Review

Neurobiological effects of the green tea constituent theanine and its potential role in the treatment of psychiatric and neurodegenerative disorders

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Theanine (*n*-ethylglutamic acid), a non-proteinaceous amino acid component of green and black teas, has received growing attention in recent years due to its reported effects on the central nervous system. It readily crosses the blood–brain barrier where it exerts a variety of neurophysiological and pharmacological effects. Its most well-documented effect has been its apparent anxiolytic and calming effect due to its up-regulation of inhibitory neurotransmitters and possible modulation of serotonin and dopamine in selected areas. It has also recently been shown to increase levels of brain-derived neurotrophic factor. An increasing number of studies demonstrate a neuroprotective effects following cerebral infarct and injury, although the exact molecular mechanisms remain to be fully elucidated. Theanine also elicits improvements in cognitive function including learning and memory, in human and animal studies, possibly via a decrease in NMDA-dependent CA1 long-term potentiation (LTP) and increase in NMDA-independent CA1-LTP. Furthermore, theanine administration elicits selective changes in alpha brain wave activity with concomitant increases in selective attention during the execution of mental tasks. Emerging studies also demonstrate a promising role for theanine in augmentation therapy for schizophrenia, while animal models of depression report positive improvements following theanine administration. A handful of studies are beginning to examine a putative role in attention deficit hyperactivity disorder, and theoretical extrapolations to a therapeutic role for theanine in other psychiatric disorders such as anxiety disorders, panic disorder, obsessive compulsive disorder (OCD), and bipolar disorder are discussed.

Keywords: Theanine, Central nervous system, Neuroprotection, Anxiety, Selective attention, Depression, Alzheimers, Anti-Psychotic, Alpha-wave

Introduction

Theanine (γ-glutamylethylamide or γ-ethylamino-L-glutamic acid), a derivative of glutamic acid, is a plant-based non proteinaceous amino acid first identified and isolated in 1949.[1] It is a major constituent of the tea plant Camellia Sinensis, comprising 50% of the plants total free amino acids, or 2% of the total content, and it is also found in smaller amounts in two other Camellia species. It is present in significant amounts in both green and black tea, highest amounts being reported in green tea, oolong tea and naturally fermented Pu-erh tea.[2] These teas in particular have long been considered to exert relaxant and calming effects, while simultaneously increasing alertness. As a result, theanine has received steadily increasing attention in recent years both in the medical literature and on popular media internet sites. It is manufactured and sold as a herbal supplement, commercially branded as *Suntheanine*. This review focuses on the known effects of theanine on brain activity and function, and discusses its potential use in the treatment of certain psychiatric, psychological, and neurodegenerative disorders.

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Biochemistry

When theanine is administered orally, it peaks in the serum within an hour of administration and presents in urine after 5 hours, its levels subsequently decreasing over the following 24 hours.[3] It reaches its maximum level in the brain within 5 hours, and gradually disappears from the liver, kidney, and brain within 24 hours.[4] It is transported through the intestinal brush border membrane and is thought to be

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metabolized to glutamate and ethylamide by renal phosphate-independent glutaminase.[3,5] It freely passes the blood–brain barrier probably via the leucine-preferring transport system in a competitive manner relative to large neutral amino acids,[6] although it has also been suggested that its ethyl base increases its fat solubility, facilitating its passage across same.[7] Theanine levels are increased in the brain within an hour of oral administration, continue to increase for a further 4 hours, and gradually decrease to non-detectable levels over the following 19 hours.[3,4] It results in an increase in the concentration of glutamate and ethylamine in the brain, and is well tolerated within all mammalian systems examined with an LD_{50} of greater than 5000 mg/kg in rats.[1]

Effect on brain chemistry and neurotransmission

One of the earliest studies of the effects of L-theanine on the central nervous system (CNS) by Kimura and Murata[7] reported increased intracerebral levels of GABA after 30 minutes intra-peritoneal injection. More recently, Yamada *et al.*[8] demonstrated a six-fold increase in glycine release from the striatum of rats following perfusion of theanine into the rat brain; however, they reported no significant effect on the release of the excitatory neurotransmitter glutamic acid, alongside a fall in aspartic acid release. They thus suggested a role for theanine in inhibitory neurotransmission. Dopamine release increased up to two-fold during the same experiment. Because glycine is known to evoke dopamine release in rat striatum,[9] the authors speculate on the possibility that the theanine-mediated dopamine release is in fact mediated by glycine.[8] *In vitro*, Kakuda *et al.*[10] demonstrated inhibition of glutamate release from cultured neurons following sustained exposure to theanine. Abnormally high levels of extracellular glutamate have been implicated in the pathophysiology of cerebral ischaemia and infarction; a therapeutic role for theanine via a potential neuroprotective effect will be further discussed below. The involvement of $GABA_{(A)}$ receptors on the neuroprotective effect of theanine following experimental middle cerebral infarction has also been investigated by Egashira *et al.*[11] They found that the protective effects of L-theanine were ablated by the GABA antagonist bicuculline, lending further support to the hypothesis that theanine exerts at least some of its psychoactive effects via an increase in brain GABA levels. In a recent study of the effects of theanine on developing rat brains, theanine was fed to pregnant females for 3 weeks and the brains of the resulting offspring were analyzed for various neurotransmitters. Glycine and GABA levels were increased in 1- to 3-week-old rats compared to controls, as were nerve growth factor and neurotrophin.[12] There is evidence that both glycine and GABA function as excitatory neurotransmitters during brain development and play a crucial role in healthy brain maturation.[13] The findings need to be replicated but suggest that the addition of L-theanine to the maternal rat diet may promote nerve maturation and enhances GABA synthesis in developing offspring.[12] Whether or not this effect occurs in humans is not known but given the established safety of L-theanine, clinical trials in humans may be worth considering. The effects of L-theanine on neurochemistry may not be confined to alterations in the release of the inhibitory neurotransmitters GABA and glycine. Kimura and Murata's[7] study also reported a decrease in brain norepinephrine levels, possibly as a result of the reported increase in GABA levels. One study has also demonstrated selective increases in serotonin and dopamine within the striatum, hypothalamus and hippocampus following theanine administration into rat brain, while levels of 5H1AA or norepinephrine were unaffected.[14] Pretreatment with the NMDA receptor antagonist AP-5 ablated the theanine-induced increase in dopamine release.[14] This led the authors to suggest that the action of theanine may be mediated by the enhancement of calcium utilization via the NMDA receptor. Further support for this proposal has been recently provided by Wakabayashi *et al.*,[15] who *inter alia*, found that L-theanine administration resulted in an increase in calcium release within cultured cortical neurons. The increase in calcium release was suppressed by both NMDA antagonists AP-5 and MIC-801. In a separate paper, Yokogoshi *et al.*'s[16] demonstrated a decrease in the overall levels of brain serotonin and 5HIAA concentrations following administration of intragastric theanine, but did not comment on the significance if any, of these apparently contradictory findings. However, one would expect that the selective increases in brain neurotransmitter activity within specific brain areas would be of greater pharmacological significance than overall levels within the brain, which may simply reflect the sum of net metabolic activity. It is also interesting that two distinct mechanisms to explain the theanine-mediated increase in striatal dopamine release have been proposed by Yamada's and Yokogoshi's groups, respectively.

It must be borne in mind that most of the above studies have focused on the acute effects of theanine on neurotransmitter levels or release. Yokogoshi and Terashima found that chronic administration of theanine over 3 months resulted in decreased serotonin and 5H1AA in the cerebral cortex (cited in[17]). Three other studies to date have investigated, *inter alia*, the effects of chronic theanine administration over 14 days, on GABA, glutamate, and serotonin

levels.[18–20] Shen *et al.*[18] reported increased brain GABA and glycine and decreased aspartate following theanine treatment over 15 days in a rat model of cerebral infarction. Schallier *et al.* reported no effect of theanine treatment for 14 days on extracellular glutamate levels in either the frontal cortex or hippocampus, and decreased cortical GABA levels with no effect on hippocampal GABA.[19] In the second study by Yamada *et al.*,[20] levels of serotonin and 5H1AA within the cortex were reduced after 3 months intake of theanine. Further studies on the longer-term effects of chronic theanine administration on global and selective brain neurotransmitter release are needed to confirm the above and clarify the CNS response to longer-term usage. Recently, theanine administration has also been shown to increase levels of the neurotrophin brain-derived neurotrophic factor (BDNF),[15,21,22], the potential significance of which will be discussed below.

The known effects of theanine on brain neurotransmitter activity is summarized in Table 1. Despite its structural similarity to glutamate, there is no evidence of any increase in glutamate levels following theanine injection or administration, thus rendering unlikely the conversion of theanine to glutamate within the CNS. To date, the bulk of the evidence on the neurochemical effects of theanine points to a possible decrease in glutamate levels, alongside increases in serotonin and dopamine in selected areas such as the midbrain. Overall brain levels of the inhibitory amino acids GABA and glycine are also increased, although there is a lack of data on selective changes in these amino acids within specific brain regions. There is however growing evidence for an increase in brain levels of BDNF following theanine administration. On the basis of current evidence, the net effects of theanaine appear to reduce excitatory activity with resultant calming and anxiolytic effects and possible down-regulation of stimulatory pathways. Its ability to selectively increase levels of serotonin and dopamine in areas such as the striatum, hypothalamus, and hippocampus suggest a further potential role for this amino acid in the modulation of mood, motivation, cognition, and memory. Limited experimental support for a role in these functions is beginning to emerge and will be outlined below. It seems likely that selective rather than global changes in brain chemistry are responsible for most of the observed effects of theanine on neurochemical dependent brain function and activity. An independent effect of theanine on brain wave activity will be outlined below based on studies to date.

Neuroprotective effects of theanine and preventative effects on cognitive dysfunction

Extracellular glutamate can cause neuronal cell death by acting as a powerful neurotoxin in the CNS.[26] Because an excess of extracellular glutamate has been implicated in the mechanism of neuronal death and toxicity as a result of transient ischaemia (ibid) a number of animal and *in vitro* studies have investigated whether theanine can exert a protective effect on cerebral infarction as a result of its ability to inhibit glutamate accumulation. These studies have all demonstrated a significant protective effect.[11,18,27–33] Initial studies of its neuroprotective effects have focused on its modulation of neurotransmission, particularly in relation to glutamate. For example, the direct binding of theanine to glutamate receptor subtypes such as the NMDA, AMPA, and kainite receptors was found to result in the inhibition of glutamate binding thus antagonising the latter's effects.[27,30] Direct evidence for this hypothesis has come from Zukhurova's group, who reported that repeated intrastriatal injections of theanine during reperfusion prevented brain injury caused by glutamate receptor agonists.[31] However, more recent studies point to more complex effects. Di *et al.* investigated the neuroprotective effect of theanine in an *in vitro* model of Alzheimers disease using the human APP transgenic SH-SY5Y cell, in which amyloid β neurotoxicity was triggered by L-glutamate. Theanine administration significantly attenuated glutamate-induced apoptosis at similar levels seen with the NMDA receptor inhibitor MK-801, and it furthermore suppressed the activation of c Jun N-terminal kinase, caspase-3, plus inducible and neuronal nitric oxide synthase (NOS) induced by glutamate.[32]

Table 1 Effect of L-Theanine on brain neurotransmitter levels

	Neurotransmitter							
Brain region	Glutamate	Glycine	GABA	Serotonin	Dopamine	Catecholamines	Aspartate	BDNF
General	$\downarrow^{10}$ $\leftrightarrow^{19*}$	$\uparrow^{12,18}$	$\uparrow^{12,18*}$	$\downarrow^{16}$	$\uparrow^{8}$	$\downarrow^{7}$	$\downarrow^{8,18}$	$\uparrow^{14,15,22}$
Midbrain/striatum	$\leftrightarrow^{8}$	$\uparrow^{8}$	ND	$\uparrow^{14,17}$	$\uparrow^{8,14,17}$ $\downarrow^{23}$§	ND	$\downarrow^{8}$	ND
Hippocampus	$\leftrightarrow^{19*}$	ND	$\leftrightarrow^{19*}$	$\uparrow^{14,17}$	$\uparrow^{14,17}$	$\uparrow^{24}$	ND	$\uparrow^{8,15,18}$
Hypothamalus	ND	ND	ND	$\uparrow^{14,17}$ $\leftrightarrow^{12}$	$\uparrow^{14,17}$ $\leftrightarrow^{12}$	ND	ND	ND
Cerebrum/cortex	$\leftrightarrow^{19*}$	ND	$\uparrow^{7}$	$\downarrow^{17,20,25*}$ $\leftrightarrow^{12}$	$\leftrightarrow^{12}$	$\uparrow^{24}$	ND	ND
Cultured neurons	$\downarrow^{10}$	ND	ND	ND	ND	ND	ND	ND

Note: Parentheses refer to reference number; ↔= no change; ND = not done; *chronic administration; §nicotine-treated.

However, the affinity of theanine for glutamate receptors is 80–30 000-fold less than that of glutamate[27] and so other mechanisms in the protective effect of theanine have also been investigated. Nagasawa *et al.* investigated the protective effect of L-theanine on primary cultured cortical neurons.[33] They reported an inhibition by theanine of delayed neurotoxicity by brief exposure to glutamate, which was abolished by group 1 mGLuR antagonists. They thus suggest that the group 1 metabotropic glutamate receptors may be involved in neuroprotection by theanine. Egashira *et al.* have suggested that the protective effect may be mediated via an indirect action on $GABA_A$ receptors during reperfusion.[27] This hypothesis is supported by the recent findings of Shen *et al.*,[18] who demonstrated an increase in glycine, GABA, and BDNF in rat brain following cerebral ischaemia-reperfusion injury in the theanine-treated group. Kakuda, on the other hand, speculates on a central role for glutamine in theanine-mediated neuroprotection.[28] Glutamine plays a crucial role in glutamate recycling within the CNS via a glutamine/glutamate cycle which acts to convert the extracellular glutamate to glutamine via glutamine synthetase after the glutamate has been transported into astrocytes. This glutamine is released back into the extracellular space, where it is taken up into glutamatergic neurons via the glutamine transporter and hydrolysed by phosphate-dependent glutaminase back to glutamate.[28] His group found a bidirectional inhibition of theanine and glutamine in isolated rat synaptosomes, theanine inducing a significant decrease in glutamine accumulation in a concentration-dependent manner. They therefore speculated that theanine could at least partly alter extraceullular glutamine levels via interaction with glutamine transporters in nearby astroglia. Alterations in glutamate transmission and the glutamate/glutamine cycle are hypothesized to contribute to the pathology of Alzheimer's disease.[26] One study has investigated the effects of L-theanine ingestion over 5 weeks on neuronal function following injection of amyloid β (1–42).[34] They reported an attenuation of amyloid β-induced memory impairment, reduced Amyloid β levels, and inhibition of Amyloid β-induced neuronal cell death in the cortex and hippocampus of mice brains. They also found a reduction in the downstream expression of extracellular signal-related kinase (ERK), p38 mitogen-activated protein kinase and transcription factor NF-kappaβ activity induced by Amyloid β. A parallel study by Cho *et al.* looked at the effect of L-theanine on neurotoxicity *in vitro* caused by rotenone and dieldrin, environmental toxins which have been implicated in the aetiology of Parkinson's disease. They found an attenuation of toxin-induced DNA fragmentation, apoptosis, and haem oxygenase up-regulation. Pretreatment with L-theanine-blocked the toxin-induced down-regulation of ERK1/2 and of BDNF in SH-SY5Y cells.[24]

It is not surprising, in light of the above findings, that intervention studies on the effect of theanine on cognitive decline in human and animal subjects have recently begun to emerge. Kakuda investigated the effects of 47.5 mg theanine daily on cognitive function in the elderly and reported significantly lower decline than in controls.[35] Kuriyama *et al.* reported lower incidences of cognitive impairments in human subjects ingesting theanine in the form of two or more cups of green tea daily.[36] Kataoka *et al.* administered theanine powder equivalent to 47.5 mg theanine per day.[37] They used the Hasegawa's dementia scale revised (HDS-R) Dementia scale to measure changes in cognitive function. The theanine-treated group scored significantly better from the seventh month onwards (apart from the eleventh month for which no explanation was offered by the authors) and suggested to the authors that the decline in the HDS-R scores was prevented by the ingestion of green tea powder over a prolonged period. However, the authors did not rule out an ancillary role for the relatively high catechin content within the formulation. Kim *et al.* investigated the effects of administration of oral theanine for 5 weeks to mice followed by injection of β-amyloid protein intracerebrally.[34] They reported a significant reduction in β-amyoid-induced neuronal cell death in the cortex and hippocampus, as well as reduction in oxidative lipid damage and of memory impairment as measured by the water maze and passive avoidance tests.

Theanine and brainwave activity

In addition to its ability to alter neurotransmitter activity, theanine has also been found to significantly alter alpha brain-wave activity both in human and animals. Alpha brain-wave activity in humans is indicative of wakeful relaxation as well as increased creativity, better performance under stress, and improved learning and concentration, as well as decreased anxiety.[38] Four studies reported increases in alpha brain wave activity in the occipital, parietal and frontal and areas following 50–250 mg theanine,[38–41] while Owen *et al.*[42] reported increasing alpha activity over time following ingestion of 50 mg theanine in human subjects. Nobre *et al.*[43] reported similar increases following ingestion of 50 mg theanine over 105 minutes both at rest and during passive activity. Just one recent study by Fox *et al.*[44] has reported a lack of effect of 100 mg theanine on alpha wave activity. While the authors postulate that the effect of theanine on alpha activity could be dose dependent, this is not borne out by the positive

correlations between lower doses of theanine and increased alpha band activity reported by the other groups. In addition, there is evidence that theanine may antagonize some of the stimulatory effects of caffeine; it has, for example, been found to antagonize the decrease in serotonin levels which have been artificially raised by caffeine administration in rats.[45] This agrees with the earlier report by Yokogoshi *et al.*[6] of an overall decrease in brain serotonin levels following theanine administration. In a study in rats, a biphasic effect of theanine on rat electroencephalogram (EEG) recordings was reported, such that 2 μmol/kg of intravenous theanine alone increased the power of beta brain wave activity in the cortex, amygdala and hippocampus, but 10 μmol or greater inhibited the excitatory effects of 5 μmol/kg caffeine on beta-wave activity.[46] Another study found that theanine antagonised the effects of caffeine on blood pressure but did not significantly affect jitteriness, alertness or other aspects of mood when given as a single bolus of 200 mg.[47] Several animal studies have also reported antagonistic effects of L-theanine on the convulsive and excitatory action caused by caffeine administration.[48–50] One of these investigated the effects of L-theanine on caffeine-induced sleep disturbances in rats and found that low doses (22.5 and 37.5 mg/kg) significantly reduced the caffeine-induced decreases in slow wave sleep while higher concentrations (75 and 150 mg/kg) had no effect.[50] The lack of effect of the higher doses is noteworthy and may be a reflection of their supra-physiological concentrations, similar to amounts typically administered to a typical 70 kg human. It also underscores the importance of establishing therapeutically optimal doses given the possibility of a plateau or biphasic effect above certain critical concentrations.

Because alpha brain wave activity is known to play an important role in critical aspects of attention, it is not surprising that several studies have investigated the effect of theanine, both with and without caffeine, on attention and cognition in healthy human subjects. In one study, 250 mg of theanine combined with 150 mg of caffeine improved simple reaction time, numeric working memory and sentence verification, and delayed word recognition reaction time.[51] It was concluded that beverages containing both theanine and caffeine have different pharmacological effects to caffeine alone. Another study of healthy humans found that combined caffeine and theanine (40 and 97 mg, respectively) improved speed and accuracy of attention switching and reduced susceptibility to distraction over 90 minutes.[52] Gomez-Ramirez *et al.*[41] reported that administration of 250 mg theanine resulted in greater resting alpha-wave activity but significant decreases in background alpha activity, particularly in the auditory modality, while subjects were actively performing a demanding attention switching task. A second study by the same group found a significant reduction in background alpha activity following treatment with 250 mg L-theanine compared to placebo during a highly demanding visuo-spatial task, which was accompanied by a shift in scalp topography indicative of treatment-related changes in the neural generators of ongoing tonic alpha activity occurring over seconds or minutes.[25] This is considered to be an EEG correlate of sustained attentional processing. They concluded that theanine plays a role in sustained attention over time during a demanding task as opposed to affecting moment-to-moment alpha wave activity. Their most recent study investigating a lower concentration of theanine (100 mg) with and without 50 mg caffeine on maintenance of vigilance during a sustained attention task reported reduced commission and omission errors with both compounds alone, while combined treatment with both conferred no further additional benefits.[44] The beneficial effect of theanine was less than that of caffeine, however, with caffeine reducing omission and commission errors by 50 and 30% respectively, compared to theanine (36 and 23%). Theanine had no effect on reaction speed in this study, even though a reduction in same was reported by them in their previous study[41] and also by Rogers *et al.*[47] They suggest that systematic does response studies on the neurophysiological effects of theanine are needed to critically unpack any differential effects of theanine on cognitive performance and attention.

Given the number of studies on the effects of theanine on brainwave activity, it is surprising that only one study has investigated the direct effects of theanine on susceptibility to epileptic seizures. Schallier *et al.* have recently investigated the effect of 4% L-theanine over a 14-day period on generalized and limbic seizures induced by pentylenetetrazol (PTZ) and pilocarpine, respectively, in mice.[19] Theanine significantly increased the threshold dose of pilocarpine for inducing body twitch, rearing and falling. However, it either had no effect on the PTZ-induced behaviours or decreased the threshold for two of them, namely tonus and death. These differential effects of theanine on pathologically induced seizures will need to be replicated but suggest a more complex role for theanine on brain-wave activity than previous studies under normal conditions would suggest.

In summary, theanine appears to exert a positive role in the focussing of selective attention and reduction of distracting stimuli, by means of its effects on alpha brain wave activity. Whether or not this effect is directly or indirectly related to its ability to selectively modulate the release of neurotransmitters such as GABA is not known. However, two

studies have found an increase in alpha brain wave activity following oral GABA administration to humans[53,54] rendering a causal link possible. Given its positive enhancement of attention, and its reduction of distracting stimuli in all of the human studies undertaken to date, an investigation of an adjuvant role for L-theanine in the treatment of attention deficit disorder (ADD) would appear to be warranted. Early trials in what is likely to become a fruitful area of investigating are now beginning to emerge and will be discussed below.

Learning and memory

Recently, attention has begun to focus on behavioural and molecular effects of theanine in learning and memory. In rats, chronic administration of L-theanine for 3 months resulted in a significant improvement in avoidance learning ability and retrieval of pellets.[20] The increase in the score of the avoidance test was accompanied by a decrease in serotonin and 5HIAA in the cerebral cortex. Theanine-fed rats also retrieved pellets more efficiently than controls and demonstrated superior memory ability as estimated by the transfer test. This replicates some of the findings of Kim *et al.*[34] Another study reported an increase in exploratory activity and object-recognition memory in newborn rats fed theanine over several weeks.[21] BDNF, which is a direct measure of hippocampal neurogenesis, was also significantly increased in weanling brains after 6 weeks of theanine administration. A second study in mice has also reported increases in hippocampal BDNF after 3 weeks theanine administration.[15] One recent study of rats has investigated the effect of theanine administration on long-term potentiation (LTP) at rat hippocampus synapses and exposure to acute stress in post partum rats.[55] They reported a reduction in serum corticosterone levels, which is known to cause inhibiton of synaptic plasticity. They thus further examined the effect of theanine on CA1 LTP in hippocampal slices following a 100-Hz and a 200-Hz stimulus for 1 second. Theanine administration resulted in significant attenuation of the 200 Hz stimulus while effecting no difference in the 100 Hz pulse. Administration of the NMDA antagonist APV resulted in significant reduction of the 200 Hz LTP pulse in the control but not theanine-fed mice. The authors concluded that theanine reduces NMDA receptor-dependent CA1 LTP while increasing NMDA independent CA1-LTP. It also prevented the attenuation in LTP following exposure to the tail suspension stress test. They conclude that the lack of NMDA receptor-dependent CA1 LTP by theanine intake is involved in ameliorating the attenuation of CA1 LTP after tail suspension. Disruption of hippocampal CA1 neuronal activity is known to be involved in the pathology of Alzheimer's, and recent experimental evidence suggests that amyloid-β disturbs NMDA receptor-dependent LTP induction in the hippocampal CA1 and dentate gyrus both *in vivo* and *in vitro*.[56] This study is therefore significant in helping to establish one of the precise molecular mechanisms by which theanine may exert neuroprotective effects on cognitive abilities. No doubt more studies will emerge to lend further support to the above findings. A recent study by Tian *et al.*[57] investigated the protective effect of L-theanine on chronic restraint stress-induced cognitive impairments in mice over 4 weeks.[57] Cognitive function was measured by the Morris water maze and step through tests, and levels of serum corticosterone, dopamine, and catecholamines were also measured. Theanine caused a reversal both of the cognitive impairment and of the increased serum corticosterone and catecholamine levels. The reduction in serum corticosterone confirms the findings of Takeda *et al.*[55]

Potential role in the treatment of anxiety

A small number of studies have begun to focus on the potential of theanine as an anxiolytic agent in light of its effects both on alpha brain wave activity and on inhibitory neurotransmitter release. Three studies to date have investigated the effects of a single dose of theanine on measures of anxiety. The first study compared the effects of 200 mg of theanine compared to 1 mg of a standard benzodiazepine, alprezolam, on measures of anticipatory anxiety in 16 healthy humans.[58] Although theanine exerted a relaxing effect during the baseline resting stage, it had no effect on the experimentally induced anxiety stage. In another once-off study the effects of theanine on physiological stress responses using a mental arithmetic test as the acute stressor was investigated.[59] Theanine was administered either at the beginning or mid-way through the trial and resulted in a reduction in heart rate and salivary immunoglobulin A, indices of psychological stress. Analysis of heart rate variability indicated that both of these reductions were attributed to an attenuation of sympathetic nervous activation. One other once-off study in rats investigated the effects a single standard (10 mg/kg) dose of theanine on its own and combined with either midazolam or the benzodiazepine antagonist flumezanil on behavioural measures of anxiety in the rat.[60] The single dose of theanine did not reduce anxiety as measured by the open maze test, but when combined with midazolam resulted in a further modest attenuation of the effects of midazolam alone. There was no difference between the theanine group and the theanine plus flumezanil group. Given the lack of effect of the single dose of theanine, this finding is to be expected. Thus to date, there appears to be evidence for only a

modest role for a single dose of theanine on the alleviation of acute anxiety.

Several recent studies have begun investigating long-term administration of theanine on chronic anxiety associated with the acute psychoses, specifically schizophrenia. In a study, 60 patients with schizophrenia or schizoaffective disorder were supplemented with 400 mg of theanine per day along with their antipsychotic treatment, for a period of 32 months.[61] Augmentation with theanine was associated with a reduction in anxiety as measured by the Hamilton anxiety rating scale (HARS) scale, and positive and general psychopathology (measured by the positive and negative syndrome scale (PANSS) three-dimensional model). These differences ranged from modest to moderate. It was also found to be safe and well tolerated. The authors concluded that L-theanine augmentation therapy can ameliorate positive, activation and anxiety symptoms in schizophrenia and schizoaffective disorders. In a follow-up study by the same authors to examine the association between circulating levels of neurochemical indicators and the positive effects of L-theanine, they found that BDNF and cortisol to dihydroepiandrosteronesulphate (DHEAS) ratio were significantly associated with the beneficial effects of theanine.[22] Both of these molecules are thought to play a role in a myriad of brain functions including neuroprotection, neuronal excitability, memory, mood regulation, and response to stress.[15] Thus, a further role for BDNF, apart from its role in hippocampal neurogenesis and memory, in theanine-mediated effects within the CNS, may also be emerging. However, there is still a dearth of studies on direct effects of theanine on measures of anxiety, including generalized anxiety disorder and panic disorder on humans; the studies inferring such an effect indirectly from alpha brain wave changes. As with ADD, given the positive effects reported so far, these studies would appear to be well warranted at this point. Finally, one recent study investigated the effect of short-term theanine administration on opioid withdrawal in morphine-dependent monkeys and reported significantly decreased withdrawal scores compared to controls.[62] They also reported anxiolytic like effects in mice given a single dose of theanine, which is at odds with others studies involving a single bolus to rats. However, it is possible that species differences may account for the difference. On the basis of their findings, they suggest that L-theanine may be useful in the treatment of opioid withdrawal as well as in anxiety disorders.

Theanine and depression

Two animal studies have begun to investigate whether theanine can exert either antidepressant effects and the findings, albeit in need of replication, are promising. In a 10-day study of rats, the antidepressant effect of 1, 4, and 20 mg/kg theanine resulted in improvements in two standard antidepressant screening tests, the forced swim test and tail suspension test, compared with controls.[63] Theanine was also found to antagonise reserpine-induced ptosis and hypothermia, suggesting mediation by the monoamine neurotransmitter system. Wakabayashi *et al.*[15] also demonstrated antidepressant effects via reduced immobility in the forced swim test, and an antipsychotic effect by means of improved pre-pulse inhibition of acoustic startle. Deficits in neural pre-pulse inhibition signify abnormalities in sensorimotor gating as found in schizophrenia and Alzheimer's disease. The same paper demonstrated an increase in calcium levels in cultured cortical neurons following theanine administration and an agonistic effect on the NMDA receptor. The authors concluded that theanine may possess both anti-psychotic and antidepressant effects. Interestingly, expression of BDNF has been found to be decreased in depression, and antidepressants up-regulate its expression.[64] In addition, suicidal behavioural is also associated with a lower expression of BDNF (ibid) leading to the suggestion that BDNF and its mediated signalling may participate in the pathophysiology of depression and suicide. To what extent BDNF levels correlate with actual measures of depression and suicidal behaviour has not been elucidated, but given the ability of theanine to up-regulate its levels, clinical trials of theanine in clinically depressed humans would appear to be well warranted at this point. Plasma BDNF levels are also known to be decreased in bipolar disorder and to correlate with the severity of the disease,[64] with some authors suggesting that it plays a critical role in the pathophysiology of this disease and in the activity of therapeutic agents used in its treatment.[65] Levels of GABA are also known to be decreased in mood disorders (for reviews see refs.[65–69]); trials of supplementary theanine both in the maintenance and during active treatment of bipolar episodes may therefore also be worth considering.

Attention deficit hyperactivity disorder and nicotine dependence

Two studies have begun to investigate a role for L-theanine in attention deficit hyperactivity disorder (ADHD). One study by Cross *et al.*,[70] albeit flawed in design, administered theanine (along with the serotonin precursor, 5-HTP) on a cohort of adapted and at-risk children demonstrating at least one clinical diagnosis of ADHS, mood disorder, attachment disorder, and pervasive developmental disorder. Treatment group was administered theanine in sublingual doses of 50 mg for 6–8 weeks; it was not specified whether this dose was given more than once daily.

They were also given 100 mg of the serotonin precursor 5-HTP daily for the same period. A delayed treatment group was given the same supplement 2 months later but it is not stated for how long they received the supplements. Urinary measurements of a range of neurotransmitters were carried out pre- and post-treatment in each group. There were significant increases in urinary serotonin, glutamate and GABA in the treatment group, the largest increase being for serotonin. The delayed treatment group also appeared to show alterations in some of the neurotransmitters measured but these are difficult to interpret given that the duration if this treatment group is not stated. Behavioural changes were measured by the child behaviour checklist questionnaire for children and adolescents. Four of the 11 subscales, namely anxious/depressed, thought problems, attention problems and other problems were statistically significantly for the treatment group with a further three subscales approaching significance. An additional limitation of this study, which the authors alluded to, was its non-randomised nature, i.e. parents knew which treatment group their child was allocated to and this may have subjectively biased their perceptions of behaviour post-treatment. The second paper studied the effects of L-theanine for 6 weeks on sleep quality on a cohort of boys formally diagnosed with ADHD.[71] Two hundred milligrams of theanine was administered twice daily. Actigraph watch data findings indicated that boys who consumed L-theanine obtained significantly higher sleep percentage and sleep efficiency scores, along with a non-significant trend for less activity during sleep compared to the placebo group. The authors concluded that because disturbed sleep may be linked etiologically with the disorder, L-theanine may represent a safe adjuvant therapy in childhood ADHD.

A recent paper has investigated effects of theanine on nicotine dependence in mice.[23] L-theanine was found to inhibit the rewarding effects of nicotine in a conditioned place preference model of the mouse. It also reduced the upregulation of α, β, and γ subunits of the nicotinic acetylcholine receptor induced by nicotine in mouse brain regions related to the dopamine reward pathway. Additionally, L-theanine inhibited nicotine-induced expression of the cellular transcription factor c-Fos in the reward areas of mouse brain, as well as nicotine-induced tyrosine hydroxylase expression and dopamine production in the midbrain of mice.

Conclusion

On the basis of biochemical and *in vitro* studies, L-theanine is now emerging as a potentially significant modulator of brain activity and function. Its ability to affect a wide range of biological processes, both synaptic and cellular, is remarkable, and its direct effects on neurotransmission appear to comprise only one aspect of its pharmacological activity. Further studies are needed to establish the extent to which theanine directly upregulates the inhibitory neurotransmitters GABA and glycine, and inhibits the excitatory neurotransmitter glutamate. Regarding its ability to reduce anxiety and increase subjective feelings of calm, its positive effects on alpha brain wave activity may yet emerge as of equal or greater physiological significance than its ability to modulate neurotransmission. It is possible that a synergistic interplay of both effects combine to produce the reported improvements in anxiety. To date, no studies have been carried out on whether L-theanine might play a role in the treatment of anxiety disorders including panic disorder or generalized anxiety disorder. However, the small number of studies concerning L-theanine supplementation in the treatment of chronic anxiety of schizophrenia is promising. In addition, there is limited evidence for an antidepressant effect of L-theanine in animals, possibly via modulation of the monoamine oxidase system and BDNF levels. Further studies in human would also now appear to be justified. Its paradoxical ability to increase alpha brain wave activity results in increased attention, alertness, and improved cognitive function in all of the human studies in normal subjects to date. However Schalliers findings of increased susceptibility to generalized seizures in animals,[19] while in need of replication with other animal models, is the only paper to report an adverse effect of theanine treatment. This may outrule theanine supplementation in human epileptics.

There already exists a significant body of literature on the potential neuroprotective effects of the green tea catechins by Youdim, Mandel, and others (for reviews, see refs.[72,73]). These effects appear to involve a myriad of cellular mechanisms including iron chelation, anti-oxidant activity via scavenging of free radicals, and modulation of specific cellular survival and signal transduction pathways such as the protein kinases. The mechanisms by which theanine exerts its neuroprotective effects appear to differ primarily from those of the catechins; there is now a significant body of evidence demonstrating the direct or indirect antagonism of glutamate metabolism and neurotransmission within the CNS, alongside a possible further up-regulation of inhibitory neurotransmitters such as GABA and glycine, and increases in BDNF. However, further cellular downstream effects of L-theanine are now beginning to emerge as a result of the recent *in vivo* and *in vitro* studies of Di, Kim, and Cho.[24,32,34] Their collective findings suggest that notwithstanding the down-regulation of brain glutamate metabolism, L-theanine is also capable of interfering

to some degree, with harmful activities such as DNA fragmentation, amyloid-induced cell death and apoptosis. Its ability to inhibit the suppression of a myriad of cellular proteins such as C Jun Kinase, Caspase 3 plus NOS and haem oxygenase production, all implicated with neurodegeneration and cell cycle regulation and survival, may suggest further means by which theanine exerts neuroprotective activity. Furthermore, its ability to inhibit the ERK and MAP kinases, would appear to play a role in its prevention of amyloid β-induced cell death. Its ability to alter the cell protein kinases is shared by the catechins; whether the overall effects within the context of green tea consumption are additive or not is presently unknown. Overall, however, the positive findings to date regarding theanine are providing additional support for the efficaciousness of green tea consumption apropos of cognitive function and neuroprotection. Given the positive experimental outcomes following treatment with theanine post-cerebral infarction in animals, further studies on L-theanine supplementation in humans following cerebral infarction and injury seem justified. There is also an increasing body of evidence to support a role for L-theanine in the partial prevention of Alzheimer's disease and cognitive decline in humans partly due to its effect on CA1 neurons. The increase in brain levels of BDNF following L-theanine administration to animals point to a further possible role for this amino acid in neurogenesis and memory formation. Given the role of BDNF in synaptic plasticity and learning via its effects on late phase LTP, one correlation that merits further attention is that between BDNF and L-theanine. Whether or not its ability to increase levels of BDNF might play a further indirect role in the treatment of psychiatric disorders such as depression, bipolar disorder, and OCD, remain speculative at present but worthy of investigation. It is worth reiterating the findings of Takeda *et al.*[55] regarding the positive effect of theanine on NMDA-independent CA1 LTP in rats. The latter phenomenon is now thought to play a part in spatial memory formation within the hippocampus.[74] In light of the positive effects of L-theanine administration on cognitive function in rats involving spatial awareness such as the water maze task, it is interesting to speculate on whether theanine may partly exert its effects via this mechanism. It is also worth noting that theanine appears capable of both NMDA receptor dependent and independent effects. BDNF has also been implicated in the aetiology of Alzheimer's disease; it has been suggested that the early memory dysfunction seen in AD may be related to BDNF levels within the hippocampus.[75] It is postulated that this may have a bearing on support for cholinergic neurons as well as a deficit in LTP. In light of the reported findings of increased BDNF levels within the hypothalamus following theanine administration,[8,15] the latter effects must be considered as potentially equally significant as its effects on glutamergic neurons and receptors. The single study to date on the positive effects of theanine on nicotine dependence[23] is in need of replication, but may suggest a previously unidentified therapeutic or adjuvant role for theanine in the adjuvant treatment of nicotine addiction. Further studies on its effects on withdrawal from other drugs such as opioids may be well worth pursuing. Apropos a role for L-theanine in the treatment of ADHD, further studies are needed on actual effects on behavioural indices. In addition, the effect of L-theanine on activity and function within the nucleus accumbens, which is thought to function abnormally in this disorder would be worth investigating. In particular, a low density of dopamine receptor sites has been found in this region in ADD and ADHS. The nucleus accumbens also contains the highest density of dopamine in the brain and is particularly sensitive to serotonin. Given that L-theanine has been shown to increase dopamine and serotonin levels in the mesolimbic area, positron emission tomography and functional magnetic resonance imaging studies following a treatment regime with L-theanine in affected individuals could provide useful information on any differential effects of L-theanine in this group.

Finally, a recent study has reported diminished serum BDNF levels in a small cohort of patients with obsessive compulsive disorder.[76] Although they did not find a correlation between BDNF and severity of symptoms, the authors suggested that a possible dysfunction of neurotrophic expression in the pathology of OCD might provide the rational for further investigations of new therapeutic strategies. Given both the positive effect of L-theanine on BDNF levels, and the predominance of anxiety in the phenotypical expression of this disorder, specific studies on the effects of L-theanine in OCD may also be worthwhile. Finally, an interesting correlation has been reported between plasma BDNF levels and clinical symptoms of anorexia nervosa, and it has been hypothesized that BDNF could be involved in the severity of this disease also via modulation of psychopathological traits that are associated with its phenotype.[77] A similar rationale for clinical trials of L-theanine supplementation in Anorexia may be equally warranted.

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