The Neuropharmacology of L-Theanine(*N*-Ethyl-L-Glutamine): A Possible Neuroprotective and Cognitive Enhancing Agent

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ABSTRACT. L-theanine (*N*-ethyl-L-glutamine) or theanine is a major amino acid uniquely found in green tea. L-theanine has been historically reported as a relaxing agent, prompting scientific research on its pharmacology. Animal neurochemistry studies suggest that L-theanine increases brain serotonin, dopamine, GABA levels and has micromolar affinities for AMPA, Kainate and NMDA receptors. In addition has been shown to exert neuroprotective effects in animal models possibly through its antagonistic effects on group 1 metabotrophic glutamate receptors. Behavioural studies in animals suggest improvement in learning and memory. Overall, L-theanine displays a neuropharmacology suggestive of a possible neuroprotective and cognitive enhancing agent

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For centuries, the Japanese have reported relaxing effects of unfermented green tea. Green tea is primarily consumed as beverage since it was brought to Japan from China in the eighth century during China's Tang Dynasty. More recently, the popularity of green tea has spread through a number of countries including North Africa and the Middle East, prompting scientific evaluation of its proposed benefits (Graham, 1992).

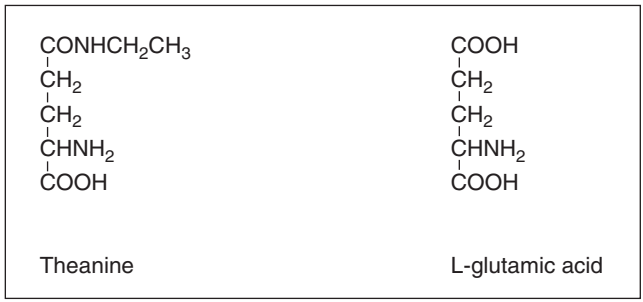
L-theanine or theanine is a major free amino acid uniquely found in green tea (Konishi & Takahashi, 1969; Neumann & Montag, 1983; Selvendran, 1970). It was first isolated and identified in green tea leaves, *Camellias sinensis*, in 1949 by Sakato (1949) and in the mushroom, *Xerocomus badius*, in the early 1950s (Casimir et al., 1960). Theanine accounts for 50% of the total amino acids in green tea leaves. It comprises about 1-2% of the total dry weight of the green tea leaves (Goto et al., 1996). L-theanine is synthesised in the roots of tea plants from glutamic acid (glutamate) and ethylamine before being transported to the leaves (Buckowski et al., 1999).

While, the historic use is well documented, the neuropharmacology of L-theanine has not been described. There have only been a few experimental studies of L-theanine and evidence from these studies suggests that L-theanine may modulate key brain neurotransmitters and their receptors, which may explain its reported benefits documented historically. The major purpose of this paper is to review the available literature on the neuropharmacology of L-theanine. The review will include studies in animals and humans describing effects on neurotransmitters levels, their receptors and behavior.

L-THEANINE CHEMISTRY

L-theanine (*N*-ethyl-L-glutamine) is structurally similar to glutamic acid, which is one of the primary excitatory neurotransmitters in the central nervous system. The structure of L-theanine and glutamic acid is shown in Figure 1.

FIGURE 1. Chemical Structure of Theanine and Glutamic Acid.



It is shown to be stable in solution over a pH ranging between 3.0 and 6.6 and shows good stability in both neutral (pH = 6.5) and acidic (pH = 3.0) conditions in 12 months storage with temperature below 25°C (Juneja et al., 1999).

THEANINE PHARMACOKINETICS

Kitaoka et al. (1996) reported that oral administration of L-theanine to guinea pigs results in absorption of L-theanine through the intestinal tract via a common Na^+-coupled co-transporter in the brush-border membrane in the same manner as glutamine. Similarly, both oral and intraperitoneal administration of L-theanine administered to rats, leads to absorption through the intestinal tract and then is subsequently hydrolysed to glutamic and ethylamine in the rat kidney (Unno et al., 1999; Desai et al., 2005). The latter finding suggests that the kidney is the most effective site for the enzymatic hydrolysis of L-theanine to glutamic acid and ethylamine (Unno et al., 1999).

Studies suggest that L-theanine also crosses the blood-brain barrier and is transported in a dose-dependent manner into the brain. Transport into the brain occurs via the leucine-preferring transport system of the blood-brain barrier (Yokogoshi et al., 1998a). The plasma concentrations of L-theanine and ethylamine reach their highest levels about 0.5 and 2 hours, respectively (Unno et al., 1999). Kimura and Murata (1971) reported that L-theanine (administered intraperitoneally) was taken up by brain tissue within 30 minutes after its administration without any metabolic change in mice. In animals, L-theanine concentrations reaches maximal levels at 1hour post-administration in blood

serum and liver, and 5 hours post-administration in the central nervous system (Terashima et al., 1999). Concentrations were shown to decline gradually over the course of 24 hours (Terashima et al., 1999). No pharmacokinetic studies have been conducted in humans.

EFFECTS ON BRAIN NEUROTRANSMITTERS

Effects on Monoamines

There are limited studies on the effects of L-theanine on brain neurotransmitters. Yokogoshi et al. (1998a) reported a dose-related increase in striatal dopamine release after both intra gastric and direct injection of L-theanine into the striatum of rats. This effect was shown to be mediated by the utilization of Ca^{2+} by simulation of NMDA receptors as the effect of L-theanine on dopamine was inhibited by the NMDA receptor antagonist AP-5 (Yokogoshi et al., 1998a). The concentrations of noradrenaline was found to be unaffected by L-theanine administration (Yokogoshi et al., 1998a), suggesting selective effects on dopamine neurons in the brain. However an earlier study in rats reported that L-theanine may affect noradrenaline-dependent signaling pathways as it was shown to inhibit the noradrenaline stimulated adenosine 3,5-monophosphate (cAMP) formation (Kimura & Murata, 1980). This could, however, be a general effect on second messenger pathways as in the same study, the histamine stimulated cAMP formation was also found to be inhibited by L-theanine.

An initial study showed that theanine was found to increase tryptophan, the precursor of serotonin, but it decreased both serotonin and the serotonin metabolite 5-hydroxy indole acetic acid (5-HIAA) in the brain (Yokogoshi et al., 1998a). However, a subsequent study by the same authors investigating specific brain regions reported that intragastric L-theanine significantly increased serotonin levels in the striatum, hippocampus and hypothalamus (Yokogoshi et al., 1998b). The discrepancy in the serotonin findings could be due to the fact that the former study reported whole brain concentrations, whereas the latter study reported changes within specific brain areas.

Effects on GABA

One study reported an increase in brain γ-aminobutyric acid (GABA) concentrations following L-theanine administration in mice (Kimura &

Murata, 1971). In the same study, the convulsive effect of caffeine was inhibited by L-theanine suggesting a possible GABA related anti-convulsive action.

EFFECTS ON NEUROTRANSMITTER RECEPTORS

A recent study suggests that L-theanine has low affinity for a number of glutamate receptors including AMPA (α-Amono-3-hydroxy-5-Methylisoxazole-4-Propionic Acid), kainate, and the glycine site of the NMDA (*N*-Methyl-D-Aspartate) receptor (Kakuda et al., 2002). The reported affinities were in the micromolar range (AMPA = 19.2 μM; Kainate = 0.373 μM; NMDA glycine site = 329 μM), with the binding activity (antagonistic) for AMPA and kainite receptors being 10-fold higher than that for NMDA receptor glycine sites. While the affinity of theanine was 80-fold lower than glutamate, the reported affinities are thought to play some role in neuroprotection (Kakuda et al., 2002). There are no studies reporting affinities for other brain neurotransmitter receptors including receptors for monoamines.

NEUROPROTECTIVE EFFECTS

Kakuda et al. (2000) examined the neuroprotective effect of L-theanine on post-ischemic neural death in field CA1 of the gerbil hippocampus. Ischemic neural death in the field CA1 of hippocampus was significantly suppressed when L-theanine was pre-treated in a dose-dependent manner. Similarly, in the middle cerebral artery occlusion model of cerebral infarction in mice, theanine (administered before and 3 hours after the occlusion) reduced the size of the cerebral infarcts (Egashira et al., 2004). The mechanism action of L-theanine on the brain remains relatively unknown. Since L-theanine is a natural glutamate analogue, one mechanism responsible for the neuroprotective effects may be related to L-theanine's affinity to glutamate receptor subtypes such as AMPA, kainite and NMDA (Kakuda, 2002). It has been suggested that L-theanine may act on glutamate receptors as an antagonist with very mild binding capacity (micromolar range), and this effect may contribute towards its neuroprotective effects (Kakuda et al., 2000). More recently Nagasawa et al. (2004) showed the theanine inhibited the delayed death of neurons caused by brief exposure to glutamate and this effects was blocked by group 1 metabotrophic glutamate receptor

(mGluR) antagonists, suggesting that group 1 mGluRs might be involved in the neuroprotective effects of theanine.

BEHAVIOURAL PHARMACOLOGY OF L-THEANINE

L-Theanine and Cognition

Studies have reported potential cognitive enhancing effects of L-theanine, particularly concerning learning and memory. Juneja et al. (1999) examined chronic effects (4 months) of L-theanine administration (180 mg/day) on memory and learning ability in rats. The study applied an operant conditioning paradigm to examine learning ability. During operant conditioning paradigm, food was delivered when rats pushed a lever and a light turned on. Learning ability was significantly improved in rats exposed to L-theanine, (i.e., rats showed greater correct responses when compared to the control rat group). Learning ability was also investigated in two avoidance tests, a passive and active avoidance test. The avoidance conditions assessed general tendency of the rats to move from a light to a dark compartment. In the passive avoidance test, the electric shock was applied immediately after a rat moved from a light to a dark compartment. Rats administered L-theanine (180 mg/day) showed greater cognitive ability, reporting hesitation to move to the dark compartment, and hence remained longer in the light compartment compared with control group. The active avoidance test examined the escape behaviour of rats from an electric shock. Rats treated with L-theanine showed an increase in avoidance behaviour, which signified an improvement of their memory ability.

Similarly, Yokogoshi and Terashima (2000) reported significant improvement of avoidance learning ability during both passive and active avoidance tasks, following long-term L-theanine administration (3 months). In addition, memory ability as estimated by the Morris Water Maze (MWM) transfer test was also improved by the chronic administration of L-theanine (Yokogoshi & Terashima, 2000). The MWM has been extensively used as an optimal preparation for assessing cognitive functions in animals. The MWM is a widely accepted test of spatial learning in rodents in which rats have to learn to find a hidden platform submerged several centimetres below the water level in a circular pool filled with water (Morris, 1981; Graziano et al., 2002; Higgins et al., 2002). Rats administered L-theanine were faster and more consistent than the control rats in finding the hidden platform in the MWM experiment.

The findings of the above studies suggest that L-theanine may have positive behavioural effects on learning and memory. These findings support the neurochemical findings showing increases in monoamines (Yokogoshi et al., 1998a,b). Given that serotonin and dopamine have been shown to improve cognitive processes such as attention, learning and memory (Andre, 2002; Menesses, 1999), it is possible that the learning and memory effects of L-theanine may be mediated via manipulation of serotonin and dopamine.

L-Theanine and Anxiety

While there is historical evidence for possible anxiolytic effects of L-theanine, very few studies have examined this experimentally in animals or humans. Ito et al. (1998) examined the effects of L-theanine (200 mg) on brain α-activity in eight human volunteers. Participants were divided into two equal groups: high anxiety and low anxiety, based on the Manifest Anxiety Scale. The study reported relaxation effects resulting from the generation of α-activity in the occipital and parietal regions of the brain. Furthermore, the increase in α-activity was generated around 30 minutes after L-theanine administration, which is consistent with the pharmacokinetic finding in rats showing that L-theanine is incorporated into the brain tissue within 30 minutes after administration (Kimura & Murata, 1971). While, L-theanine may modulate α-activity, this measure is not a direct marker of anxiolytic potential as similar effects on α-activity are observed with non-anxiolytics (i.e., amphetamines) (Montagu, 1968). Thus modulating α-activity may not be a direct measure of possible anxiolytic effects. In addition the latter study failed to examine subjective anxiety levels and hence it is unclear if theanine has direct effects on anxiety. However, given that L-theanine increases both serotonin and GABA (Kimura & Murata, 1971; Yokogoshi et al., 1998a,b), and both these neurotransmitters play an important role in the pathophysiology of anxiety disorders and anxiolytic effects (Gonzalez et al., 1998; Graeff, 2002; Kent et al., 2002), it is possible L-theanine may have anxiolytic effects.

We recently examined the acute effects of L-theanine (200 mg) in comparison with the benzodiazepine, alprazolam on anticipatory anxiety in humans (Lu et al., 2004). The effects were assessed under a relaxed and experimentally induced anxiety (anticipation of an electric shock) condition. The findings suggested that while L-theanine may have some relaxing effects under resting conditions as evidence by reduction in anxiety level on the tranquil-troubled subscale of the visual

analogue mood scale (VAMS), both L-theanine and alprazolam did not demonstrate any acute anxiolytic effects under conditions of increased anxiety in the AA model. These findings suggest that studies examining the chronic effects of L-theanine of anxiety are warranted.

TOXICOLOGY OF L-THEANINE

There are no reported side effects in studies investigating L-theanine within animals (Kakuda et al., 2000) or humans (Ito et al., 1998). L-theanine has also shown to produce no drowsiness in humans (Ito et al., 1998). Sudzuka et al. (1996) showed studies on cancer chemotherapy demonstrating that L-theanine enhanced the therapeutic efficacy of doxorubicin without inducing side effects.

SUMMARY

Pre-clinical studies suggest that L-theanine increases a number of neurotransmitters including serotonin, dopamine and GABA levels and has micromolar affinities for AMPA, kainate and NMDA receptors. Behavioural studies in animals demonstrate improvements in learning and memory following L-theanine administration. In addition L-theanine has been shown to exert neuroprotective effects in animal models possibly through its antagonistic effects on group 1 metabotrophic glutamate receptors. While studies in humans are lacking, there is some evidence for a possible anxiolytic effect. Further studies are warranted in both animals and humans to examine L-theanine's efficacy as a neuroprotective and cognitive enhancing agent as well as a possible anxiolytic.

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