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Review

Neuroprotective effects of theanine and its preventive effects on cognitive dysfunction

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ABSTRACT

Theanine (γ-glutamylethylamide) characteristically present in tea leaves (*Camellia sinensis*). It has a similar chemical structure to glutamate, which is a neurotransmitter related to memory. Theanine passes through the blood–brain barrier and has been shown to have a cerebroprotective effect and a preventive effect on neuronal cell death after transient cerebral ischemia. The neuroprotective effect is partly due to the antagonistic action of theanine on glutamate receptor subtype AMPA and kainate receptors, but the affinity is very low. Theanine also acted on glutamine (Gln) transporter strongly and inhibited the incorporation of extracellular Gln into neurons, which in turn suppressed the conversion of Gln to glutamate by glutaminase, a reaction required for condensation into synaptic vesicles to form a neurotransmitter pool responsible for subsequent exocytotic release upon stimuli. In an investigation of elderly persons with normal or slight cognitive dysfunction, volunteers who ingested powdered green tea containing a high theanine concentration (equivalent to 47.5 mg day^{-1} of theanine) showed significantly lower decline in cognitive function compared with that of the placebo group. This result suggested that theanine might have improved a slight cognitive dysfunction in elderly persons.

1. Introduction

There are estimated to be approximately 2 million dementia patients in Japan. Given the country's rapidly aging society, the number of dementia patients is likely to increase further in the near future. Such patients will become an enormous burden on care givers and a huge financial strain on society. The most common causes of dementia in the elderly include Alzheimer's disease, cerebrovascular disease, and dementia with Lewy bodies. Of these, Alzheimer's disease is the leading cause, followed by cerebrovascular disease, and their mixed dementia is also found in Japan.

It has been reported that cerebrovascular dementia [1] is associated with blood vessel disorders of the brain caused by cerebral infarction [2], diabetes [3], high blood pressure [4], cardiac vascular disease [5], etc. The Framingham study in the U.S.A. reported that stroke increased a subject's risk of dementia compared with that in age- and sex-matched controls. Primary and secondary prevention of stroke should significantly decrease the risk of dementia [6]. Other studies have also reported that stroke is a significant risk factor of cognitive impairment and dementia [7,8]. Glutamate can cause neuronal cell death by acting as a powerful neurotoxin in the central nervous system when its extracellular concentration is elevated because of cerebral ischemia such as stroke. In order to diminish glutamate toxicity, the extracellular concentration must be decreased, for example, by postsynaptic glutamate receptor antagonists in glutamatergic neurons [9,10]. However, although there are reports of neuroprotectants in stroke, such as *N*-methyl-D-aspartate (NMDA) receptor antagonists [11,12], all have been subject to side effects. Therefore, there is a need to develop useful medicines, preventive medical supplies, and/or neuroprotective supplements.

Kuriyama et al. [13] reported that high consumption of green tea (≥2 cups per day) is associated with lower prevalence of cognitive impairment in humans in epidemiology study of Tsurugaya project in Japan. An attractive quality ingredient of green tea leaves is theanine, which has an analogous chemical structure to glutamate and glutamine (Gln) (important neurotransmitters related to memory) (Fig. 1). There are several reports on the neuroprotective effects of theanine on ischemic neuronal cell death [14–16], and also on its action mechanisms [17,18] and metabolism [19,20]. Kataoka et al. [21] showed that long-term ingestion of a high concentration of theanine in powdered green tea suppressed the progression of cognitive dysfunction and suggested a preventive effect on dementia in the elderly.

2. Metabolism of theanine

Common-grade green tea leaves contain 0.2–2.4% (w/w) theanine [22]. Kitaoka et al. [23] reported that intestinal absorption of theanine and Gln was mediated by a common Na^+-coupled

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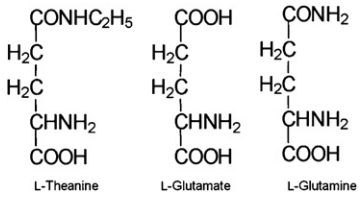

Fig. 1. Chemical structure of L-theanine (γ-glutamylethylamide), L-glutamate, and L-glutamine.

co-transporter in the brush-border membrane, the affinity of which was lower for theanine than for Gln. Unno et al. [19] showed that when 200 mg of theanine was orally administered to rats, the plasma concentrations of theanine and ethylamine reached their highest levels approximately 0.5 and 2 h after administration, respectively. Within the rat kidney, theanine is hydrolyzed to glutamate and ethylamine. Tsuge et al. [20] reported that theanine was hydrolyzed by phosphate-independent glutaminase in the kidney and proposed that the glutamyl moiety was transferred by means of a γ-glutamyl transpeptidase reaction to other peptides in vivo. In contrast, Yokogoshi et al. [24] reported that theanine was transported into the brain through the blood–brain barrier via a leucine-preferring transporter system. In this way, orally administered theanine was easily absorbed from the intestinal tract and partially transported into the brain through the blood–brain barrier.

3. Neuroprotective effect of theanine

3.1. Neuroprotective effect of theanine on delayed neuronal cell death after transient brain ischemia

Theanine has an inhibitory effect on the stimulation of the central nervous system by caffeine [25,26], a reduction effect on blood pressure [27], a relaxation effect [28], and an enhancing human γδ T lymphocyte function [29]. The chemical structure of L-theanine is a similar to that of glutamate, which is a very important neurotransmitter related to memory. The neuroprotective effects of L-theanine on delayed neuronal cell death following transient ischemia were elucidated using an animal model [14]. Transient forebrain ischemia was induced by bilateral occlusion of the common carotid arteries for 3 min in gerbils. Seven days after ischemia, pyramidal neurons in the hippocampal CA1 region degenerated or disappeared (Fig. 2b and e). No change in the number of CA1 pyramidal neurons was observed in the sham-operated group (Fig. 2a and d). On the other hand, most CA1 pyramidal neurons were preserved in animals that were administered 1 μL of 500 μM theanine solution 30 min before ischemia (Fig. 2c and f). Theanine pretreatment significantly inhibited ischemic neuronal cell death in the hippocampal CA1 field in a dose-dependent manner. Lowering the intraischemic brain temperature by 2 °C has been shown to significantly reduce the extent of ischemic neuronal damage [30]. MK801, an NMDA-type glutamate receptor antagonist, exerts protective effects when the brain temperature is lowered [31]. However, some NMDA-type glutamate receptor antagonists have been shown to exert their protective effects without a lowering of the brain temperature [32]. In the animal model described above cerebral ischemic experiments were performed during brain regulation at an ischemic insult temperature and continuous monitoring [33]. The tests were performed while maintaining the brain temperature at 37 °C; this suggests that the neuroprotective effect of theanine does not depend on lowering of the brain temperature but may be due to a direct effect on neurons [14]. Egashira et al. [34] reported that theanine significantly prevented impairment of spinal memory in rats subjected to repeated cerebral ischemia at 7 days after the second reperfusion. They further reported that theanine significantly inhibited the decrease in the number of surviving cells in the hippocampal CA1 field of the same rats [34]. These results indicate that theanine may prevent cerebrovascular disease by mitigating cognitive dysfunction through inhibition of ischemic neuronal cell death.

3.2. Neuroprotective effect of theanine on middle cerebral artery occlusion

Kakuda et al. [15] reported that theanine significantly prevented neuronal cell death in rats using an occluded middle cerebral artery (MCA) model similar to the clinical model described above. They further suggested a cerebral protective action of theanine. Theanine (125 μM, 250 μM, and 500 μM) was injected through the lateral ventricle 30 min before the onset of MCA occlusion under controlled body temperature (37 °C) and anesthesia. Focal cerebral ischemia was induced by temporary MCA occlusion for 1 h with a suture technique. After 24 h, the brains were removed, and infarct volumes were measured. The infarct volume was significantly reduced by treatment with 250 μM and 500 μM theanine in a dose-dependent manner. Egashira et al. [35] reported that theanine (1 mg kg^{-1}) was injected i.p. 3 h after occlusion or immediately before occlusion significantly decreased the size of cerebral infarcts 1 day after the occlusion. Thus, theanine has a neuroprotective effect on MCA occlusion, which is often observed clinically and might be clinically useful for preventing cerebral infarction.

4. Mechanisms underlying of the neuroprotective effects of theanine

4.1. The neuronal excitotoxin glutamate and neuronal cell death

The human brain is thought to contain approximately 100 billion neurons. These neurons form complicated neural networks from several thousands to several tens of thousands of synapses. It is thought that information entering the human body through vision, audition, gustation, olfaction, and/or tactile sense is transferred to the hippocampus, where it is consolidated and stored temporally as short-term memory. Potent information is finally fixed in the hemisphere as long-term potentiation (LTP). The memorized information is judged in an association cortex and is thought to be used functionally.

Glutamate is an important neurotransmitter concerned with memory and is present at a concentration of approximately 10 mM in the glutamatergic neurons. When electric information is transmitted to the synapse via an axon, glutamate is discharged in the synaptic cleft from synaptic vesicles in pre-synaptic terminals. Glutamate receptors in the post-synaptic membrane are activated, and information is transmitted as chemical information (Fig. 3). When the transfer of information is complete, glutamate is eliminated from the synaptic cleft and taken into glial cells and neurons by glutamate transporter [36,37]. A low extracellular glutamate concentration (<2 μM) is maintained to avoid excessive excitement [38]. This process is very short term. However, when the supply of oxygen and glucose (a nutrient source) stops because of cerebral infarction or cardiac arrest, the electric potential of the cell membrane depolarizes, glutamate is excessively released into the extracellular space, and glutamate receptors are excited in a disorderly manner [39]. There is a particularly vulnerable region in the brain [40,41], in which neuronal cell death occurs approximately 2 days after 5 min of cerebral ischemia [42]. Cerebral ischemia results in excessive stimulation of glutamate receptors and abundant flow of Ca^{2+} ions into neurons through NMDA receptors [43]. Such an excessive flow of Ca^{2+} ions into neurons results in excessive