Neuroprotective effects of theanine on ischemic delayed neuronal death.

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Summary
Vascular dementia patients have increased in number in Japan's increasingly aging society. The incidence of stroke during a 4-year follow-up of a study population was reportedly higher in those who consumed less green tea (Camellia sinensis) than in those who drank more. Theanine (γ-glutamylethylamide) is contained in green tea as a natural glutamate analog. The neuroprotective effect of theanine on ischemic delayed neuronal death was examined in the present study. Theanine was administered through the lateral ventricle 30 minutes before ischemia in gerbil. Transient forebrain ischemia was induced by bilateral occlusion of the common carotid arteries for 3 minutes in the gerbil under careful control of the intracerebral temperature at 37°C. Seven days after ischemia, the number of intact CA1 neurons in the hippocampus was assessed. Ischemia-induced neuronal death in the hippocampal CA1 region was significantly prevented in a dose-dependent manner in the theanine-pretreated groups. These findings indicate that consumption of theanine rich green tea might be useful to prevent ischemic neuronal damage.

Keywords
Theanine, Dementia, Neuroprotection, Delayed neuronal death

Introduction
In an increasingly aging society, the number of senile dementia patients is steadily rising in Japan. Many of these cases are cerebral vascular dementia, in which neuronal death follows transient lack of oxygen or ischemia, which is caused by stroke. The neuronal death is believed to arise from glutamate neurotoxicity with an excessive release of glutamate, the principal excitatory neurotransmitter in the brain. Epidemiological studies have shown that the incidence of stroke is significantly lower in those who consume more than 5 cups of tea.1)

A glutamate analog, γ-glutamylethylamide (theanine), which is responsible for a distinctive component of the taste of green tea leaves (Camellia sinensis), is found at levels as high as 3% by weight in high-quality green tea. Lower amounts of theanine are also found in lower-quality green tea, black tea and oolong tea. Because of its solubility in water, theanine can be easily transported in the bloodstream and can also cross the blood brain barrier, presumably because of its simple, partially hydrophobic...
chemical structure. We, therefore, were interested in the action of theanine in protecting against ischemic damage, in particular because of its structural similarities to glutamate, which may enable it to modulate glutamate receptors. In the present study, we tested for a protective effect of theanine on delayed ischemic neuronal death in the gerbil.2)

Material and Methods

Under anesthesia with halothane, 1 μl of 50-500 μM theanine was given through the lateral ventricle in gerbils. Thirty minutes later, transient forebrain ischemia was induced by bilateral occlusion of the common carotid arteries for 3 min under careful control of brain temperature at approximately 37°C. Animals in the control group were identically given an equal volume diluent before ischemia, while animals in the sham-operated group were administered the theanine solution and were not subjected to ischemia. After the survival period of 7 days, brains were removed and embedded in paraffin, and hippocampal slices were made. The sections were then, stained with cresyl-violet and the neurons in field CA1 of the hippocampi were enumerated.

The statistical significance of differences in cell counts was analyzed by one-way ANOVA and the values were expressed as mean ± S.D.

Results and discussion

The sham-operated group was not changed in field CA1 (Fig. 1, a, d). Almost all CA1 neurons had degenerated or disappeared in the control group (Fig. 1, b, e). However, in the 500 μM theanine-treated groups, ischemic neuronal death of CA1 neurons was significantly prevented (Fig. 1, c, f), and neuroprotective effect of theanine was in a dose-dependent manner (Fig. 2).

Fig. 1. Photographs showing neuroprotective effect of theanine against ischemic delayed neuronal death in the gerbil hippocampus. Brain sections were obtained 7 days after 3-min transient ischemia. Lower photographs show the higher magnification of the field CA1 in each experimental group. (Kakuda et al, Neurosci. Lett., 289, 189, 2000).
This evidence indicates that theanine is highly effective in protecting neurons against ischemic damage, and suggests that theanine might be useful clinically for preventing the onset of vascular dementia. Cerebral protective effects were also observed in the theanine-pretreated groups in a dose-dependent manner, and corrected infarct volume was significantly reduced in the 250 µM and 500 µM theanine-treated groups. These findings indicate that consumption of theanine-rich green tea might be useful to prevent ischemic neuronal damage.

Though the mechanism by which theanine acts on the brain is largely unknown, our experimental studies to date demonstrated that more than 400 µM of theanine prevented neuronal death induced by the application of 50 µM of glutamate on cultured rat cortical neurons. Though the transient increase of intracellular Ca\(^{2+}\) was observed using fura-2 when 800 µM of theanine was exposed to cultured rat cortical neurons, this increase was suppressed by exposure to theanine in the presence of 50 µM of D-APV. We also demonstrated the neuroprotective effect of theanine on neuronal death in the rats which were given Kainate. In the present study, the lowering brain temperature was not shown by administration of theanine.

These results suggest that theanine might at least act on the glutamate receptor subtype NMDA (N-methyl-D-aspartate) receptor and Kainate receptor. Further studies on the mechanism of theanine actions on glutamate receptors are necessary.

![Figure 2: Effect of theanine on ischemic delayed neuronal death in gerbil hippocampal CA1 neurons.](image)

Fig. 2. Effect of theanine on ischemic delayed neuronal death in gerbil hippocampal CA1 neurons. The point and bar show the mean ± SD for each group. Asterisks indicated a significant difference, compared to the control group, at p<0.01 using Scheffé's multiple comparison procedure.

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References

1) Y. Sato, H. Nakatsuka, T. Watanabe, S. Hisamichi, H. Shimizu, S. Fujisaku, Y.
2) Kakuda, T., Yanase, H., Utsunomiya, K., Nozawa, A., Unno, T. and Kataoka, K.,
3) Kakuda, T., Yanase, H., Nozawa, A., Unno, T. and Kataoka, K., Nippon
4) Nozawa, A., Umezawa, K., Kobayashi, K., Kawahara, M., Muramoto, K., Kakuda,
5) Nozawa, A., Umezawa, K., Kobayashi, K., Muramoto, K., Kawahara, M.,
Mizutani, A., Kakuda, T. and Kuroda, Y., Society for Neuroscience, 335.9
6) Kakuda, T., Nozawa, A., Kubo, T., and Tsukamoto, S., Nippon Nogeikagaku Kai,
Abstract 2M6a17 (2001).