory (BAI: Beck and Steer, 1987) consists of 21 items assessing anxiety symptoms, especially focuses on those symptoms that are distinct from depressive symptoms. Each item is rated on a 4-point scale ranging from 0 'not at all' to 3 'severely, I could barely stand it', with a total score ranging from 0 to 63.

Statistical analysis

The data were analysed using the Statistical Package for the Social Sciences (SPSS for Windows, version 11, SPSS Inc., Chicago II USA). The maximum effects of each treatment on anxiety were calculated as the difference between the mean scores at post drug (maximum value regardless of time, i.e. 2½ or 5 h) relative to the baseline mean score and the baseline mean score itself. The drug conditions (L-theanine, alprazolam, placebo) and time (baseline testing, post-treatment maximum score) were the independent variables, and behavioural measures (VAMS subscales, BAI and STAI) were the dependent variables. The data were analysed using a 3 (drug) by 2 (time) repeated measures multivariate analysis of variance (MANOVA), conducted separately for both the relaxed (baseline) and AA task conditions. *Post hoc* analyses were carried out on significant interactions between drug and time in order to examine the specific effects of each drug. STAI Trait anxiety was also used as a covariate in a multivariate analysis of covariance (MANCOVA), in order to examine the influence of trait anxiety on the drug induced changes in behavioural anxiety in the AA model. In addition, to examine the effects of the task conditions (relaxed vs AA) on measures of anxiety, a paired-sample *t*-test was conducted for each behavioural anxiety measure using the pre-drug baseline scores.

RESULTS

The BDI scores ($M = 3.81$, $SD = 4.78$) and trait anxiety scores ($M = 33.44$, $SD = 7.14$) indicated that all subjects' scores were within the normal range. Paired-sample *t*-test showed that all behavioural measures were associated with increased subjective anxiety in the AA task condition relative to the relaxed condition. However, only scores on the BAI ($t(47) = 3.61$, $p < 0.01$) and tranquil–troubled

Table 1. Mean and standard deviation (SD) for behavioural measures of anxiety in both relaxed and AA condition

Behavioural measures	Relaxed condition	AA condition
	Mean (SD)	Mean (SD)
BAI	1.48 (2.73)	3.04 (3.45)[a]
VAMS		
Calm	9.83 (11.33)	10.75 (9.01)
Relaxed	10.13 (12.33)	12.60 (10.43)
Tranquil	10.71 (13.31)	14.73 (13.15)[a]

[a] $p < 0.05$ for the AA relative to relaxed condition.

subscale of in the VAMS ($t(47) = 2.28$, $p > 0.05$) showed significant differences between the two task conditions. The means and standard deviations (SD) for the behavioural measures of anxiety in both relaxed and AA conditions are shown in Table 1.

In the relaxed task condition, the repeated measures MANOVA revealed a significant drug × time interaction for two of the dependent variables, STAI state anxiety and the tranquil–troubled subscale of the VAMS. The means and standard deviations, and the associated significant levels for the drug × time interaction on each measure of anxiety in the relaxed condition are shown in Table 2. Further post hoc comparisons were performed for both subjective measures of anxiety. Alprazolam significantly increased STAI state anxiety scores in the relaxed condition in comparison with placebo ($F(1, 15) = 6.11$, $p < 0.05$). L-theanine had no significant effect in comparison with placebo ($p > 0.05$). With regard to the tranquil–troubled subscale of the VAMS, L-theanine significantly reduced subjective anxiety in comparison with alprazolam ($F(1, 15) = 5.37$, $p < 0.05$) and placebo ($F(1, 15) = 4.73$, $p < 0.05$). In the AA condition, the repeated measures MANOVA failed to reveal any significant drug × time interactions. The means and standard deviations and the associated significant levels for the drug × time interaction on each measure of anxiety in the AA condition are shown in Table 3. The results were also re-analysed using trait anxiety scores on the STAI as a covariate in both the relaxed and anxious conditions. Subsequent repeated measures MANCOVA failed to show any significant drug × time interactions for any of the anxiety measures. In addition no significant adverse effects were found between the treatment conditions (i.e. L-theanine or alprazolam) in comparison with placebo.

Table 2. Effects of placebo, L-theanine and alprazolam on subjective anxiety in the relaxed condition. Results are expressed as mean and standard deviation (SD). The F and p values refer to drug × time interaction for the repeated measures MANOVA

Anxiety measures	Placebo		L-Theanine		Alprazolam		Significance	η^2
	Baseline Mean (SD)	Post-drug Mean (SD)	Baseline Mean (SD)	Post-drug Mean (SD)	Baseline Mean (SD)	Post-drug Mean (SD)		
BAI	1.19 (2.20)	1.75 (3.51)	1.81 (2.81)	1.69 (2.91)	1.44 (3.22)	2.19 (2.40)	$F = 1.17$; $p = 0.32$	0.24
STAI State anxiety	26.50 (6.92)	26.12 (6.66)	24.56 (5.56)	25.25 (5.75)	26.50 (4.68)	31.50 (9.23)	$F = 4.54$; $p = 0.03$[a]	0.63
VAMS								
Calm	10.56 (14.49)	13.94 (12.34)	9.94 (9.20)	10.56 (10.31)	9.00 (10.30)	16.69 (14.46)	$F = 1.49$; $p = 0.24$	0.29
Relaxed	9.06 (11.90)	16.50 (18.24)	11.62 (11.94)	12.69 (14.67)	9.69 (13.73)	17.94 (17.36)	$F = 0.88$; $p = 0.43$	0.19
Tranquil	8.37 (7.81)	16.44 (14.71)	13.19 (12.99)	9.94 (7.06)	11.87 (15.58)	20.00 (20.80)	$F = 4.05$; $p = 0.03$[b]	0.68

$n = 16$; [a] $p < 0.05$ for alprazolam vs placebo (Greenhouse-Geisser); [b] $p < 0.05$ for L-theanine vs placebo and L-theanine vs alprazolam.

Table 3. Effects of placebo, L-theanine and alprazolam on subjective anxiety in the AA condition. Results presented as mean and standard deviation (SD). The F and p values refer to drug × time interaction for the repeated measures MANOVA

Anxiety measures	Placebo		L-Theanine		Alprazolam		Significance	η^2
	Baseline Mean (SD)	Post-drug Mean (SD)	Baseline Mean (SD)	Post-drug Mean (SD)	Baseline Mean (SD)	Post-drug Mean (SD)		
BAI	2.00 (2.83)	1.87 (2.80)	4.13 (4.36)	1.94 (2.72)	3.00 (2.80)	2.00 (2.71)	$F = 1.69$; $p = 0.21$	0.1
VAMS								
Calm	15.25 (17.86)	17.19 (15.91)	11.31 (9.74)	14.19 (13.73)	11.00 (8.58)	19.06 (15.69)	$F = 0.87$; $p = 0.43$	0.05
Relaxed	13.62 (13.53)	18.94 (14.70)	11.37 (7.35)	15.31 (22.17)	12.81 (10.06)	17.94 (18.31)	$F = 0.04$; $p = 0.96$	0.01
Tranquil	14.88 (14.15)	15.31 (11.08)	14.81 (13.22)	11.37 (13.20)	14.50 (12.92)	17.37 (13.20)	$F = 0.86$; $p = 0.43$	0.05

$n = 16$.

DISCUSSION

To our knowledge this is the first study to examine the anxiolytic effects of L-theanine. The study examined the acute effects of L-theanine in comparison with the benzodiazepine, alprazolam and placebo on behavioural measures of anxiety in healthy human subjects under both a relaxed (baseline) state and during an experimentally induced anxiety state (i.e. anticipation of a mild electric shock). The findings provide some evidence to support a relaxing effect of acute L-theanine administration during the relaxed (baseline) experimental state, with subjects reporting to be more tranquil in the tranquil–troubled dimension of the VAMS compared with placebo. In comparison, alprazolam did not exert any anxiolytic effects when compared with placebo on any of the measures during the relaxed state. In addition, neither L-theanine nor alprazolam demonstrated significant anxiolytic effects during the experimentally induced anxiety state suggesting that under conditions of increased anxiety, neither drug had measurable anxiolytic effects.

The finding of some calming or relaxing effect of L-theanine in the resting (baseline) state is consistent with a previous report indicating that such effects may correspond with increases in alpha band electrocortical activity in the occipital and parietal cortex (Ito *et al.*, 1998). Furthermore the current findings support the historical use of green tea as a relaxing agent (for review see; Lu *et al.*, 2004). However, in all cases the evidence for this effect is not strong. For example, in our study L-theanine was only found to affect one of the anxiety subscales (i.e. tranquil–troubled subscale of the VAMS), while in the study of Ito *et al.* (1998), the measure of anxiety indexed by changes in alpha band activity would be considered an indirect and possibly a crude measure of the anxious state. Surprisingly, alprazolam did not exert anxiolytic or calming effects in the relaxed or baseline experimental condition. Paradoxically there was an increase in subjective reports of anxiety in the STAI state anxiety measure. While it is difficult to explain this increase in state anxiety with alprazolam, it should be noted that there have been inconsistencies in the literature with regard to the effects of benzodiazepines on subjective reports of anxiety under resting conditions. Some studies have reported an anxiolytic effect of alprazolam (Riba *et al.*, 2001) and also other benzodiazepines (McNair *et al.*, 1982; Guimaraes *et al.*, 1989), while other studies suggest a lack of effect of a number of benzodiazepines (Baas *et al.*, 2002) on state anxiety under resting conditions. Similar discrepancies have been noted with serotonergic anxiolytics with findings suggesting that drugs that increase serotonin, enhance conditioned fear responses while inhibiting unconditioned fear (for review see Graeff, 2002, 2003). The differential effects of pro-serotonergic compounds have been hypothesized to reflect region-specific changes in the pre-frontal cortex versus the periaqueductal grey (Deakin and Graeff, 1991). It is possible that benzodiazepines may also have region-specific effects (depending on the anxiety model or state), which could account for varying behavioural changes in state anxiety.

An important observation in the current study was that neither L-theanine nor alprazolam demonstrated any effects on subjective anxiety levels under the experimentally induced anxiety condition. While the reason for the lack of effect of L-theanine during the experimentally induced anxiety condition (in comparison with the mild anxiolytic effects reported under resting conditions) is not known, it is likely that the intensity of anxiety was too strong in the experimental anxiety condition, leading to an ineffective anxiolytic effect. In addition, given that L-theanine only demonstrated weak anxiolytic effects on the resting (baseline) state, it is not surprising that no anxiolytic effects were observed under conditions of increased anxiety. The absence of an anxiolytic effect of alprazolam is consistent with a number of studies, which fail to show an anxiolytic effect of benzodiazepines during experimentally induced anxiety (for review see; Graeff, 2003). For example, a number of benzodiazepines have been shown to have no effects on self-reported anxiety in the stimulated public speaking model (Graeff *et al.*, 1985; McNair *et al.*, 1982), and the Stroop colour-word test (Tulen *et al.*, 1991). However, it should be noted that the latter studies, including our current study used behavioural (subjective) measures of anxiety and a growing body of research suggests that benzodiazepines may demonstrate anxiolytic effects when measured objectively using physiological measures (i.e. skin conductance, startle response, brain electrical activity). For example, a number of studies have suggested that benzodiazepines including alprazolam could inhibit the fear, darkness or context potentiated startle response (Patrick *et al.*, 1996; Bitsios *et al.*, 1999; Riba *et al.*, 2001) and benzodiazepines have been shown to be sensitive in a model of aversive conditioning of the skin conductance responses to tones (Hellewell *et al.*, 1999). Similarly, our recent findings using SSPT suggests that the electrophysiological responses in the frontal and temporal cortex during anticipatory anxiety are attenuated with a number of anxiolytics including alprazolam following acute administration

(Gray *et al.*, 2002). These findings suggest that while subjective measures may not be sensitive in detecting drug induced anxiolytic effects, the effects of L-theanine and alprazolam may be observed or detected in models of anxiety incorporating objective physiological measures of anxiety such as startle, skin conductance response or electrophysiological imaging. This is not surprising given that the neural networks/circuits involved in the physiological measures (i.e. startle) may be modulated differentially by neurochemicals compared with networks involved with subjective self reports. For example, the startle response originates from structures located in the brain stem and is modulated by descending pathways (Davis, 1984), while subjective changes during anticipatory anxiety are thought to involve a neuroanatomical circuitry that includes the prefrontal, anterior temporal and occipital cortex and the insula (Chua *et al.*, 1999; Gray *et al.*, 2003). Alternatively, anxiolytic effects may be observed in pharmacological models of anxiety, such as the CCK-4 induced panic model in which benzodiazepines, including alprazolam have been shown to reduce CCK-4 induced panic (de Montigny, 1989; Zwanzger *et al.*, 2003a). The latter findings may also be related to specific modulation of neural networks (within the neocortical and limbic areas, which have been shown to co-localize both CCK and GABA) (Somogyi *et al.*, 1984).

Previous studies have suggested that the effects of anxiolytics may depend on the trait anxiety level of subjects. Alprazolam has been shown to exert a greater anxiolytic effect in subjects with high trait anxiety compared with low trait anxiety in the Stroop colour-word test (Nakano *et al.*, 1978). Similarly, L-theanine has been shown to have greater effects with regard to the generation of α activity in a high anxiety group compared with the low anxiety group (Ito *et al.*, 1998). It is unlikely that trait anxiety influenced the findings of the current study, as a separated analysis with trait anxiety as a covariate also showed that neither L-theanine nor alprazolam demonstrated any anxiolytic effects.

It is likely that the lack of effect of both L-theanine and alprazolam may be related to drug dose and treatment duration. Alprazolam has been shown to exert dose related effects in a number of human models of anxiety including the simulated public speaking model (McNair *et al.*, 1982) and the fear potentiated startle model (Patrick *et al.*, 1996). Furthermore, the panic induced by CCK-4 was only partially attenuated by the acute dose of alprazolam (Zwanzger *et al.*, 2003a) suggesting that a more prolonged dosing may be necessary for optimal pharmacological effects. This is supported by the clinical studies that demonstrate anxiolytic effects of alprazolam in anxiety disorders (including generalized anxiety disorder and panic disorder) only following chronic administration (Laakmann *et al.*, 1998; Lydiard *et al.*, 1997; Sheikh and Swales, 1999). Hence one cannot rule out a possible anxiolytic effect of both L-theanine and alprazolam in the anticipatory anxiety model, with higher doses or following chronic administration.

In summary, the findings of the current study suggest that under conditions of experimentally induced anticipatory anxiety, neither L-theanine nor alprazolam had any acute anxiolytic effects in healthy subjects as measured by behavioural measures of anxiety. However, there was some evidence for a calming or relaxing effect of L-theanine under resting conditions providing some support for the historical use of green tea as a relaxing agent. Further studies are warranted to examine the effects of L-theanine in comparison with alprazolam using objective physiological measures of anxiety. In addition, dose response studies or chronic dosage studies are required to further examine the possible efficacy of L-theanine as an anxiolytic.

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