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Polyphenol-Induced Inhibition of the Response of Na^+/Glucose Cotransporter Expressed in *Xenopus* Oocytes

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To study the effects of polyphenols on the Na^+/glucose cotransporter (SGLT1) response, SGLT1 was expressed in *Xenopus* oocytes by injecting cRNA synthesized from the cloned cDNA of the small intestine cotransporter of rats, and the electrical response elicited by glucose or galactose was measured by a voltage clamping method. Most phenol derivatives had no effect on the response. However, the polyphenols (+)-catechin, (−)-epicatechin gallate (ECg), and (−)-epigallocatechin gallate (EGCg), which are components of green tea, caused an inhibition of the response, which was almost independent of glucose concentration. The inhibition constants were estimated to be 2.3 mM for (+)-catechin and 0.45 mM for both ECg and EGCg, assuming the noncompetitive inhibition mechanism. Saponin prepared from tea seeds also inhibited the response significantly. Tannic acid and aqueous extracts of teas induced nonspecific electrical responses in both cRNA-injected and noninjected oocytes at lower concentrations than those that caused an inhibition of the SGLT1 response when their dose-dependent effects were examined. These results are possibly helpful in the development of a dietary supplement for diabetic patients.

KEYWORDS: Catechin; Na^+/glucose cotransporter; noncompetitive inhibition; polyphenol; *Xenopus* oocyte

INTRODUCTION

Tea is one of the major beverages consumed by humans. Enormous amounts of tea (*Camellia sinensis*) processed to green tea, black tea, or oolong tea are consumed all over the world to relieve drowsiness, stress, and neuralgia in humans (*1*). Chemical analyses of tea components have revealed that tea contains health-promoting compounds: amino acids such as γ-aminobutyric acid (GABA) or theanine, vitamins such as vitamin C or E, caffeine, catechin, and pigments (*2*). Reportedly, various components of tea cause alertness, heart stimulation, antioxidation, anticancer, or antibiotic effects (*1*, *2*). It is also known that some tea components decrease blood pressure and blood sugar or control the amount of cholesterol in the blood. Especially large amounts of various polyphenol derivatives are present in tea, and they act as antioxidants when they are absorbed into the blood (*2*). The decrease of blood sugar may result from the decrease of the absorption of the sugar in the small intestine caused by these compounds.

The Na^+-dependent glucose cotransporter (SGLT1) is expressed in the jejunum and transports glucose into epithelial cells. The glucose is then transported into the blood via the facilitated glucose transporter (GULT). Inhibition of glucose uptake via SGLT1 in the small intestine may prevent hyperglycemia, which causes diabetes. Herbs such an Indian herb (*3*), *Gymnema sylvesta*, have been used as a health-promoting supplement for reducing the weight of overweight people. It has also been reported that saponins, for example, from the fruit of the Japanese *Kochia scoparia* (*4*), inhibit the increase of serum glucose in glucose-loaded rats. Welsh et al. (*5*) reported that polyphenolic compounds such as tannic acid and chlorogenic acid inhibited intestinal glucose uptake. Recently Kobayashi et al. (*6*) reported that polyphenols which are present in teas inhibit intestinal glucose uptake in a competitive manner in experiments utilizing brush-border membrane vesicles or everted sacs of the jejunum and radiolabeled glucose. However, the mechanisms of such inhibition by these substances have not been investigated in detail. Therefore, it is important to examine whether these effects are due to the direct inhibition of SGLT1 activity.

In previous studies, SGLT1 was expressed in *Xenopus* oocytes by injection of rat small intestine poly(A)$^+$RNA, and the responses of the oocytes were measured electrophysiologically (*7*). A cDNA coding for the high-affinity Na^+-dependent glucose cotransporter, SGLT1, was isolated from rabbit small intestines by expression cloning employing *Xenopus* oocytes (*8*). We

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cloned a cDNA for rat SGLT1 from a rat jejunum cDNA library, examined the sugar specificity of the encoded transporter (*9*), and compared the specificity with that of the H^+/hexose cotransporter of *Chlorella* (*10*).

In the present study, we expressed rat SGLT1 in *Xenopus* oocytes by injecting cRNA synthesized from cloned cDNA coding SGLT1 and measured the electrical responses of the oocytes elicited by glucose or galactose in the presence and absence of various compounds such as polyphenol derivatives present in teas. As expected, (−)-epicatechin gallate (ECg) and (−)-epigallocatechin gallate (EGCg) inhibited the SGLT1 response strongly. (+)-Catechin also caused significant inhibition of the response. Saponin prepared from tea seeds inhibited the response slightly. Other compounds tested, including (−)-epicatechin (EC), (−)-epigallocatechin (EGC), chlorogenic acid, and monophenols, induced little effect on the response of SGLT1 expressed in *Xenopus* oocytes. These results will be helpful in the development of foods to control the blood glucose level in the postprandial state in diabetic patients.

MATERIALS AND METHODS

Materials. (+)-Catechin, EC, EGC, ECg, and EGCg were supplied from Tokyo Food Techno Co. Ltd., Tokyo, Japan. (+)-Catechin and EGCg were also purchased from Nacalai Tesque, Kyoto, Japan, and Sigma Chemical Co., St. Louis, MO, respectively. Tannic acid was purchased from Nacalai Tesque. D-(+)-Galactose and chlorogenic acid were purchased from Sigma Chemical Co. d-(+)-Glucose, 2,2-bis(4-hydroxyphenyl)propane (bisphenol A), and saponin from tea seeds were purchased from Wako Pure Chemical Industries, Osaka, Japan. All chemicals were of guaranteed reagent quality. Green tea was a gift from the foundation of Kyoto Green Tea. Black tea (Nittoh, Tokyo, Japan) and oolong tea (Kotanikokuhunn, Kochi, Japan), were purchased from a local shop.

One gram of teas was extracted in 20 mL of hot normal frog Ringer solution (115 mM NaCl, 1 mM KCl, and 1.8 mM $CaCl_2$ in 5 mM Tris at pH 7.2) for 3 min. The precipitate was removed from the extract by filtration with a filter paper. The effect of the extract on 0.5 mM glucose-elicited response of SGLT1 was examined by the addition of the extract (2−10 μL/mL) to the glucose solution used to treat oocytes.

Preparation of cRNA and *Xenopus* Oocytes. The cRNA of the rat jejunum SGLT1 was synthesized from the cloned cDNA of the SGLT1 using RNA polymerase according to the standard procedures.

Adult female frogs (*Xenopus laevis*) were purchased from Hamamatsu Seibutsu Kyozai, Co., Hamamatsu, Japan. The oocytes were dissected from the ovaries of adult female frogs that had been kept in ice for 1 h. They were manually detached from the inner ovarian epithelium and follicular envelope after incubation in a collagenase (type I, 1 mg/mL; Sigma) solution for 1 h using the procedure of Kusano et al. (*11*). The oocytes were microinjected with cRNAs in sterilized water and then incubated in modified Barth solution [88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.33 mM $Ca(NO_3)_2$. and 0.41 mM $CaCl_2$ in 5 mM Tris at pH 7.6] containing 25 mg/L penicillin and 50 mg/L streptomycin at 15−18 °C for 2−7 days before the electrophysiological measurements.

Electrophysiological Measurements. The membrane current of the receptors evoked by glucose or galactose was measured by the voltage clamping method with a voltage clamp amplifier (CEZ-1100; Nihon Kohden Kogyo, Tokyo, Japan). An oocyte was placed on the net of a small chamber (of ∼0.3 mL volume) and impaled with two microelectrodes filled with 3 M KCl, one for monitoring the membrane potential and the other for passing current for clamping the membrane potential, usually at −40 mV. The oocyte placed on the net was continuously perfused from the bottom with the normal frog Ringer solution by using a gravity feed system, usually at a flow rate of ∼2 mL/min (*12*).

Measurement of the SGLT1 Response. Glucose was dissolved in the normal frog Ringer solution. To examine the effect of the extract or tea components on the glucose-elicited response, each test compound was added to the solution. One or other of the solutions was selected by switching a cock in the flow system. The control response was obtained by perfusing glucose solution without any compound and was taken as 100%. The effect of a given compound on the response of SGLT1 was measured by using a mixture of glucose and the compound. In some cases, galactose was used instead of glucose. The measurement was repeated several times with the same oocyte, and control values were measured after every two or three measurements. Values of data were usually the means from four experiments. The oocyte was washed for 5 min in normal frog Ringer solution before the next measurement. Student's *t* test was used to evaluate the significance of differences between the mean values and the control values.

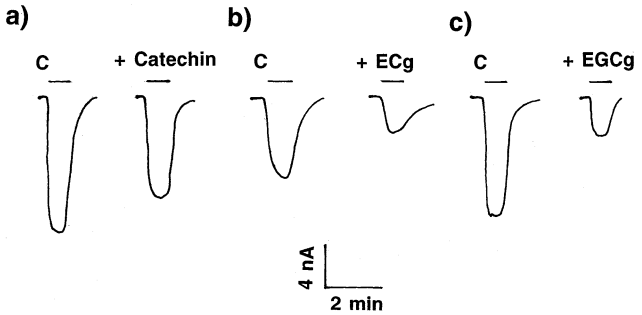

Figure 1. Effects of 0.5 mM (+)-catechin (a), 0.5 mM ECg (b), or 0.5 mM EGCg (c) on 0.5 mM glucose-elicited current of SGLT1 expressed in *Xenopus* oocytes. All traces were obtained with a voltage clamp (usually at –40 mV). An inward current is shown as a downward curve. The upper bars show when glucose or a mixture of glucose and the indicated compound was applied. Both responses in a given panel were obtained for the same injected oocyte, but the responses in panels a–c are for different injected oocytes. C, 0.5 mM glucose; + (+)-catechin, a mixture of 0.5 mM glucose and 0.5 mM (+)-catechin; + ECg, a mixture of 0.5 mM glucose and 0.5 mM ECg; + EGCg, a mixture of 0.5 mM glucose and 0.5 mM EGCg.

RESULTS

SGLT1 was expressed in *Xenopus* oocytes by injecting the cRNA synthesized from the cDNA for rat SGLT1 from a rat jejunum and used for examining the effects of phenol derivatives on SGLT1 response. **Figure 1** shows some typical membrane currents (∼10 nA) of SGLT1 elicited by glucose and the inhibition of these currents caused by (+)-catechin (a), ECg (b), and EGCg (c). The effects of some phenol derivatives on SGLT1 response caused by 0.5 mM glucose were measured and are shown in **Figure 2a**. The effects of some compounds found to influence the effect of glucose were also examined in oocytes exposed to 1 mM galactose (**Figure 2b**), because SGLT1 transports not only glucose but also galactose. These compounds caused similar effects on the responses induced by both glucose and galactose. Saponin from tea seeds at 0.02 mg/mL showed a slight inhibition (to 79.5 ± 4.7% of the control value for 0.5 mM glucose and to 75.7 ± 10.0% for 5 mM glucose, $p < 0.01$). Because bisphenol A inhibits the response of ionotropic γ-aminobutyric acid receptors expressed in *Xenopus* oocytes (*13*), its effect on the response of SGLT1 was also examined. However, it showed only a slight tendency to cause an inhibition of the response. Monophenol compounds such as acetylphenol showed no effects on the response (data not shown). Although inhibition of glucose uptake by tannic acid and chlorogenic acid was reported by Welsh et al. (*5*), these compounds did not inhibit the response of SGLT1 at the concentration of 0.01 mg/mL. Most polyphenol derivatives, especially saponin (>0.05 mg/mL) and tannic acid (>0.2 mg/mL), caused large electrical responses even in noninjected

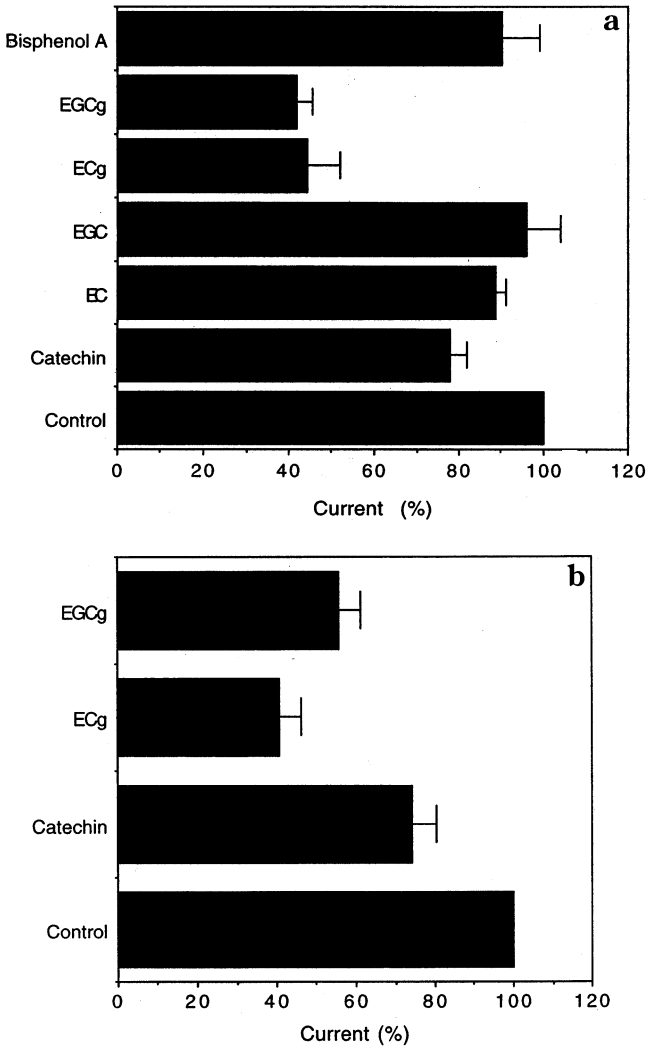

Figure 2. (a) Effects of various components at 0.5 mM on the response of SGLT1 elicited by 0.5 mM glucose. The response induced by 0.5 mM glucose without any other compound was taken to be 100%. $p < 0.05$ between the control and the value in the presence of the components [EGCg, ECg, EC and (+)-catechin] by Student's *t* test. (b) Effects of various components at 0.5 mM on the response of SGLT1 elicited by 1 mM galactose. The response induced by 1 mM galactose without any other compound was taken to be 100%. $p < 0.05$ between the control and the value in the presence of the components.

oocytes at high concentrations (**Figure 3**), possibly because they perturbed the oocyte membrane nonspecifically. ECg and (+)-catechin also caused a little current in noninjected oocyte (0.44 mg/mL ECg, 4.0 ± 0.9 nA; 0.58 mg/mL (+)-catechin, 0.48 ± 0.42 nA). Saponin and tannic acid induecd large nonspecific electrical responses in both cRNA-injected and noninjected oocytes, which prevented the sufficient assessment of the effect of these compounds on the SGLT1 response. Aqueous extracts of green tea, black tea, and oolong tea also caused membrane currents even in noninjected oocytes at high concentrations, so it was difficult to quantitate the effects of these extracts, which must contain polyphenol derivatives, on the SGLT1-mediated response. The diluted tea extract (0.5 mg of dry tea/mL), which did not cause nonspecific membrane current and was estimated to contain about 1 μM EGCg and 0.5 μM ECg from their contents in a dry tea and their extracted percentages in an aqueous solution (*1*, *2*), did not cause significant inhibition of the current elicited by 0.5 mM glucose.

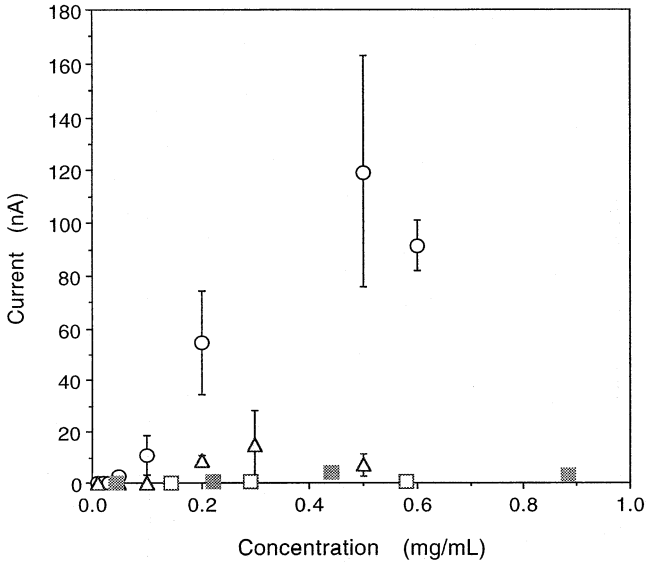

Figure 3. Nonspecific current in noninjected *Xenopus* oocyte caused by polyphenols. Peak currents were measured by applying various concentrations of saponin (○), tannic acid (△), (+)-catechin (□), and ECg (■) in frog normal Ringer solution to noninjected oocytes for 1 min.

Because (+)-catechin, ECg, and EGCg showed clear inhibition of the response of SGLT1, the dependence of the concentrations of these polyphenols and glucose on the inhibition was examined and is shown in **Figure 4**. It has been reported that these polyphenol derivatives inhibited the sodium-dependent glucose transporters of intestinal epithelial cells by a competitive mechanism (*6*). However, in the present case, the inhibition of the response of the Na^+/glucose transporter expressed in *Xenopus* oocytes was almost independent of the glucose concentration, suggesting a noncompetitive mechanism of the inhibition. The inhibition constant of (+)-catechin was estimated to be 2.3 mM, for which the noncompetitive mechanism was assumed. ECg and EGCg inhibited the response of SGLT1 similarly, and their inhibition constants were estimated to be ~0.45 mM.

DISCUSSION

It is known that polyphenols are present in tea, cocoa, or wine and are good for health because they act as antioxidants (*14*) when they are absorbed in the body. Reportedly, catechin derivatives reduce the lipid hydroperoxide level in the blood of rats or humans when they are added to food (*15*, *16*). They scavenged free radicals such as superoxide anion radicals, hydroxyl radicals, and lipid peroxyl radicals both in vivo and in vitro (*2*).

Inhibition of glucose uptake in the small intestine may be helpful for reducing the blood glucose level in diabetic patients. In fact, various herbs are commercially supplied as health-promoting dietary supplements to reduce patients' weight and maintain their health. It is most important to identify the components in ordinary foods in the diet that inhibit glucose uptake in the small intestine. Reportedly, some polyphenols that are present in teas inhibit glucose uptake in the small intestine, as shown using brush-border membrane vesicles or everted sacs of jejunum (*6*). Therefore, we examined their effects on glucose uptake mediated by SGLT1 expressed in *Xenopus* oocytes.

None of the monophenols examined caused any inhibition, but some of the polyphenols inhibited the response of SGLT1 expressed in *Xenopus* oocytes. As reported before (*6*), catechin

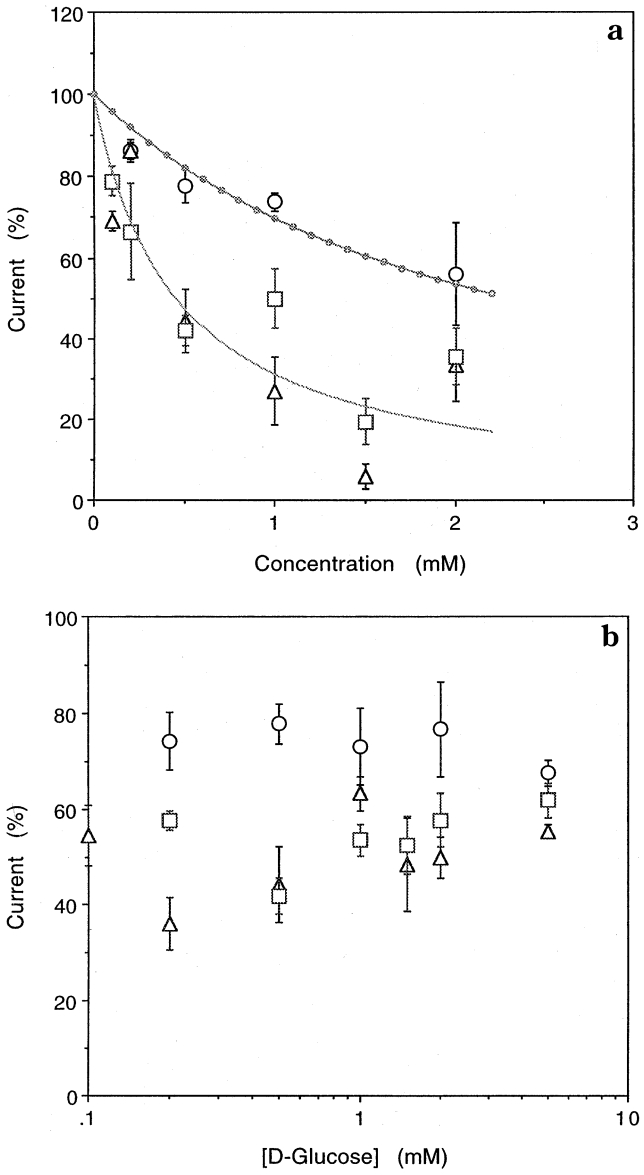

Figure 4. (a) Dose–response of inhibition by (+)-catechin (○), ECg (△), and EGCg (□). The effects of these compounds were measured in the presence of 0.5 mM glucose, and theoretical curves were drawn by using the noncompetitive inhibition constants of 2.3 mM for catechin and 0.45 mM for ECg and EGCg. The response induced by 0.5 mM glucose without any other compound was taken to be 100%. $p < 0.05$ between the control and the value in the presence of the component, by Student's *t* test. (b) Effects of glucose concentration on the inhibition of SGLT1 mediated-response in the presence of 0.5 mM (+)-catechin (○), ECg (△), and EGCg (□). The response induced by glucose without any other compound was taken to be 100%. $p < 0.05$ between the control and the value in the presence of the component, by Student's *t* test.

derivatives, especially ECg and EGCg, inhibited the response strongly and similarly, which indicates the importance of the galloyl residue in the inhibition of the SGLT1 response. However, the inhibition of SGLT1 response was independent of glucose concentration, suggesting a noncompetitive inhibition mechanism, in contrast to the findings reported by Kobayashi et al. (*6*), who proposed a competitive inhibition mechanism. It is difficult to explain the reason for the discrepancy regarding the inhibition mechanism in the two different experiments, that is, our measurement of SGLT1-electrical responses using the *Xenopus* oocyte expression system and glucose uptake using rabbit intestinal brush-border membrane vesicles studied by Kobayashi et al. (*6*). However, most reported competitive inhibitors of SGLT1 are substrate derivatives, that is, aglycons (*17*). ECg and EGCg, the major tea polyphenols with a triphenol structure, generally have relatively strong biological functions (*5*). These compounds are also reported to bind to the lipid bilayers with high affinity and to perturb the membrane structure (*18*). Therefore, it is unlikely that these compounds bind specifically to the glucose binding site in SGLT1. Thus, these catechin derivatives in teas, wine, or cocoa act possibly not only as antioxidants but also as inhibitors of glucose uptake in the small intestine, which may be helpful to diabetic patients. Because (+)-catechin inhibited glucose uptake weakly (K_i = 2.3 mM), it is unlikely that it suppresses glucose uptake in the small intestine under physiological condition (*6*). Tea catechin derivatives have also been reported to inhibit intestinal α-amylase or sucrase, which may be the main mechanism for the suppression of plasma glucose increase after a meal (*19*, *20*). Further biological experiments are necessary to clarify how catechin derivatives work as a dietary supplement.

The *Xenopus* oocyte expression system combined with electrophysiological measurement is very useful for examining the effect of various compounds on SGLT1 response, because *Xenopus* oocytes, which are globular with a diameter of >1 mm, are more stable, larger, and simpler in shape than epithelial cells in the small intestine, and therefore electrophysiological measurements of the transporters expressed in the oocytes can be made easily and repetitively for a long period. However, some compounds such as saponin, known to work as a surfactant, or tannic acid, used to tan animal skins, at high concentrations prevented glucose uptake measurements because of nonspecific current induced possibly by membrane perturbation, which was also observed in noninjected oocytes. Aqueous extracts of teas also induced some electrical responses in noninjected oocytes, which prevented exact measurements of the inhibition of the SGLT1 response by these extracts. Therefore, it remains necessary to fractionate the extracts before the application to the oocytes in order to identify the compounds in these extracts responsible for the effects on the SGLT1 responses. These nonspecific currents, possibly through membrane perturbation by these compounds, may destroy the membrane potential of epithelial cells in the small intestine and disturb the uptake of nutrients. In the future, it will be necessary to clarify whether saponin, tannic acid, and tea extracts also induce nonspecific current in epithelial cells of the stomach or small intestine under physiological conditions, which may be detrimental to health.

In conclusion, we showed inhibition of the response of SGLT1 expressed in *Xenopus* oocytes by some polyphenols and tried to clarify the mechanism of the inhibition at the molecular level. In the future, it will be necessary to examine whether glucose uptake in the small intestine is reduced when foods such as teas, chocolate, or wine, which contain these active polyphenols, are consumed. It is also necessary to clarify not only the dietary effect of polyphenols but also their toxicity to cells when they are supplied as a dietary supplement.

ABBREVIATIONS USED

Bisphenol A, 2,2-bis(4-hydroxyphenyl)propane; EC, (−)-epicatechin; ECg, (−)-epicatechin gallate; EGC, (−)-epigallocatechin; EGCg, (−)-epigallocatechin gallate; GABA, γ-aminobutyric acid; SGLT1, Na^+/glucose cotransporter (sodium-dependent glucose transporter).

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