Anxiolytic Effects of L-theanine – a Component of Green Tea – When Combined With Midazolam, in the Male Sprague-Dawley Rat

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The purpose of the study was to investigate the anxiolytic effects of L-theanine and its potential interaction with the $GABA_A$ receptor in Sprague-Dawley rats. L-theanine is a major component of green tea, which has traditionally been used as an herbal remedy in the treatment of many medical conditions, including anxiety. Herbals and supplements and their potential interactions perioperatively are a concern to anesthetists.

Fifty-five rats were divided into 5 groups: control (saline); L-theanine (positive control); flumazenil (a known benzodiazepine receptor antagonist) and L-theanine; and midazolam and L-theanine. The behavioral component of anxiety was evaluated using the elevated plus-maze and calculated by the time spent in the open arm of the maze divided by total time in the maze.

Data were analyzed using a 2-tailed multivariate analysis of variance and Sheffé posthoc test. The data suggest that L-theanine does not produce anxiolysis by modulation of the $GABA_A$ receptor; however, in combination with midazolam, a synergistic or additive effect was demonstrated by decreased anxiety and both fine and basic motor movements. These data may provide direction for further studies examining L-theanine and its effects on anxiety and motor activity.

Keywords: Anxiolysis, elevated plus-maze, green tea, L-theanine, rats.

The prevalence of anxiety disorders in American culture is estimated at approximately 40 million people over the age of 18 years, or 16% of the adult population.[1] This statistic makes anxiety and its sequelae the object of intense scrutiny by medical practitioners and researchers: those treating the psychological and physical aspects of anxiety, and those administering medical care to patients who may be affected by symptoms of anxiety, as in the operative environment.

Anxiety is a natural, adaptive mechanism that prepares the body to react to danger or threats that lie ahead; if anxiety becomes pathological, it exerts serious emotional and physical effects on the individual.[2] Selye[3] described an organism's stress response to anxiety that includes the release of endocrine and neurotransmitter mediators that ultimately cause an increase in corticosteroid levels, and a sympathetic nervous system response that elevates circulating levels of epinephrine and norepinephrine. Perioperative patients may have a variety of symptoms, including heart palpitations, chest pain, stomach upset, frequent urination or diarrhea, shortness of breath, headaches, impaired concentration, irritability, and confusion.

Medications used to attenuate the symptoms of anxiety, such as benzodiazepines, have been shown to be effective in improving postoperative outcomes such as nausea or vomiting and pain.[4] One early study on preoperative anxiety found that patients with decreased anxiety required less pain medication and were discharged an average of 2.7 days sooner.[5] Because of these and other positive outcomes, the use of anxiolytics has become quite common in the preoperative environment.

In addition to traditional medications used in a controlled environment, anesthesia providers need to be aware that many patients self-medicate for anxiety using herbal preparations. Recent studies demonstrate that there continues to be substantial use of herbal medications in the United States. Prevalence of herbal therapy increased from 12% in 1997 to more than 18% in 2002.[6] Furthermore, a 2004 study of active duty–enlisted personnel found a much higher rate of supplement use among that population, with more than 60% of survey participants using supplements at least once per week.[7] Finally, a 2000 survey by Tsen and colleagues[8] found that 22% of presurgical patients reported the use of herbal remedies.

The lack of reporting by the patient population of herbal medications is of clinical significance. A 2006 review of the literature by Robinson and McGrail[9] indicates that failure to disclose the use of alternative or herbal medications may be as high as 77%. Finally, the safety, efficacy, or quality control of supplements is not regulated by the US Food and Drug Administration, making it difficult to generalize study outcomes. The concern surrounding herbal self-medication and lack of disclosure by patients is that these preparations may have side effects or mechanisms of action that interfere with or have interaction effects with anesthetic medications.[10,11] Little research exists as to the efficacy, side effects, or interactions of most herbal therapies.

One herbal medication that has gained recent popularity for self-treatment of anxiety is the green tea component L-theanine. Green tea has been a popular beverage in many cultures for thousands of years. Although it has long been surmised that green tea has multiple health benefits such as relaxation and anxiolysis, it is only in the last 50 years that these effects have been linked to L-theanine.[12] Theanine, a derivative of glutamate, is absorbed in the small intestine, is metabolized by the kidney, and crosses the blood-brain barrier.[13] Effects of L-theanine on the brain are not fully understood; however, animal studies show evidence for multiple pharmacologic effects. These effects include: (1) inhibition of glutamate reuptake, (2) increase in γ-aminobutyric acid (GABA), (3) increase in dopamine release in the striatum, and (4) increase in serotonin levels.[14] In the instance of the L-theanine component of green tea, only one study has been completed regarding its anxiolytic effects.[15] In addition, no published studies exist on the possible interactions between L-theanine and benzodiazepines or other anxiolytics.

The purpose of this study was 2-fold. The first objective was to determine if the green tea component L-theanine has anxiolytic effects in the rat model. The second objective was to investigate possible modulation of the $GABA_A$ receptor by L-theanine in the rat central nervous system (CNS).

Materials and Methods

Fifty-five male Sprague-Dawley rats (Harlan Sprague Dawley Laboratories) weighing 200 to 250 g were used. They were housed in groups of 3 in polycarbonate "shoebox-sized" cages lined with bedding. The animals went through a 14-day adaptation period in a temperature-controlled environment (22 ± 1°C, 60% humidity) with a light-dark cycle, receiving 12 hours of light (6 AM to 6 PM) and 12 hours of dark (6 PM to 6 AM). They were allowed free access to food and water. The animals were handled only for weighing, drug administration, and cleaning of cages and were naive to the elevated plus-maze (EPM). The use of laboratory rats in this protocol was in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and received approval from the Institutional Animal Care and Use Committee at Brooke Army Medical Center, San Antonio, Texas.

Rats were randomly divided into 5 treatment groups of 11 rats per group. The groups were as follows: (1) vehicle (control, saline); (2) L-theanine, 10 mg/kg[16] (Sigma Chemical Co, St Louis, Missouri); (3) midazolam, 1.5 mg/kg; (4) flumazenil, 3 mg/kg, and L-theanine, 10 mg/kg; and (5) midazolam, 1.5 mg/kg, and L-theanine, 10 mg/kg. The group receiving flumazenil (a known benzodiazepine receptor antagonist)[14,17] and L-theanine was used to evaluate the potential modulation of the benzodiazepine receptor site on the $GABA_A$ receptor by L-theanine. Each animal received 2 intraperitoneal (IP) injections. Depending on the group, the first injection was either flumazenil or dimethyl sulfoxide (vehicle for flumazenil). The second injection was also group-dependent according to group assignments. All animals received 2 intraperitoneal injections, each containing 1 mL of the total volume.

Additionally, all experimentation occurred on a timed schedule between 9 AM and 3 PM over 2 consecutive days to control for the circadian rhythm of the animals.

Thirty minutes after drug administration, each animal was placed in the center of the EPM, facing the open arm, with the maze located in a lightened room. The EPM is a widely used instrument to measure anxiety in the rodent model and has been validated by Pellow and colleagues,[18] based on the previous work by Montgomery.[19] Research on this instrument has supported its use as a standard measurement of anxiety, specifically benzodiazepine-induced anxiolysis in rodents.[18]

Each rat was evaluated for 5 minutes on the EPM for the behavioral response to anxiety. Each test run was video recorded for verification of data validity. The EPM was cleaned with soap and water and dried between each animal run to limit variability. Immediately after the 5-minute test on the EPM, the animals were removed from the testing room and returned to their respective cages.

The EPM was networked with MotorMonitor software (version 5.0, Kinder Scientific Co, Poway, California) that tracked the number of entries into each type of arm (open vs closed), time spent in the open arms expressed as a percentage of the total time, and fine and basic motor movements. Basic motor movements are defined as the simple count of beam breaks in the EPM. Each interruption of a photo beam increased the basic movement count. These movements are a calculation of gross motor locomotion but do not distinguish the type of activity performed. Fine motor movements are a compilation of small-animal movements: grooming, head weaves, or head bobs. Data were analyzed using a 2-tailed multivariate analysis of variance (MANOVA) and Sheffé posthoc test.

Results

Data were collected from 55 subjects, with 1 rat withdrawn from the study for unanticipated olfactory stimulation in the testing environment during the time on the EPM. Inadvertent odor from an outside food source resulted in observable behavioral change and subsequent result error.

Analysis of the ratio of open arm time vs total time spent in the EPM revealed no statistically significant difference between the control group and the L-theanine, midazolam, and flumazenil groups. Significant differences were found between the control group and the midazolam plus L-theanine group (P = .005) in the open arm, a measurement of behavioral changes associated with anxiolysis (Table and Figure 1).

As mentioned in the Materials and Methods section, the MotorMonitor software also tracked the total number of basic (gross) and fine motor movements. Analysis of these data indicated a significant decrease in basic movements of rats in the midazolam plus L-theanine group (P = .006) and the midazolam group (P = .034) compared with the control group (Table and Figure 2).

Discussion

Green tea has been used for centuries in Asian countries as a means of relaxation therapy. This study investigated the purported anxiolytic properties of L-theanine, a component of green tea, and its potential interaction with the GABA receptor site.

Few research studies currently exist that provide statistically supported data on the effects of L-theanine on anxiety or established dosing regimens. Lu and colleagues[15] investigated the effects of a one-time, 200-mg/kg, oral dose of L-theanine on anticipatory anxiety in human subjects. Their research suggests there was a decrease in baseline anxiety, but minimal or no effects on anticipatory anxiety was observed.[15] Juneja and colleagues[12] investigated the relaxation properties of L-theanine using physiologic measurements. Monitoring alpha-wave stimulation, a measurement for relaxation, they concluded that the increase in alpha waves supported their hypothesis that L-theanine can be related to relaxation.[12] After extensive research by Sugiyama and Sadzuka,[16] intraperitoneal administration of 10 mg/kg of L-theanine has become an accepted route of administration in the rat model.

Historically, behavioral data suggest that rats prefer closed arms of the maze as opposed to the open arms.[18] Increases in the percentage of time spent in the open arms of the maze indicate anxiolytic effects. Our current research findings comparing the ratio of open arm time to total time spent in the EPM suggest that L-theanine alone at the 10-mg/kg dose may not produce anxiolysis. Furthermore, there was no difference between the flumazenil (benzodiazepine receptor antagonist) plus L-theanine group and the group receiving L-theanine alone at the 10-mg/kg dose, which suggests it does not modulate the $GABA_A$ benzodiazepine receptor site.

Further evaluation of the data indicated that there was a significant increase in time spent in the open arm vs total time spent in the maze when L-theanine was combined with midazolam. This suggests a possible synergistic or additive effect between the 2 agents, with L-theanine acting at an unidentified receptor site. Further evidence that supports a possible interaction between mi-

Group	Control	L-theanine (L)	Midazolam (Mid)	Flumazenil + L-theanine (Flum + L)	Midazolam + L-theanine (Mid + L)
Ratio open arm time: total time	7.7 ± 1.57	8.9 ± 2.93	14.6 ± 3.86	7.9 ± 1.18	22.6 ± 6.05*
Basic movements	1,000.0 ± 50.0	970.6 ± 86.94	724.7 ± 127.2*	963.1 ± 69.44	636.2 ± 95.04*
Fine movements	708.9 ± 31.32	691.5 ± 57.03	482.9 ± 85.43*	663.2 ± 40.78	446.4 ± 66.49*

Group	Posthoc analyses	
Ratio open arm time: total time	Mid + L vs Control and Flum + L: P = .005	Mid + L vs L: P = .01
Basic movements	Mid vs Control: P = .034	Mid + L vs Control: P = .006 Mid + L vs L: P = .011 Mid + L vs Flum + L: P = .013
Fine movements	Mid vs Control: P = .01 Mid vs L: P = .016 Mid vs Flum + L: P = .037	Mid + L vs Control: P = .003 Mid + L vs L: P = .005 Mid + L vs Flum + L: P = .013

Table. Ratio of Open Arm Time to Total Maze Time (in seconds) and Number of Motor Movements on Elevated Plus-Maze per Group

Data are presented as mean ± standard error of the mean (SEM).
*Statistically significant difference of P < .05.

dazolam and L-theanine is that both fine and basic motor movements also decreased with the combination of L-theanine and midazolam. Although our data did not support our hypothesis that L-theanine has anxiolytic effects in the rat model, our findings do suggest that L-theanine in combination with midazolam modulates the CNS by increasing anxiolysis and decreasing fine and basic motor movements.

A possible explanation for our findings may be explained by research conducted by Yokogoshi and colleagues.[20-22] According to their findings, L-theanine caused a statistically significant increase in dopamine levels in the striatum and in serotonin levels in the hippocampus, striatum, and hypothalamus following intragastric administration of L-theanine at various dosages as well as after microinjection of L-theanine directly into the brain.[20-22]

Future investigations might explore the motor effects of L-theanine using other balance and locomotion instruments such as the rotarod. It is important to determine the molecular site of action of L-theanine in order to understand the biochemical and pharmacologic effects of this herbal extract. Additional studies might include those designed to determine effects of L-theanine at various other CNS receptor sites, such as cholinergic, dopaminergic, or glutamate receptors (eg, *N*-methyl-D-aspartate, or NMDA) and peripherally at the neuromuscular junction. Once the molecular mechanism of action is clearly defined, work may then focus on studying the important clinical interactions of L-theanine and other pharmaceuticals. Further research could then explore the possible efficacy of L-theanine as an anesthesia adjunct or whether it has adverse interactions with anesthesia medications in the perioperative period.

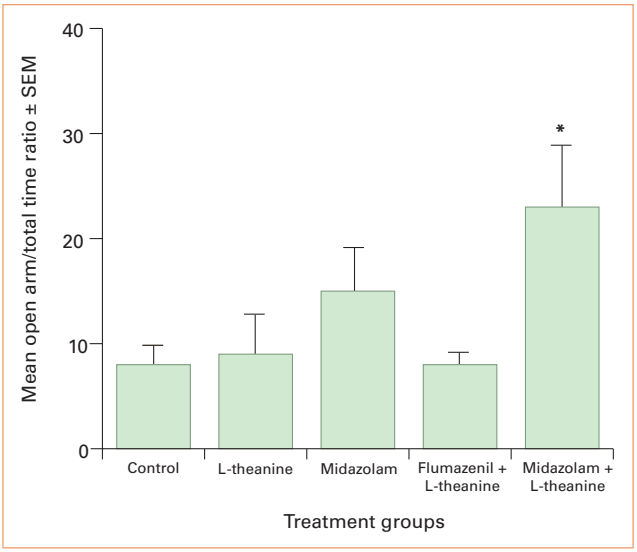

Figure 1. Ratio of Open Arm Time/Total Time (in seconds) on Elevated Plus-Maze
Drugs were injected 30 minutes before testing the rats on the elevated plus-maze. Asterisk indicates statistically significant difference of $P < .05$; SEM, standard error of the mean.

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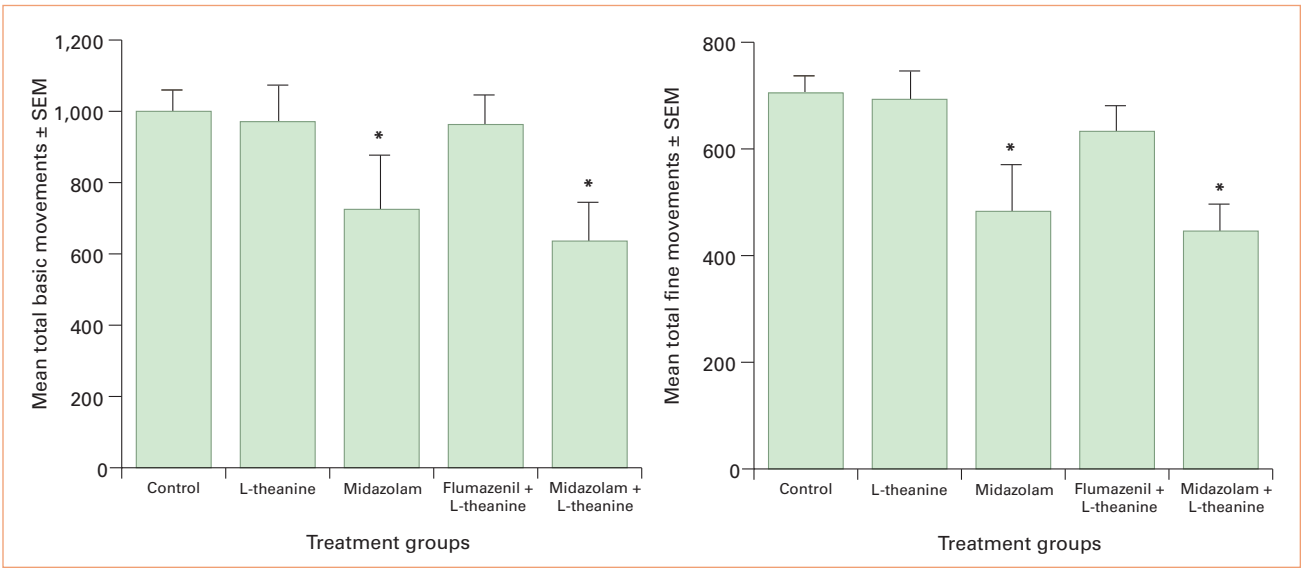

Figure 2. Basic and Fine Motor Movements on Elevated Plus-Maze
Drugs were injected 30 minutes before testing the rats on the elevated plus-maze. Asterisk indicates statistically significant difference of $P < .05$; SEM, standard error of the mean.

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