**Original research paper**

**L-Theanine intake increases threshold for limbic seizures but decreases threshold for generalized seizures**

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L-Theanine, an ethylamide derivate of glutamate found in abundance in green tea, has been shown to exert beneficial actions in animal models for several neurological disorders. We here investigated for the first time the effect of L-theanine intake on seizure susceptibility using acute pilocarpine and pentylenetetrazol (PTZ) mouse models for studying, respectively, limbic seizures or primarily generalized seizures. Moreover, we studied the effect of L-theanine intake on extracellular hippocampal and cortical glutamate and gamma-aminobutyric acid (GABA) levels, using *in vivo* microdialysis. Feeding mice with a 4% L-theanine solution significantly decreased their susceptibility to pilocarpine-induced seizures whereas susceptibility to PTZ-induced seizures was increased. The latter effect was linked to decreased extracellular GABA concentrations in frontal cortex.

**Keywords:** L-Theanine, Glutamate, GABA, Limbic seizures, Generalized seizures

**Introduction**

Epilepsy is a neurological disorder that is characterized by sudden, recurrent seizures. It is commonly accepted that an imbalance between excitation and inhibition in the brain might be the culprit triggering seizures or convulsions. Although a large number of drugs have been developed to treat seizures, the disease is poorly controlled in approximately 30% of epileptic patients.

Despite a case report by Kaufman and Sachdeo¹ showing that excessive caffeine ingestion from tea increased seizure frequency in a patient with mixed seizure disorder (grand mal seizures, absence seizures, atonic seizures, and myoclonic seizures), only very few reports investigated the link between tea consumption and seizure susceptibility. Kabuto *et al.*² described in a mouse model that mimicks the situation in humans with posttraumatic epilepsy, that catechins, the most important polyphenol antioxidants in tea extract, can prevent or diminish the occurrence of epileptic discharges induced by iron ions. Contrarily, it has been reported that black as well as green tea extract can increase chemically induced seizure sensitivity in several mouse models for generalized seizures.³ In the latter study, and in contrast to the previously mentioned studies, equivalent concentrations of crude polyphenols or caffeine were ineffective in these same mouse models. Therefore, the authors attributed the effects of tea to one or more unknown constituents of the tea extracts.

L-Theanine is an amino acid present in species of *Camellia sinensis* that contributes to the umami taste of green tea. L-Theanine is a natural ethylamide analogue of glutamate that crosses the blood–brain barrier and is available as a food supplement.⁴ It acts as an antagonist, although with very low affinity, at ionotropic glutamate receptors.⁵ Moreover, L-theanine can affect presynaptic release of glutamate by inhibiting glutamine transporters.⁶ Its presumed beneficial actions have been investigated and confirmed in animal models for several neurological disorders, such as Parkinson’s disease, Alzheimer’s disease, and ischaemic stroke. Indeed, it has been shown that intraperitoneal (i.p.) administration of L-theanine results in a reduction of the size of the cerebral infarct in a rat model for cerebral ischaemia.⁷ Moreover, oral treatment...
of mice with L-theanine reduced Aβ1–42-induced memory impairment in a model for Alzheimer’s disease. In an in vitro study, the neuroprotective effect of L-theanine against neuronal damage caused by Parkinson’s disease-inducing toxins was demonstrated. Surprisingly, and in spite of the known interaction with glutamate receptors and glutamine transporters as well as the reported effects of L-theanine on intracerebral gamma-aminobutyric acid (GABA) concentrations, the effect of L-theanine on seizure susceptibility was never investigated.

We here describe for the first time the effect of chronic L-theanine intake on seizure susceptibility in the acute pentylenetetrazol (PTZ) and pilocarpine mouse models for, respectively, primarily generalized seizures and limbic seizures with secondary generalization. Using in vivo microdialysis, we measured the impact of L-theanine intake on extracellular glutamate and GABA concentrations in frontal cortex and hippocampus, the respective sites of focus in the above-described mouse models.

Materials and methods

Animals

All experiments were performed on male NMRI mice weighing 30–45 g, according to national guidelines on animal experimentation, and were approved by the Ethical Committee for Animal Experimentation of the Faculty of Medicine and Pharmacy of the Vrije Universiteit Brussel. Mice had ad libitum access to drinking water and food. Mice with L-theanine treatment received 4% (w/V) L-theanine (Suntheanine, provided by Taiyo Europe, Filderstadt, Germany) as their only drinking solution for 14 days prior to the experiments (according to Kim et al.).

In vivo microdialysis

Microdialysis was performed as described previously. Briefly, microdialysis guides (CMA/7, CMA/Microdialysis, Solna, Sweden) were implanted in the hippocampus (coordinates relative to bregma: L +3.0, AP –2.7, and DV –1.5) and in the frontal cortex (L –0.5, AP +1.1, and DV 0.5). Animals were placed in a microdialysis cage and the next day CMA/7 (membrane 2 mm for hippocampus and 1 mm for cortex) microdialysis probes were inserted. Six baseline samples were collected over a 120-minute period (20 minutes/sample) at a flow rate of 2 μl/minute. Glutamate and GABA content was determined in each sample by HPLC, as described previously. Probe positioning was histologically verified at the end of the experiments.

Chemoconvulsant models

The threshold for different phases of pilocarpine- and PTZ-induced seizure activity was determined by intravenous (i.v.) infusion of pilocarpine (24 mg/ml) or PTZ (7.5 mg/ml) in the lateral tail vein at a constant rate of 150 μl/minute. Methylscopolamine (1 mg/kg, subcutaneously) was injected 30 minutes before pilocarpine infusion to prevent peripheral cholinergic symptoms. During the experiment, the animal could freely move in a Plexiglas cage. The endpoints that were used to determine the seizure threshold and the equation used to determine seizure threshold are as described in Schallier et al. and De Bundel et al. Briefly, time was measured from the start of the PTZ or pilocarpine infusion until the onset of the different consecutive typical behaviours. The following endpoints were used to determine the seizure threshold: (1) myoclonic twitch, (2) onset of generalized tonic–clonic phase (falling), (3) onset of generalized tonic phase (tonus), (4) death for the PTZ model and (1) first body twitch, (2) rearing, (3) tonic–clonic phase (falling), (4) onset of generalized tonic phase (tonus), (5) death for the pilocarpine model. Seizure threshold was determined for each animal according to the following equation: dose (mg/kg) = (duration of infusion (s) × rate of infusion (ml/minute) × drug concentration (mg/ml) × 1000)/(60 s × weight of mouse (g)).

Statistics

Data are expressed as means ± standard error of the mean (SEM). The non-parametric two-tailed Mann–Whitney test (α = 0.05) was used to evaluate the effect of L-theanine intake on seizure threshold and on extracellular glutamate/GABA levels.

Results

The effect of L-theanine intake on the convulsion threshold

Infusion of pilocarpine or PTZ into the lateral tail vein produced an array of rapidly progressing behaviours. Both chemoconvulsants induced seizures in mice though with different behavioural convulsion patterns. A significantly higher dose of pilocarpine was necessary to induce pilocarpine-evoked behaviours in mice that received 4% L-theanine in their drinking bottles for 14 days prior to the experiments compared to mice that received tap water (Fig. 1A). We revealed a significant effect of L-theanine intake upon the threshold dose of pilocarpine for inducing body twitch (water: 255 ± 26 mg/kg; L-theanine: 315 ± 36 mg/kg), rearing (water: 287 ± 23 mg/kg; L-theanine: 343 ± 35 mg/kg), and falling (water: 328 ± 25 mg/kg; L-theanine: 386 ± 38 mg/kg). For the last two behaviours that were scored, i.e. tonus and death, we detected a trend towards an increased threshold after L-theanine intake, though no significance was reached (tonus: water: 364 ± 40 mg/kg; L-theanine: 398 ± 37 mg/kg; death: water: 376 ± 39 mg/kg; L-theanine: 419 ± 40 mg/kg).
Conversely, whereas 1-theanine intake had no effect on the first two behaviours in the PTZ model, i.e. myoclonic twitch (water: 36 ± 5 mg/kg; 1-theanine: 41 ± 13 mg/kg) and falling (water: 41 ± 8 mg/kg; 1-theanine: 48 ± 17 mg/kg), the threshold dose was significantly decreased for the last two behaviours, i.e. tonus (water: 91 ± 10 mg/kg; 1-theanine: 60 ± 14 mg/kg) and death (water: 107 ± 18 mg/kg; 1-theanine: 76 ± 9 mg/kg) (Fig. 1B).

The effect of 1-theanine intake on cortical and hippocampal extracellular glutamate and GABA levels

1-Theanine uptake had no effect on extracellular glutamate levels in frontal cortex (water: 0.230 ± 0.040 μM; 1-theanine: 0.245 ± 0.030 μM) (Fig. 2A) or hippocampus (water: 0.335 ± 0.066 μM; 1-theanine: 0.366 ± 0.098) (Fig. 2B).

Whereas 1-theanine intake did not affect hippocampal GABA dialysate levels (water: 0.0046 ± 0.0009 μM; 1-theanine: 0.0058 ± 0.0012 μM) (Fig. 2D), cortical GABA levels were significantly decreased (water: 0.0064 ± 0.0020; 1-theanine: 0.0015 ± 0.0004 μM) (Fig. 2C).

Discussion

We here show for the first time that daily intake of 1-theanine can significantly affect the convulsion threshold. Some of the observed effects might, at least in part, be explained by the decreased extracellular cortical GABA levels that were observed after chronic 1-theanine intake. It should be noted that, based on our data, we cannot make any statement about the effect of drinking tea on seizure susceptibility. The daily dose of 1-theanine that was consumed by the mice corresponded to the dose that resulted in reduction of Aβ1−42-induced memory impairment in a model for Alzheimer’s disease and largely passes the daily intake of 1-theanine by a heavy tea drinker. Moreover, many other constituents of tea, such as caffeine, can affect seizure threshold.

The current research is nevertheless very relevant since 1-theanine is available as a food supplement.

Daily intake of 1-theanine significantly increased the seizure threshold, and thus decreased the seizure susceptibility, in the acute pilocarpine mouse model. Although it had been reported that 1-theanine can inhibit the uptake of glutamine in neurons and as such suppress the synthesis of glutamate in presynaptic terminals, we could not attribute these anticonvulsive effects to changes in hippocampal extracellular glutamate or GABA concentrations as they were unaffected by chronic 1-theanine intake. Though, 1-theanine can still interfere with glutamatergic neurotransmission as an antagonist of ionotropic glutamate receptors.

Indeed, 1-theanine has been shown to protect cells against glutamate-induced excitotoxicity via inhibition of the NMDA receptor pathway. This could possibly explain the anticonvulsive effects in the pilocarpine model.

Cortical glutamate levels were not changed by 1-theanine intake, contrary to cortical GABA levels. Feeding mice for 2 weeks with 4% 1-theanine in their drinking water resulted in decreased extracellular GABA levels in frontal cortex. These decreased GABA levels might contribute to the proconvulsive effect of 1-theanine that was observed in the acute PTZ model. Indeed, PTZ acts by antagonizing GABAAergic inhibition via an effect at the picrotoxin site of the chloride ionophore of the GABA_A receptor. Decreased extracellular GABA levels could thus potentiate the effect of PTZ.

It has been described that green as well as black tea extract potentiated PTZ-induced convulsions in mice.

In these experiments, PTZ was administered subcutaneously, resulting in complete tonic extension of the hindlimbs. Administration of tea extract accelerated the onset of convulsion and increased the mortality of the mice, in accordance to the increased susceptibility to tonus and death that we observe in...
our PTZ model. Since this effect could not be linked to polyphenols or caffeine, it was attributed to an unknown constituent of tea extracts. We here provide evidence that this unknown constituent, that decreases the threshold for PTZ-induced seizures, might be L-theanine. The same authors described that both black and green tea extract are unable to alter total GABA brain levels. Using in vivo microdialysis we showed that, although hippocampal extracellular GABA levels were unaffected, cortical extracellular GABA levels were significantly decreased by daily L-theanine intake. This is in sharp contrast to the observations of Kimura and Murata11 who described that total brain GABA levels are unaltered by i.p. L-theanine injection.

Taken together, our data suggest that L-theanine might be useful in the development of new drugs for reducing limbic seizures but certainly not for primarily generalized seizures. This is in line with some of the existing antiepileptic drugs, such as carbamazepine and oxcarbazepine, which can be used for partial epilepsy, though as an unwanted side effect could worsen generalized epilepsies, even leading to status epilepticus.

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References


