Green Tea Polyphenols Inhibit the Sodium-Dependent Glucose Transporter of Intestinal Epithelial Cells by a Competitive Mechanism

Yoko Kobayashi,[†] Miho Suzuki,[†] Hideo Satsu,[†] Soichi Arai,[†] Yukihiko Hara,[‡] Koichi Suzuki,[§] Yusei Miyamoto,[†] and Makoto Shimizu*,[†]

Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan; Food Research Laboratories, Mitsui Norin Company Ltd., 223-1 Miyabara, Fujieda-shi, Shizuoka 426-0133, Japan; and Graduate School of Food and Nutritional Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan

Intestinal glucose uptake is mainly performed by the sodium-dependent glucose transporter, SGLT1. The transport activity of SGLT1 was markedly inhibited by green tea polyphenols, this inhibitory activity being most pronounced in polyphenols having galloyl residues such as epicatechin gallate (ECg) and epigallocatechin gallate (EGCg). Experiments using brush-border membrane vesicles obtained from the rabbit small intestine demonstrated that ECg inhibited SGLT1 in a competitive manner, although ECg itself was not transported via SGLT1. The present results suggest that tea polyphenols such as ECg interact with SGLT1 as antagonist-like molecules, possibly playing a role in controlling the dietary glucose uptake in the intestinal tract.

Keywords: *Green tea; polyphenol; glucose transporter; intestinal cell; diabetes*

INTRODUCTION

Diabetes is a disease characterized by chronic hypoglycemia, which can lead to a number of microvascular and macrovascular complications. Hyperglycemia is associated with an increased risk of cardiovascular diseases (Balkau et al., 1998; Harris and Eastman, 1998). Inhibiting the glucose uptake in the intestines may be beneficial for diabetic patients to control the blood glucose level in the postprandial state. Substances that inhibit amylase and glycosidases have therefore been analyzed, and some of them have been modified to develop drugs for treating diabetes (Clissold and Edwards, 1988; Yoshikuni, 1988).

Tea polyphenols have been reported to inhibit intestinal α-amylase or sucrase (Hara and Honda, 1990; Honda and Hara, 1993; Matsumoto et al., 1993). This may be the main mechanism for the suppressive effect of polyphenols on the increase in plasma glucose level after a meal (Honda and Hara, 1993; Matsumoto et al., 1993; Valsa et al., 1997). However, the inhibition of intestinal glucose transporters by tea polyphenols may also participate in reducing the blood glucose level. The major component of glucose transport in the intestines is the Na^+-dependent glucose transporter (SGLT1). SGLT1 can be blocked by the plant-derived glycoside phloridzin (Alvarado, 1967). Phloridzin has been reported to excrete glucose into the urine by reducing the SGLT-mediated reabsorption of glucose in retinal epithelial cells and to lower the blood glucose level in several diabetic animal models upon its subcutaneous injection (Khan and Effendic, 1995; Krook et al., 1997)

In the present study, by using a rat everted sac, we show that the tea polyphenol potently inhibited the functional activity of SGLT1. Detailed studies on the effect of tea polyphenols were performed by using rabbit intestinal brush-border membrane vesicles (BBMVs) and by an electrophysiological method. BBMVs comprise spherical membrane vesicles on which SGLT1 is expressed, the outer surface representing the intestinal lumen and the core of the vesicle representing the intestinal cell. The advantage of using BBMVs in such studies is that SGLT1-mediated glucose transport can be studied without any interference from the cytoplasmic glucose metabolism. Welsh et al. (1989) have reported that catechin, one of the major tea polyphenol derivatives, inhibited SGLT1. Because epicatechin gallate (ECg) and epigallocatechin gallate (EGCg), the major tea polyphenols with a triphenol structure (Figure 1), generally have stronger biological functions than catechin, ECg and EGCg were expected to have a stronger effect on glucose transport inhibition than catechin. We therefore investigated the effect of ECg and EGCg on the intestinal glucose uptake via SGLT1.

MATERIALS AND METHODS

Chemicals. Catechin (C), epicatechin gallate (ECg), epigallocatechin (EGC), and epigallocatechin gallate (EGCg) were obtained from Mitsui Norin Co. (Shizuoka, Japan). d-[6-^{3}H]-Glucose (specific radioactivity = 33.0 Ci/mmol) was obtained from Amersham Radiochemical Center (London, U.K.); all other reagents and chemicals were commercially available extra-pure-grade products.

Uptake Experiments Using Everted Sacs. The incorporation of D-glucose into everted jejunal sacs was determined as originally described by Lostao et al. (1998). Briefly, 6-week-old male Wistar rats (from Nisseizai, Tokyo, Japan) were anesthetized, and a segment of the jejunum was quickly excised, rinsed with an ice-cold saline solution, everted, and

* Corresponding author (telephone +81-3-5841-5127; fax +81-3-5841-8026; e-mail ams316@mail.ecc.u-tokyo.ac.jp).
[†] The University of Tokyo.
[‡] Mitsui Norin Co. Ltd.
[§] University of Shizuoka.

10.1021/jf0006832 CCC: $19.00
Published on Web 10/10/2000

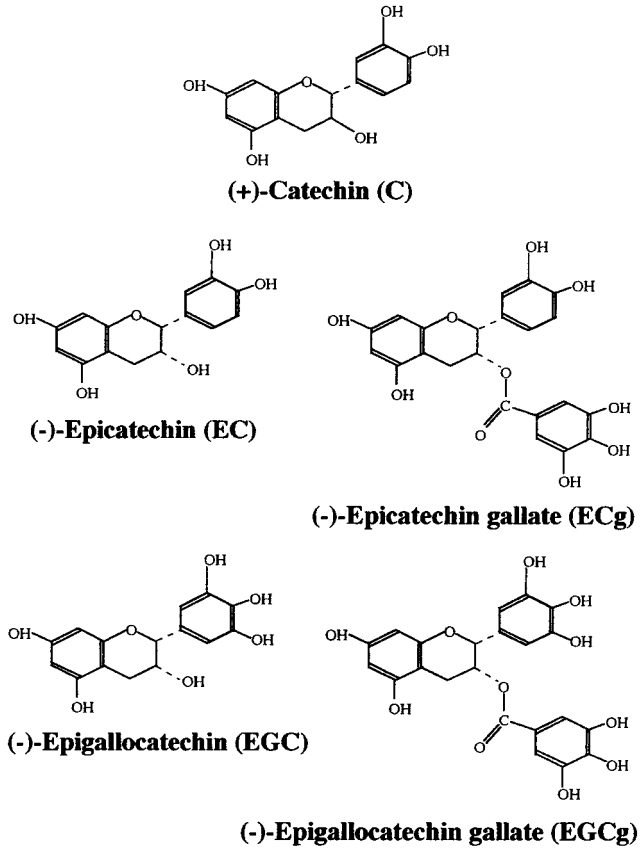

Figure 1. Structures of major green tea polyphenols.

cut into 3-cm pieces. Groups of seven to eight intestinal sacs were incubated at 37 °C in the uptake buffer (the uptake buffer contained 140 mM KCl or NaCl, 10 mM $KHCO_3$, 0.4 mM KH_2PO_4, 2.4 mM K_2HPO_4, 1.2 mM $CaCl_2$, and 1.2 mM $MgCl_2$ at pH 7.4) containing 1.0 mM D-glucose and 0.1 μCi/mL of d-[^{3}H]-glucose in the absence (control) or presence of tea polyphenols. To measure the sodium-dependent glucose uptake, the uptake buffer containing 140 mM NaCl instead of KCl was used. At the end of the incubation period, the tissues were washed in an ice-cold saline solution, blotted carefully to remove the excess moisture, weighed wet, and extracted by shaking for 12 h in 12 mL of a liquid scintillation cocktail; the radioactivity was counted with an LSC 5100 liquid scintillation analyzer (Aloka, Tokyo, Japan).

BBMVs. *Preparation of the BBMVs.* BBMVs were prepared from rabbit jejunum (male rabbits, 3 months old, from Nisseizai, Tokyo, Japan) by the Mg^{2+}-precipitation method in the presence of ethylene glycol bis(β-aminoethyl ether) *N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) (Tiruppathi et al., 1988; Miyamoto et al., 1985). The small intestine was excised, rinsed with an ice-cold KCl solution, and everted on a stainless steel wire. Mucosal scrapings from two rabbits were homogenized with a 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)/tris(hydroxymethyl)aminomethane (Tris) buffer at pH 7.5 containing 300 mM mannitol and $MgCl_2$ added to a final concentration of 10 mM. The mixture was stirred continuously for 1 min and subsequently centrifuged at 3000*g* for 15 min. The supernatant was decanted and centrifuged at 60000*g* for 30 min. The resulting BBMVs were resuspended in a 10 mM HEPES/Tris buffer (pH 7.5) containing 300 mM mannitol to a final protein concentration of 10 mg/mL. BBMVs were stored in liquid nitrogen until needed.

Uptake Experiments with BBMVs. Measurement of the glucose uptake by BBMVs was performed at room temperature by the rapid filtration technique using a Millipore filter (DAWP type, 0.65 μm pore size) (Ganapathy et al., 1981). The rate of glucose uptake was determined by incubating BBMVs in a 10 mM HEPES/Tris buffer (pH 7.5) containing 0.1 μM D-[^{3}H]-glucose and 150 mM NaCl. The diffusional glucose uptake was determined by replacing NaCl with an equimolar amount of KCl in the reaction mixture. The uptake was initiated by mixing 40 μL of a membrane suspension with 160 μL of the uptake buffer containing labeled glucose and was terminated by adding 5 mL of an ice-cold stop buffer with subsequent filtration under vacuum. The stop buffer was 10 mM HEPES/Tris (pH 7.5) containing 150 mM KCl. The filter was washed three times with 5 mL of the stop buffer and then transferred to a counting vial. The radioactivity associated with the filter was counted with an LSC 5100 liquid scintillation analyzer.

HPLC Analysis of ECg. Uptake of ECg by BBMVs was measured by using the same method as that already described for glucose uptake, except for replacing D-[^{3}H]glucose with ECg (0.5 mM final concentration). To the washed filter (washed three times with the stop buffer), which had adsorbed BBMVs, was added 1 mL of ethanol to extract ECg from BBMVs. The mixture was then centrifuged for 15 min at 100000*g*, and the resulting supernatant was filtered with Chromatodisc 4A (0.45 μm pore size, Kurabou, Tokyo, Japan), the filtrate being injected into an HPLC column (ODS-H-1251, 4 × 250 mm, Senshu-Kagaku, Tokyo, Japan). The eluent was 0.3% phosphoric acid/acetonitrile (75:25, v/v) at a flow rate of 0.8 mL/min (PU-1580 pump with an LG-1580-02 gradient unit, Jasco, Tokyo, Japan). Tea polyphenols were detected with UV at 280 nm (Jasco UV-1575 UV−vis detector).

Electrophysiological Measurement. Male ddy mice (8−9 weeks old, Japan SLC, Hamamatsu, Japan) were anesthetized, and segments of the ileum were removed. They were rinsed with buffered saline and everted. The everted intestine filled with a serosal solution (21 mM $NaHCO_3$, 2.4 mM K_2HPO_4, 0.6 mM KH_2PO_4, 119 mM NaCl, 1.2 mM $CaCl_2$, and 1.2 mM $MgCl_2$) was placed inside a glass tube containing the same solution, which was bubbled continuously with 95% O_2 and 5% CO_2 at 37 °C. The potential difference was measured with a high-input impedance electrometer (SS-1934, Nihon Kohden Co., Tokyo, Japan) through calomel half-cells (Kitaoka et al., 1996). 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. Glucose or ECg was added to the apical side from a concentrated aqueous stock solution, and the potential difference was registered with a chart recorder (R-5-101G, Yanaco Co., Tokyo, Japan).

Statistics. Each result is shown as the mean ± SE. The kinetic constants were calculated by a linear regression of the Eadie−Hofstee plot. The statistical difference between two groups was analyzed by Student's *t* test.

RESULTS

Effect of Tea Polyphenols on Glucose Uptake in Everted Sacs. Figure 2 shows time course plots of glucose uptake by everted sacs in the presence and absence of Na^+. The difference between the glucose uptake in the presence of Na^+ and that in the absence of Na^+ represents the Na^+-dependent glucose uptake by SGLT1, whereas the glucose uptake in the presence of Na^+ represents the total glucose uptake by the everted sac. The Na^+-dependent glucose uptake rate was linear within the first 5 min and was ∼55% of the total glucose uptake after 2.5 min of incubation (Figure 2).

The Na^+-dependent glucose uptake by everted sacs was inhibited by ECg and EGCg (Figure 3). The uptake of glucose after 4 min of incubation decreased from 15.1 to 9.7 μmol/g of wet tissue (36% inhibition) when 1 mM ECg was added to the uptake medium. The uptake also decreased to 11.2 μmol/g (25% inhibition) in the presence of 1 mM EGCg. On the other hand, 1 mM catechin did not affect the glucose uptake.

Effect of Tea Polyphenols on Glucose Uptake by BBMVs. *Glucose Transport in BBMVs.* Figure 4 shows

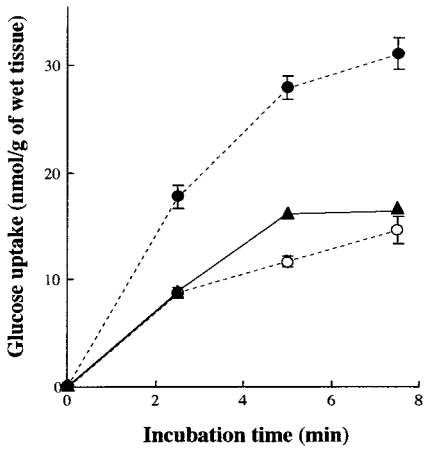

Figure 2. Time course plots of glucose uptake by rat intestinal everted sacs. The uptake buffer contained either 140 mM NaCl (●) or 140 mM KCl (○). To this buffer were added 1.0 mM D-glucose and 0.1 mCi/mL of D-[^{3}H]glucose. The difference of glucose uptake in the presence and absence of NaCl is plotted (▲) as the Na^+-dependent glucose transport. Each value is the mean ± SE (n = 8).

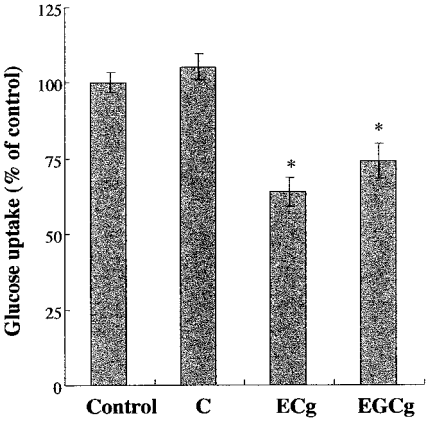

Figure 3. Effect of ECg on glucose uptake by rat intestinal everted sacs. Intestinal sacs were incubated for 4 min in the uptake buffer containing 1 mM D-glucose with or without 1 mM C, ECg, or EGCg. Each data value is expressed as a percentage of the control (uptake in the absence of tea polyphenols) and is represented as the mean ± SE (n = 8). An asterisk (*) indicates significant difference (at $P < 0.01$) from the control.

typical time course plots of glucose uptake by BBMVs. Under an NaCl gradient directed from the outside (high) to the inside (low), a very rapid uptake of glucose was observed, whereas the uptake was very small without Na^+, indicating that BBMVs used SGLT1 as the major mechanism for glucose uptake. Because the Na^+-dependent glucose uptake rate was almost linear for the initial 30 s, the uptake experiment was carried out for 15 s unless otherwise stated.

Effect of Tea Polyphenols on Glucose Uptake in BBMVs. ECg and EGCg at 1 mM concentration reduced the glucose uptake by 53 and 35%, respectively, whereas the inhibitory effects of C and EGC were not significant (Figure 5). These data imply that a galloyl ester group may be important for blocking glucose uptake. In another experiment, we have previously observed that gallic acid itself also inhibited the glucose uptake, although the inhibitory activity was much lower than that of ECg (32% inhibition by 1 mM gallic acid, compared with 62% inhibition by 1 mM ECg). The glucose uptake inhibition by ECg occurred in a concentration-dependent manner, the IC_{50} value being ~1 mM under these conditions (Figure 6).

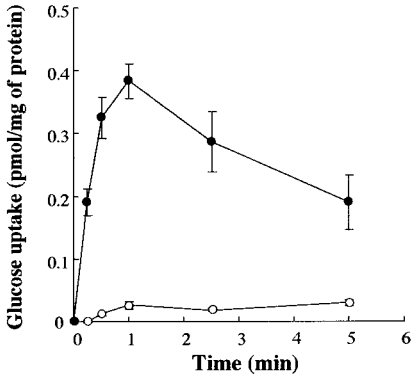

Figure 4. Time course plots of glucose uptake by rabbit intestinal BBMVs. Vesicles were equilibrated with a 10 mM Hepes/Tris buffer (pH 7.5) containing 300 mM D-mannitol before the uptake experiment. The uptake buffer contained 100 nM D-[^{3}H]glucose, 10 mM Hepes, and either 150 mM NaCl (●) or 150 mM KCl (○). Each value is the mean ± SE (n = 6).

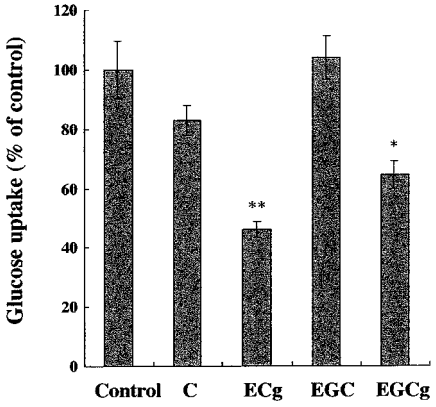

Figure 5. Effect of the polyphenols on the Na^+-dependent glucose uptake by rabbit intestinal BBMVs. The uptake was terminated after 15 s. Each data value is expressed as a percentage of the control (uptake in the absence of tea polyphenols) and is represented as the mean ± SE (n = 6). An asterisk (*) indicates significant difference from the control (at $P < 0.005$); two asterisks (**) indicate significant difference from the control (at $P < 0.001$).

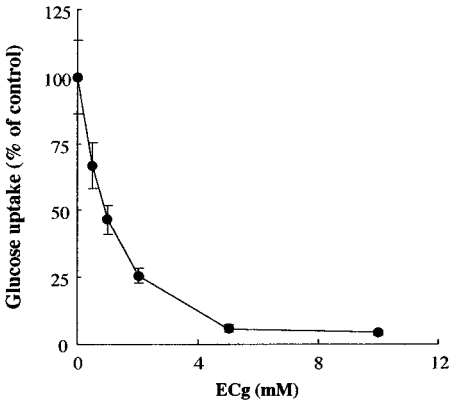

Figure 6. Effect of the concentration of ECg on the Na^+-dependent glucose uptake by rabbit intestinal BBMVs. The uptake buffer contained 100 nM D-[^{3}H]glucose and an increasing concentration of ECg. The uptake experiment was carried out for 15 s. Each data value is expressed as a percentage of the control (uptake in the absence of ECg) and is represented as the mean ± SE (n = 6).

Determination of K_i Value. To further define the inhibition pattern by ECg and to determine the K_i value

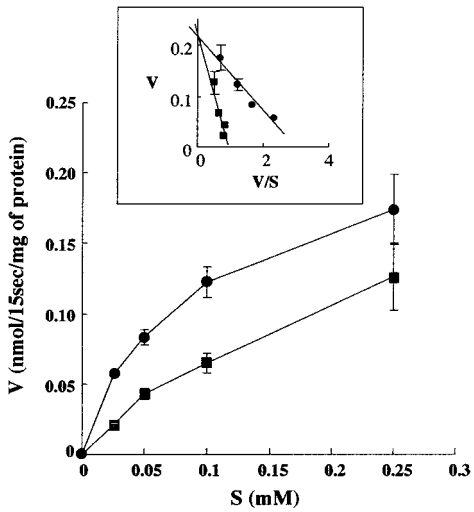

Figure 7. Concentration dependence of glucose uptake by rabbit intestinal BBMVs. The uptake experiment was carried out for 15 s in the presence (■) or absence (●) of 1 mM ECg. The initial rate of glucose uptake was plotted against an increasing concentration of the glucose medium (0.025−0.25 mM). K_i was determined by using an Eadie−Hofstee plot as shown in the inset. Each value represents the mean ± SE (n = 6).

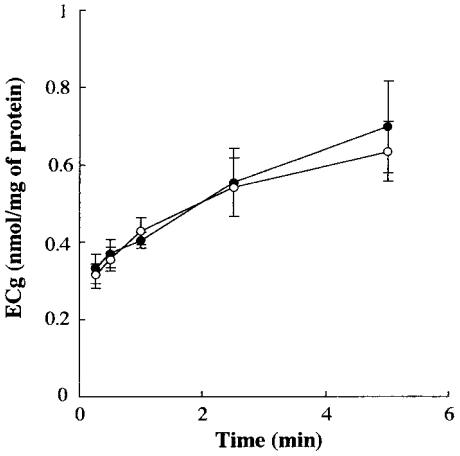

Figure 8. Incorporation of ECg in rabbit intestinal BBMVs. BBMVs were incubated in a 0.5 mM ECg solution for 0.25, 0.5, 1, 2.5, and 5 min in the presence (●) or absence (○) of 150 mM NaCl. The incorporated ECg in BBMVs was quantified by HPLC.

for ECg, kinetic analyses of the inhibition were performed. ECg inhibited the transport of glucose as shown in Figure 7, and the K_i value for ECg was calculated to be ~0.39 mM by using an Eadie−Hofstee plot. The plot suggests that ECg was a competitive inhibitor of glucose transport in rabbit intestinal BBMVs. ECg is likely to have interacted with SGLT1 and blocked the binding of glucose to this protein.

Incorporation of ECg in BBMVs. A new question arises from this finding as to whether ECg itself can be transported as a substrate of SGLT1 or not. The content of ECg incorporated into BBMVs was therefore measured after incubation of BBMVs with ECg in the NaCl or KCl buffer. Although the amount of incorporated ECg gradually increased with increasing incubation time, there was no difference between the values obtained in the presence or absence of Na^+ (Figure 8). This suggests that ECg itself was not transported into BBMVs via SGLT1.

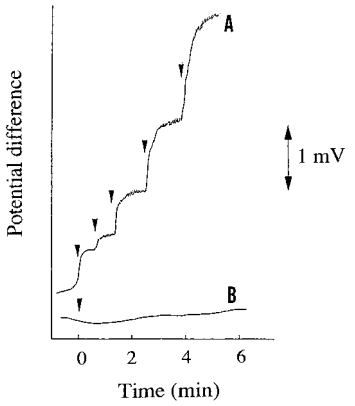

Figure 9. Effect of glucose and ECg on the transmural potential difference (PD) of the mouse intestinal wall: (A) glucose-evoked change in the transmural PD (concentrated glucose stock solution was added to the apical side of the everted sac to give a cumulative dose of glucose of 0.25, 0.5, 1, 2, or 5 mM, and the PD change was monitored); (B) effect of ECg on transmural PD (ECg at 0.25 mM was added to the apical side in the absence of glucose).

Electrophysiological Analyses. The addition of glucose to the apical side of the intestinal mucosa increased the transmural potential difference (PD) across the mouse ileal mucosa in a concentration-dependent manner (Figure 9). The transmural PD change induced by glucose was decreased when glucose was added in the presence of ECg (data not shown), in good agreement with the previous results showing that ECg inhibited the SGLT1 activity in rat everted sacs (Figure 3) and in rabbit intestinal BBMVs (Figure 5). When only ECg was added to the apical side in the absence of glucose, no transmural PD change was apparent (Figure 9). This result also supports the previous finding that ECg was not a substrate of SGLT1.

DISCUSSION

The intestinal Na^+/glucose transporter, SGLT1, was expression-cloned in 1987 (Heidiger et al., 1987), and its transmembrane structure was revealed. SGLT1 has one Na^+-binding site, and the binding of Na^+ induces a conformational change in SGLT1, by which glucose becomes accessible to its binding site. Glucose and Na^+ are then transported to the other side of the membrane (Wright et al., 1998). Tea polyphenols are thought to inhibit this process.

Substances that inhibit SGLT1 have been found in other polyphenol compounds and saponins. Recently, Vedavanam et al. (1999) have reported that a soybean extract containing isoflavones inhibited glucose transport by BBMVs. Polyphenolic compounds such as tannic acid and chlorogenic acid have also been reported to have such activity (Welsh et al., 1989). A crude saponin fraction of *Gymnema sylvesta* has been shown to inhibit glucose absorption in the small intestine of rats in a glucose tolerance test (Murakami et al., 1996; Yoshikawa et al., 1997a). Other saponins isolated from various sources have also been found to exhibit hypoglycemic activity in the oral glucose test (Yoshikawa et al., 1996, 1997b). However, the mechanisms for the inhibition by these substances have not previously been investigated in detail. The present results suggest that tea polyphenols such as ECg bind to the glucose transporter as an antagonist-like molecule and inhibit the transport of glucose, although it has not yet been revealed whether

ECg binds to the glucose-binding site or to the Na^+-binding site.

The inhibitory activity of tea polyphenols was observed only with certain types of tea polyphenols such as ECg and EGCg, suggesting that the galloyl ester group was essential for this activity. The inhibitory activity of gallic acid was, however, much weaker than that of ECg, suggesting that not only the galloyl residue but also the catechin structure would play a role in this inhibition. Because tea polyphenols lacking the galloyl residue such as C, EC, and EGC did not show significant inhibition at the concentration of 1 mM, the catechin structure may be involved in the increased accessibility of the galloyl residue to the glucose/Na^+-binding site in SGLT1.

Polyhydroxyl phenols are known to precipitate proteins rather indiscriminately. Therefore, a general precipitation of BBMV proteins, including SGLT1, by tea polyphenols could be the inhibitory mechanism. To examine this possibility, precipitability of bovine serum albumin in the presence of tea polyphenols was investigated. There was a slight occurrence of precipitation of serum albumin in the uptake buffer when tea polyphenols (1 mM) were added to the protein solution (1 mg/mL), but the degree of precipitate formation by various polyphenols did not correlate with their glucose-uptake inhibitory activity (data not shown). For example, the degree of precipitate formation caused by EGCg was almost same as that by EGC, which did not inhibit SGLT1. We have also observed that tea polyphenols such as ECg at concentrations of <1 mM did not inhibit amino acid and peptide transporters expressed on the human intestinal Caco-2 cells (unpublished results). These results indicate that the inhibition of SGLT1 by ECg and EGCg is not due to a simple protein precipitation but a specific mechanism.

It is unknown to what extent tea polyphenols inhibit intestinal glucose transport in vivo. In the present study with an intestinal everted sac, 1 mM ECg reduced the uptake of glucose (1 mM) to 65% of the control value (Figure 3). The practical intestinal glucose level would, however, reach a level much higher than 1 mM after a meal. As the inhibition of glucose uptake by ECg is competitive, ECg may not significantly inhibit the glucose uptake when the intestinal glucose level increases after a meal. Nevertheless, orally administered ECg/EGCg may inhibit the glucose uptake to some extent because ECg and EGCg are thought to be highly interactive with mucosa, the local concentration of EGCg at the intestinal mucosa being very high. Suganuma et al. (1998) and Nakagawa and Miyazawa (1997) have reported that most of the ingested EGCg was detected at the intestinal mucosa. Furthermore, the inhibition of α-amylase and α-glycosidases by tea polyphenols (Hara and Honda, 1990; Honda and Hara, 1993; Matsumoto et al., 1993) would slow the hydrolysis of polysaccharides and oligosaccharides, resulting in a reduced rate of glucose production in the intestinal tract. Under such conditions, the inhibitory activity of tea polyphenols toward SGLT1 could play a part in the reduction of intestinal glucose absorption. Thus, tea polyphenols may act as multifunctional substances, synergistically inhibiting the intestinal glucose absorption.

Glucose uptake in various cells, other than intestinal and kidney cells, is performed by Na^+-independent glucose transporters, GLUTs. We have observed that ECg inhibited GLUTs 1 and 3, which were expressed on human intestinal Caco-2 cells (unpublished results). Compared with SGLT1, GLUT was more sensitive to ECg, the IC_{50} value being 20 μM. However, it is unlikely that ingested tea polyphenols would interfere with the glucose uptake in various tissues in the body. Although polyphenols can be absorbed to some extent through the intestinal epithelium and are detectable in various organs, the concentration of polyphenols in the blood plasma and organs is very low. Nakagawa et al. (1997) have reported that the plasma polyphenol concentration after the ingestion of a normal amount of tea (375 mg of EGCg intake) was only 4.3 μM, this being much lower than the IC_{50} value. Furthermore, the majority of ingested polyphenols undergoes structural modification (conjugation) such as methylation, sulfation, and glucuronidation before finally being excreted in the urine and bile (Piskula and Terao, 1998; Okushio et al., 1998). These modifications may also reduce the bioactivity of polyphenols as reported by Nanjo et al. (1996), who observed that the antioxidative activity of flavonoids in vitro was drastically reduced by modification of the hydroxyl groups. Because tea polyphenols are present in an unconjugated form and also in a high content in the intestinal tract, it is feasible that tea polyphenols express their suppressive function only in intestinal glucose absorption.

In this study, tea polyphenols were found to inhibit glucose uptake in the intestine. The inhibitory activity of tea polyphenols, together with their intestinal α-glycosidase inhibitory activity, may suppress the increase in the blood glucose level after a meal. Phloridzin, a classic SGLT1 inhibitor, has been investigated as a drug for diabetic patients (Oku et al., 1999). It may also be possible that tea polyphenols are a good model material for new drugs as well as for a functional food ingredient.

ABBREVIATIONS USED

BBMVs, brush-border membrane vesicles; C, catechin; EC, epicatechin; ECg, epicatechin gallate; EGC, epigallocatechin; EGCg, epigallocatechin gallate; GLUT, glucose transporter; SGLT, sodium-dependent glucose transporter; PD, potential difference.

LITERATURE CITED

Alvarado, F. Hypothesis for the interaction of phloridzin and phloretin with membrane carriers for sugars. *Biochim. Biophys. Acta* **1967**, *135*, 483−495.

Balkau, B.; Shipley, M.; Jarrett, R. J. High blood glucose concentration is a risk factor for mortality in middle-aged nondiabetic man. *Diabetes Care* **1998**, *21*, 360−367.

Clissold, S. P.; Edwards, C. Acarbose. A preliminary review of its pharmacodynamic and pharmacokineticproperties, and therapeutic potential. *Drugs* **1988**, *35*, 214−243.

Ganapathy, V.; Mendicino, J. F.; Leibach, F. H. Transport of glycyl-L-proline into intestinal and renal brush-border vesicles from rabbit. *J. Biol. Chem.* **1981**, *256*, 118−124.

Hara, Y.; Honda, M. The inhibition of α-amylase by tea polyphenols. *Agric. Biol. Chem.* **1990**, *54*, 1939−1945.

Harris, M.; Eastman, R. C. Is there a glycemic threshold for mortality risk? *Diabetes Care* **1998**, *21*, 331−333.

Hediger, M. A.; Coady, M. J.; Ikeda, T. S.; Wright, E. M. Expression cloning and cDNA sequencing of the Na^+/glucose cotransporter. *Nature* **1987**, *330*, 379−381.

Honda, M.; Hara, Y. Inhibition of rat small intestinal sucrase and α-glucosidase activities. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 123−124.

Khan, A.; Efendic, S. Evidence that increased glucose cycling in islets of diabetic *ob/ob* mice is a primary feature of the disease. *Am. J. Physiol.* **1995**, *269*, E623−E626.

Kitaoka, S.; Hayashi, H.; Yokogoshi, H.; Suzuki, Y. Transmural potential changes associated with the in vitro absorption of theanine in the guinea pig intestine. *Biosci., Biotechnol., Biochem*. **1996**, *60*, 1768−1771.

Krook, A.; Kawano, Y.; Song, X. M.; Efendic, S.; Roth, R. A.; Wallberg-Henriksson, H.; Ziearath, J. R. Improved glucose tolerance restores insulin-stimulated Akt kinase activity and glucose transport in skeletal muscle from diabetic Goto-Kakizaki rats. *Diabetes* **1997**, *46*, 2110−2114.

Lostao, M. P.; Urdaneta, E.; Martinez-Anso, E.; Barber, A.; Martinez, J. A. Presence of leptin receptors in rat small intestine and leptin effect on sugar absorption. *FEBS Lett.* **1998**, *423*, 302−306.

Matsumoto, M.; Ishigaki, F.; Ishigaki, A.; Iwashina, H.; Hara, Y. Reduction of blood glucose levels by tea catechin. *Biosci., Biotechnol., Biochem*. **1993**, *57*, 525−527.

Miyamoto, Y.; Ganapathy, V.; Leibach, F. H. Proton gradient-coupled uphill transport of glycylsarcosine in rabbit renal brush-border membrane vesicles. *Biochem. Biophys. Res. Commun.* **1985**, *132*, 946−953.

Murakami, N.; Murakami, T.; Kadoya, M.; Matsuda, H.; Yamahara, J.; Yoshioka, M. New hypoglycemic constituents in "gymnemic acid" from *Gymnema sylvestre*. *Chem. Pharm. Bull.* **1996**, *44*, 469−471.

Nakagawa, K.; Miyazawa, T. Absorption and distribution of tea catechin, (−)-epicatechin-3-gallate, in the rat. *J. Nutr. Sci. Vitaminol.* **1997**, *43*, 679−684.

Nakagawa, K.; Okuda, S.; Miyazawa, T. Dose-dependent incorporation of tea catechins, (−)-epigallocatechin-3-gallate and (−)-epicatechin, into human plasma. *Biosci., Biotechnol., Biochem*. **1997**, *61*, 1981−1985.

Nanjo, F.; Goto, K.; Seto, R.; Suzuki, M.; Sakai, M.; Hara, Y. Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. *Free Radical Biol. Med*. **1996**, *21*, 895−902.

Oku, A.; Ueta, K.; Arakawa, K.; Ishihara, T.; Nawano, M.; Kuronuma, Y.; Matsumoto, M.; Saito, A.; Tsujihara, K.; Anai, M.; Asano, T.; Kanai, Y.; Endou, H. T-1095, an inhibitor of renal Na^+-glucose cotransporters, may provide a novel approach to treating diabetes. *Diabetes* **1999**, *48*, 1794−1800.

Okushio, K.; Suzuki, M.; Matsumoto, N.; Nanjo, F.; Hara, Y. Identification of (−)-epicatechin metabolites and their metabolic fate in the rat. *Drug Metab. Dispos.* **1998**, *27*, 309−316.

Piskula, M. K.; Terao, J. Accumulation of (−)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugation enzymes in rat tissues. *J. Nutr.* **1998**, *128*, 1772−1778.

Suganuma, M.; Okabe, S.; Oniyama, M.; Tada, Y.; Ito, H.; Fujiki, H. Wide distribution of [^{3}H](−)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. *Carcinogenesis* **1998**, *19*, 1771−1776.

Tiruppathi, C.; Miyamoto, Y.; Ganapathy, V.; Leibach, F. H. Fatty acid-induced alterations in transport systems of the small intestinal brush-border membrane. *Biochem. Pharmacol.* **1988**, *37*, 1399−1405.

Valsa, A. K.; Sudheesh, S.; Vijayalakshmi, N. R. Effect of catechin on carbohydrate metabolism. *Indian J. Biochem. Biophys.* **1997**, *34*, 406−408.

Vedavanam, K.; Srijayanta, S.; O'Relly, J.; Raman, A.; Wiseman, H. Antioxidant action and potentional antidiabetic properties of an isoflavonoid-containing soybean phytochemical extract (SPE). *Phytother. Res.* **1999**, *13*, 601−608.

Welsh, C. A.; Lachance, P. A.; Wasserman, B. P. Dietary phenolic compounds: inhibition of Na^+-dependent D-glucose uptake in rat intestinal brush border membrane vesicles. *J. Nutr.* **1989**, *119*, 1698−1704.

Wright, E. M.; Loo, D. D. F.; Panayatova-Heiermann, M.; Hirayama, B. A.; Turk, E.; Eskandari, S.; Lam, J. Structure and function on the Na^+/glucose cotranporter. *Acta Physiol. Scand.* **1998**, *163* (Suppl. 643), 257−264.

Yoshikawa, M.; Murakami, T.; Harada, E.; Murakami, N.; Yamahara, J.; Matsuda, H. Bioactive saponins and glycosides. VII. On the hypoglycemic principles from the root cortex of *Aralia elata* SEEM: Structure related hypoglycemic activity of oleanolic oligoglycoside. *Chem. Pharm. Bull.* **1996**, *44*, 1923−1927.

Yoshikawa, M.; Murakami, T.; Kadoya, M.; Li, Y.; Murakami, N.; Yamahara, J.; Matsuda, H. Medicinal Foodstuffs. IX. The inhibitors of Glucose Absorption from the Leaves of *Gymnema sylvestre* R. BR. (Asclepiadaceae): Structures of Gymnemosides a and b. *Chem. Pharm. Bull.* **1997a**, *45*, 1671−1676.

Yoshikawa, M.; Shimada, H.; Morikawa, T.; Yoshizumi, S.; Matsumura, N.; Murakami, T.; Matsuda, H.; Hori, K.; Yamahara, J. Medicinal Foodstuffs. VII. On the saponin constituents with glucose and alcohol absorption-inhibitory activity from a food garnish "Tonburi", the fruit of Japanese *Kochia scoparia* (L.): Structures of scoparianosides A, B, and C. *Chem. Pharm. Bull.* **1997b**, *45*, 1300−1305.

Yoshikuni, Y. Inhibition of intestinal α-glucosidase activity and postprandial hyperglycemia by moranoline and its N-alkyl derivatives. *Agric. Biol. Chem.* **1988**, *52*, 121−128.

Received for review June 2, 2000. Revised manuscript received August 25, 2000. Accepted August 25, 2000.

JF0006832