Transmural Potential Changes Associated with the \textit{in Vitro} Absorption of Theanine in the Guinea Pig Intestine

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Theanine, \(\text{L}-\text{N}	ext{-ethylglutamine}\), is one of the major components of amino acids in Japanese green tea. To characterize the mode for intestinal absorption of theanine, the tonic dependency and kinetic properties of the theanine- and glutamine-evoked transmural electrical potential difference changes (APD) were investigated \textit{in vitro} by using everted sacs prepared from the guinea pig ileum. Both theanine and glutamine applied to the luminal side induced dose-dependent increases in APD (increase in serosal positive value). The theanine- and glutamine-evoked APD values conformed to the Michaelis-Menten relationship, with APD, not being different, whereas the half-saturation concentration was lower for glutamine (3.1 ± 0.2 mM) than for theanine (21.4 ± 0.6 mM). The theanine-evoked APD value was much smaller when theanine was applied in the presence of glutamine than when applied alone. The theanine- and glutamine-evoked APD values were both inhibited by removing \(\text{Na}^+\) from the luminal solution. These results suggest that the intestinal absorption of theanine and glutamine is mediated by a common \(\text{Na}^+\)-coupled co-transporter in the brush-border membrane, the affinity of which is lower for theanine than for glutamine.

Key words: brush-border membrane; intestinal absorption; Japanese green tea; amino acid absorption; co-transport

Theanine, \(\text{L}-\text{N}	ext{-ethylglutamine}\), is one of the major components of amino acids in Japanese green tea.\(^{1,2}\) Several physiological and pharmacological actions of theanine have been reported such as reduced blood pressure\(^{3,4}\) and reduced brain serotonin and norepinephrine levels.\(^{5,6}\)

The mode of absorption of theanine from the gastrointestinal tract is not clear. Theanine is an amino acid derived from glutamine by ethylation of the \(\gamma\text{-carbamoyl} \) moiety, so theanine could be absorbed by transport systems common to glutamine in the small intestine. Glutamine absorption across the brush-border membrane is probably mainly mediated by the \(\text{Na}^+\)-coupled, electrogenic co-transporter that is the major transport system for dipolar amino acids.\(^{4,7}\) The purpose of the present study was to investigate whether theanine and glutamine transport across the brush-border membrane occurs through the same \(\text{Na}^+\)-coupled transporter by measuring the theanine- and glutamine-evoked transmural potential changes, using everted ileal sacs from the guinea pig \textit{in vitro}. Recording the transmural potential changes is technically simple, and quantitative analyses of the changes have been shown to be useful for characterizing the electrogenic co-transport systems in the brush-border membrane of the enterocyte.\(^8,9\)

Materials and Methods

Preparation and incubation media. Male Hartley-strain guinea pigs weighing 250–800 g (Japan SLC, Hamamatsu) were allowed free access to food and water until the time of the experiments. After anesthetizing the animals by an intraperitoneal injection of urethane (1 g/kg body weight as a 25% aqueous solution), a segment of the intestine was removed from the ileum, rinsed with buffered saline and everted. The everted sac for measuring the transmural potential change was prepared as reported previously.\(^8,10\) Briefly, the everted intestine was tied over a multiple-fenestrated area of a polyethylene tube of 6 mm in outer diameter and 10 cm in length. One end of the tube, near the fenestrated area, was made blind. After being introduced by a serosal solution, the tube fixed with the everted intestinal sac was positioned inside a glass tube containing 20 ml of the luminal solution, which was bubbled continuously with 95% \(\text{O}_2\) and 5% \(\text{CO}_2\). The experiments were performed at 37°C in a water bath. The solution contained (in mM): \(\text{NaCl}, 119; \text{NaHCO}_3, 21; \text{K}_2\text{HPO}_4, 2.4; \text{KH}_2\text{PO}_4, 0.6; \text{CaCl}_2, 1.2; \text{MgCl}_2, 1.2\). In addition, glutamine (2.5), glucose (5), and \(\beta\)-hydroxybutyrate (Na salt; 0.5) value in mm, were added to the serosal solution as metabolic substrates, while 8.5 mM mannitol was added to the luminal solution to keep it isotonic with the serosal solution. In a Na⁺-free solution, NaCl was replaced by choline chloride. Theanine was purchased from Tanyo Kagaku (Yokkaichi), and glutamine and mannitol were purchased from Nacalai Tesque (Kyoto).

Recording the transmural potential difference. The potential difference was measured by a high-input impedance electrometer (SS-1394, Nihon Kohden, Tokyo) through calomel half-cells. A pair of polyethylene bridges filled with 2% agar in 1 M KCl were used to lead out the potential difference across the intestinal wall. The potential difference was recorded with a chart recorder (R-S-101G, Yanaco, Tokyo) and is referred to the luminal side. Amino acids were added to the luminal solution from concentrated aqueous stock solutions. The transmural potential difference change induced by applying mannitol to the luminal side was also determined for every sample to evaluate the potential change associated with the change in osmolality of the luminal solution due to adding the amino acids. Mannitol evoked a positive lumen (opposite in polarity to those evoked by amino acids) potential change, its absolute magnitude always being less than 10% of the potential induced by the same concentration of theanine. The potential values compensated for the cosmically induced potentials thus evaluated were used to determine the kinetic parameters and Na⁺ dependency, and are shown in the Results.

Statistics. Results are shown as means ± SE. \(n\) representing the number of animals. The kinetic constants were calculated by a linear regression of the Eadie-Hofstee plot. Statistical analyses were done by Student’s \(t\)-test, \(p < 0.05\) being considered significant.

Results

The application of theanine to the luminal solution

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Intestinal Absorption of Theanine

Fig. 1. Dose Dependency of the Theanine- and Glutamine-evoked Changes in Transmural Potential (ΔPD).

(A) Two recordings were made from the same preparation with washing intervening. Amino acid-evoked potential changes have been measured as described in Materials and Methods. Upward changes are an increase in serosal positivity. (B) Eadie Hofstee plot of the data.

Table  Kinetic Parameters of the Amino Acid-evoked Potential Change

<table>
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<tr>
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<th>ΔPD_max (mV)</th>
<th>K_m (mM)</th>
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<tbody>
<tr>
<td>Theanine</td>
<td>10.5 ± 2.3</td>
<td>21.4 ± 0.6</td>
</tr>
<tr>
<td>Glutamine</td>
<td>9.2 ± 2.5</td>
<td>3.1 ± 0.2*</td>
</tr>
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Values are means ± SE. The maximum potential change (ΔPD_max) and the half saturation concentration (K_m) was determined by the luminal addition of cumulative doses of substrates as shown in Fig. 1. Both the theanine- and glutamine-evoked potential difference changes were determined in the same preparation, which were repeated on four preparations obtained from different animals. *p < 0.05.

 increased the transmural potential difference (increase in positive serosal value) across the everted sac of the guinea pig ileum (Fig. 1A). An increase in potential difference was also evoked by the luminal application of glutamine (Fig. 1A). The concentration dependency of the increase in potential difference conformed to the Michaelis-Menten relationship for both theanine and glutamine (Fig. 1B). When the kinetic parameters were compared, the maximum potential difference changes (ΔPD_max) were no different between theanine and glutamine, whereas the half-saturation concentration (K_m) was significantly lower for glu-

Fig. 2. Na^+ Dependency of the Theanine- and Glutamine-evoked Potential Changes (ΔPD).

ΔPD was measured in the presence (solid bar, n=4) or absence (open bar, n=3) of Na^+ in the luminal solution.
When Na$^+$ was removed from the luminal solution, the change in potential difference induced by theanine and glutamine was largely removed, indicating that theanine- and glutamine-evoked potential responses depended on luminal Na$^+$ (Fig. 2).

The additivity of the theanine- and glutamine-evoked potential changes was next examined. As shown in Fig. 3, the transmural potential difference changes induced by theanine were markedly attenuated when glutamine was added in the presence of glutamine. In two preparations, the potential changes induced by 5 mM theanine were attenuated from 0.89 mV and 0.31 mV without glutamine to 0.10 (11%) (Fig. 3) and 0.08 mV (26%), each in the presence of 5 mM glutamine. These values agree well with that predicted (17%, see the Appendix) with the assumption that theanine and glutamine share a common, electrogenic co-transporter.

**Discussion**

This study has aimed to characterize the intestinal absorption, and specifically the brush-border membrane transport process, of theanine by recording the co-transport-related transmural potential changes in the everted intestinal sac preparations. It has been demonstrated that the transport activity of an electrogenic co-transporter in the brush-border membrane was intimately reflected in transmural potential difference changes.

The results of the present study show that theanine- and glutamine-evoked potential changes conformed to the Michaelis–Menten relationship with ΔPD max not being different. We have also shown that the theanine-evoked potential change was much smaller when theanine was applied in the presence of glutamine than in its absence; i.e., the theanine- and glutamine-evoked potential changes were not additive. In addition, the theanine- and glutamine-evoked potential changes were both inhibited by removing Na$^+$ from the luminal solution. These findings suggest that the intestinal absorption of theanine and glutamine is mediated by a common, Na$^+$-coupled co-transporter in the brush-border membrane. From the $K_m$ values, the affinity of the co-transporter for theanine may be 7 fold lower than that for glutamine.

Previous studies on the brush-border membrane of the rat and human small intestine have demonstrated the existence of two carrier-mediated transport processes for glutamine: one is Na$^+$-dependent while the other is Na$^+$-independent. For the Na$^+$-dependent process, the $K_m$ value for glutamine has been shown to be 1.9–3.5 mM in agreement with the $K_m$ value for glutamine evaluated by the present method for the guinea pig ileum (3.1 mM). The Na$^+$-dependent glutamine transporter in the rat and human has been shown to be electrogenic, which also agrees with the present findings for the guinea pig. Multiple transport systems for amino acids with overlapping substrate specificity are known to exist in the intestinal brush-border membrane. The major transport system responsible for transporting nearly all dipolar amino acids that possess the amino group in the $z$-position ("system B") is probably mainly responsible for the Na$^+$-dependent transport of both theanine and glutamine. It cannot be excluded, however, that other Na$^+$-dependent amino acid transport systems (such as "system B"+') could also be involved in glutamine and theanine absorption. In addition, it cannot be determined from the present electrical methods whether theanine enters across the brush-border membrane via Na$^+$-independent, electro-neutral transport systems in addition to electrogenic Na$^+$-coupled systems.

Several transport systems in the basolateral membrane of enterocytes have been shown to accept glutamine as a substrate. It remains to be investigated whether theanine also uses these systems to exit across the basolateral membrane.

**References**

Appendix

When theanine and glutamine share a common co-transport system, the simultaneous application of theanine and glutamine would produce potential changes as follows:

\[
\Delta P_{D(G+T)} = \frac{\Delta P_{D_{\text{max}}} \left( \frac{[T]}{K_m(T)} + \frac{[G]}{K_m(G)} \right)}{1 + \frac{[T]}{K_m(T)} + \frac{[G]}{K_m(G)}}
\]

where \([T]\) and \([G]\) represent the concentrations, and \(K_m(T)\) and \(K_m(G)\) represent the half-saturation concentrations, of theanine and glutamine, respectively. Equation (1) can be reduced to a simple Michaelis-Menten equation when theanine or glutamine is applied singularly as a substrate (\(\Delta P_{D(T)}\) or \(\Delta P_{D(G)}\)).

The ratio \(R\) of the theanine-induced changes in potential difference in the presence and absence of glutamine can be given as \(\frac{\Delta P_{D(G+T)} - \Delta P_{D(G)}}{\Delta P_{D(T)}}\). When 5 mM theanine is applied in the presence of 5 mM glutamine (the condition of Fig. 3), \(R\) can be calculated to be 0.17 by using the \(K_m\) values given in the Table.