Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical Pharmacology 70 (2005) 1560–1567
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Inhibitory modulation of ATP-sensitive potassium channels by gallate-ester moiety of (−)-epigallocatechin-3-gallate

Won-Ki Baek [b,1], Byeong-Churl Jang [c,1], Jun Hee Lim [d], Taeg-Kyu Kwon [d], Hyun-Young Lee [a], Chi-Heum Cho [e], Dae-Kwang Kim [e], Dong-Hoon Shin [e], Jong-Gu Park [c], Jeong-Geun Lim [e], Ji-Hyun Bae [f], Jae-Hoon Bae [a], Sun Kyun Yoo [g], Won-Kyun Park [a], Dae-Kyu Song [a,*]

[a] *Department of Physiology, Keimyung University School of Medicine, 194 Dongsan-Dong, Jung-Gu Daegu 700-712, Republic of Korea*
[b] *Department of Microbiology, Keimyung University School of Medicine, Daegu 700-712, Republic of Korea*
[c] *Department of Medical Genetic Engineering, Keimyung University School of Medicine, Daegu 700-712, Republic of Korea*
[d] *Department of Immunology, Keimyung University School of Medicine, Daegu 700-712, Republic of Korea*
[e] *Chronic Disease Research Center, Keimyung University School of Medicine, Daegu 700-712, Republic of Korea*
[f] *Department of Food Science and Nutrition, Keimyung University, Daegu 704-701, Republic of Korea*
[g] *Department of Oriental Medicine and Food Biotechnology, Joongbu University, Kumsan-gun, Chungnam 312-702, Republic of Korea*

Received 9 August 2005; accepted 9 September 2005

Abstract

(−)-Epigallocatechin-3-gallate (EGCG), a major polyphenolic substance found in green tea, is well recognized to be beneficial for human health. However, it is still controversial as to what dose of this compound is indeed good for human health. Though some recent studies have interestingly reported various beneficial effects of EGCG in cell culture system, however, plasma levels of EGCG attainable by oral regular intake in humans are normally in nanomolar range. However, potential side effects of EGCG when administered parenterally at higher concentration have not been thoroughly tested. Here, we evaluated the effect of EGCG on ATP-sensitive potassium (K_{ATP}) channels expressed in *Xenopus* oocytes. EGCG inhibited the activity of the Kir6.2/SUR1 and Kir6.2ΔC36 channels with IC_{50} of 142 ± 37 and 19.9 ± 1.7 μM, respectively. Inhibition of EGCG was also observed in Kir6.2/SUR2A or Kir6.2/SUR2B channels. Notably, (−)-epicatechin-3-gallate (ECG), another major polyphenolic substance in green tea, was found to reduce the channel activity with greater potency than EGCG. In contrast to EGCG and ECG, which have the gallic acid-ester moiety in their own structures, (−)-epigallocatechin and (−)-epicatechin exhibited very weak inhibition of the K_{ATP} channel. Collectively, these results suggest that the gallate-ester moiety of epicatechins may be critical for inhibiting the K_{ATP} channel activity via the pore-forming subunit Kir6.2 and this may be a possible mechanism by which green tea extracts or EGCG may cause unexpected side effects at micromolar plasma level.

Keywords: EGCG; ATP-sensitive potassium channel; Gallate-ester moiety; Kir6.2; *Xenopus* oocyte; Pancreatic β-cell

1. Introduction

Green tea (*Camellia sinensis*) is widely ingested, in part due to its known health benefits [1]. The *cis*-catechins are major biological components of green tea. These are characteristic polyphenolic compounds [2] that include (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG) and (−)-epicatechin (EC). Structurally, EGCG has both pyrogallol and gallate-ester moieties at the 2 and 3 positions of its ring, whereas EGC and ECG have only the pyrogallol moiety and the gallate-ester moiety, respectively (Fig. 1) [3,4].

EGCG is the main tea catechin, and its biological effect and molecular action mechanism has been widely studied.

* Corresponding author. Tel.: +82 53 250 7490; fax: +82 53 252 1605.
E-mail addresses: dksong@kmu.ac.kr, dksong@dsmc.or.kr (D.-K. Song).
[1] Authors equally contributed.

0006-2952/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2005.09.005

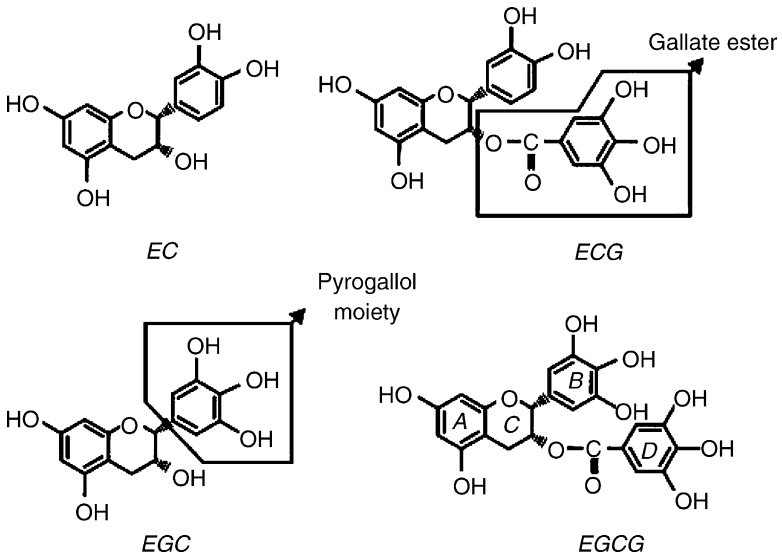

Fig. 1. Structure of epicatechins.

Many in vitro and ex vivo studies have revealed that EGCG and green tea have beneficial biological effects, such as inhibition of carcinogenesis [5,6], atherosclerosis [7] and lipogenesis [8], and treatment of diabetes [9], inflammation [10] and infections [11]. However, epidemiological studies in humans have not always shown positive results [12]. A possible major reason for this discrepancy appears to be related with the dose of catechins used in cell culture studies; many of the effects of green tea or EGCG are observed at much higher concentrations than can be attained in human plasma by regular oral intake of green tea [13,14]. Although there are considerable individual variations in plasma levels of EGCG after oral ingestion, it is unlikely that the concentration rises above 5 μM [15]. Unfortunately, this indicates that some of the benefits shown at higher concentrations (tens of micromolar) of EGCG in vitro are not directly relevant to the human body. In fact, some over-the-counter polyphenol supplements result in approximately 100-fold higher intake than the level ingested in a typical western diet [16]. Whereas EGCG has been emphasized on its positive effects, however, there are some concerned data showing that green tea catechins (1% or 0.1% of the diet for 33 weeks) implicate in tumor development in the colon of male F344 rats [17], and in the risk of iron depletion in humans with marginal iron status [18]. Despite the fact that membrane ion channels are central in signaling pathways controlling many biological functions [19], the effects of polyphenols on these channels are essentially little known. The ATP-sensitive K^+ (K_{ATP}) channel is one of K^+ channels in an octamer structure composed of two subunits (the sulfonylurea receptor (SUR) and the inwardly rectifying potassium channel (Kir)) and reported to be closely related with cell proliferation and cancer promotion [20,21].

In this study, we have evaluated whether EGCG interacts with membrane K_{ATP} channels. Here, we report that EGCG at micromolar concentrations inhibits the K_{ATP} channel activity via Kir6.2. Here, we report that EGCG at micromolar concentrations inhibits the K_{ATP} channel activity via Kir6.2. Considering that K_{ATP} channels are widespread in human tissues and share a common pore-forming subunit, Kir6.2, the present findings may suggest that any trial to evaluate the effects of EGCG at concentrations above the normal permissive plasma level (~1 μM) should be carefully taken.

2. Materials and methods

2.1. Molecular biology

Mouse Kir6.2 (GenBank D50581; [22]), rat SUR1 (GenBank L40624; [23]), rat SUR2A (GenBank D83598; [24]) and rat SUR2B (GenBank D86038; [25]) cDNAs were cloned in the pBF vector (kindly provided by Professor F.M. Ashcroft, University of Oxford, UK). Mutagenesis of individual amino acids was performed using the Altered Sites II System (Promega, Madison, WI). To obtain currents with Kir6.2 alone, a truncated form of Kir6.2 (Kir6.2ΔC36), in which the last 36 C-terminal amino acids were deleted, was generated by introducing a stop codon with site-directed mutagenesis. Capped mRNA was prepared using the mMESSAGE mMACHINE large-scale in vitro transcription kit (Ambion, Austin, TX) as previously described [26].

2.2. Oocyte collection

Female *Xenopus laevis* were anesthetized with MS222 (2 g/l added to the water, pH 7.4). One ovary was removed via a mini-laparotomy. The incision sutured and the animal was allowed to recover. All procedures were approved by

the Institutional Animal Care and Use Committee at the Dongsan Medical Institute for Life Sciences in Daegu, Korea. Immature stages V–VI oocytes were incubated for 60 min with 1.0 mg/l collagenase (type V; Sigma, St. Louis, MO) and their follicles were manually removed. Oocytes were co-injected with 0.1 ng of Kir6.2 or its mutant form and 2 ng of mRNA encoding SUR1, SUR2A or SUR2B. To express the Kir6.2ΔC36 channel, oocytes were injected with approximately 0.1 ng of its mRNA alone.

2.3. Oocyte electrophysiology

Patch electrodes were pulled from borosilicate glass (Harvard Apparatus, Kent, UK) and had resistances of 250–500 kΩ when filled with pipette solution. Macroscopic currents were recorded from giant excised inside-out patches at a holding potential of 0 mV. Currents were evoked by repetitive 3-s voltage ramps from −110 to +100 mV and recorded using a GeneClamp 500 patch-clamp amplifier (Axon Instruments, Forster, CA). The signals were filtered at 10 kHz, digitized at 1 kHz using a Digidata 1200 Interface and analyzed using pClamp8.2 software (Axon Instruments). The pipette (external) solution consisted of (mM): 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 10 HEPES, pH 7.4. The intracellular (bath) solution consisted of (mM): 107 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES, pH 7.2.

2.4. Oocyte data analysis

The conductance was determined from the slope of the plot of current versus voltage between −20 and −100 mV. The average slope from five consecutive ramps was calculated in each solution. The conductance in the presence of EGCG (G) was expressed relative to that in control solution before the application of drug (G_c). Dose–response curves for EGCG were fitted to the following equation:

$$\frac{G}{G_c} = \left[1 + \left(\frac{[\text{EGCG}]}{\text{IC}_{50}}\right)^h\right]^{-1} \quad (1)$$

where [EGCG] is the EGCG concentration; IC_{50} the concentration at which inhibition is half maximal; h is the Hill coefficient (slope factor).

2.5. Collection of single β-cells

Islets of Langerhans were isolated from the pancreas of male Sprague–Dawley rat by a collagenase digestion technique. Briefly, the animals were first anaesthetized with Nembutal. Collagenase (Type V; Sigma) dissolved at 1 mg/ml in Hank's Balanced Salts solution was transfused at the common bile duct retrogradely into the pancreatic ducts. The dissected pancreas was incubated for 15 min at 37 °C in a shaking water bath. The islets were placed into Krebs Ringer Bicarbonate Buffer (KRBB) solution containing 10% BSA, penicillin (100 U/ml) and streptomycin (0.1 mg/ml). To harvest single islet cells, the islets were further triturated and incubated in RPMI-1640 media with 11.1 mM glucose, 10% FBS and antibiotics in a humidified incubator at 37 °C with 5% CO_2 and balanced air.

2.6. Single β-cell electrophysiology

The inside-out configuration of the conventional patch-clamp technique was used. Patch pipettes were pulled from thin-walled borosilicate glass capillaries (World Precision Instruments Inc., Sarasota, USA), and had a resistance between 3 and 5 MΩ. Single islet cells on a cover glass were mounted on an inverted microscope (Axiovert 135; Carl Zeiss, Jena, Germany) and perfused with bath solution. The single channel currents were recorded using an Axopatch 200B patch-clamp amplifier (Axon Instruments, Forster, USA), passed onto an A/D converter (Digidata 1312; Axon), and later analyzed with Pclamp 8.2 software (Axon). Data were filtered at 1 KHz and sampled at 5 KHz. In inside-out experiments, cells were bathed in a solution composed of (mM) 107 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 10 EGTA, adjusted to pH 7.2 with NaOH. The pipette solution contained (mM) 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 10 HEPES, adjusted to pH 7.4 with KOH. Epicatechins were purchased from Sigma and dissolved in deionized water as a stock solution (10 mM) and then added at indicated concentrations in the bath solution just prior to the experiments.

2.7. Statistical analysis

Results are expressed as means ± S.E.M., and significance of differences was determined by ANOVA. $p < 0.05$ was considered significant.

3. Results

3.1. Direct effect of EGCG on K_{ATP} channel currents in oocyte or native β-cell membranes

We first added EGCG to the bath solution of the inside-out membrane patch to determine its direct effect on the β-cell-type K_{ATP} channel Kir6.2/SUR1 expressed in oocyte membranes (Fig. 2A). Intracellular EGCG inhibited the K_{ATP} channel current, however, there was incomplete channel recovery after washing out EGCG during the period of experiment, consistent with previous result with rat brain Kv1.5 channels [27]. The calculated IC_{50} for EGCG inhibition, as determined by Eq. (1), was 142 ± 37 μM, and the Hill coefficient (h) was 0.5 ± 0.1 (n = 5; Fig. 2B). EGCG also inhibited cardiac-type (Kir6.2/SUR2A) and smooth muscle-type (Kir6.2/SUR2B) K_{ATP}

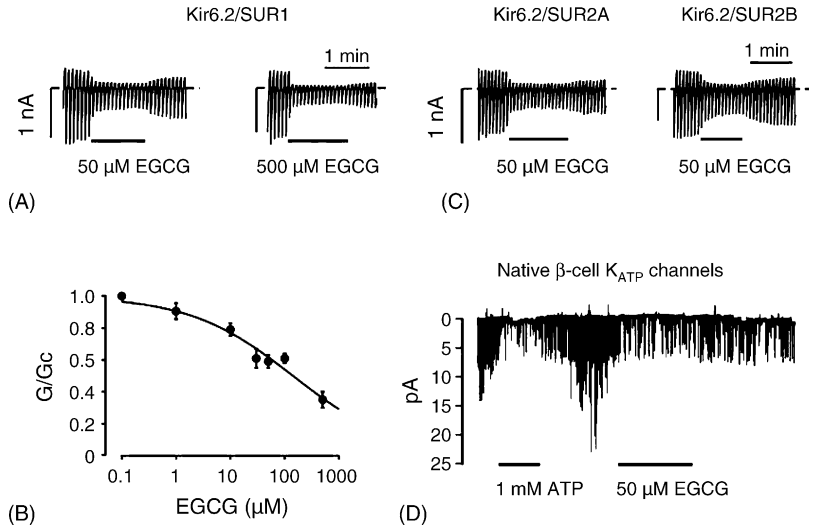

Fig. 2. Effect of EGCG on cloned and native K_{ATP} currents. Macroscopic currents recorded from inside-out patches in response to a series of 3-s voltage ramps from −110 to +100 mV from oocytes co-expressing Kir6.2 and either SUR1 (A), SUR2A or SUR2B (C). The dashed line indicates the zero current level. Holding potential is 0 mV. Downward deflections were inward currents. EGCG was added as indicated by the bars. (B) Concentration–inhibition relationship for blockage of Kir6.2/SUR1 currents by EGCG. The conductance in the presence of EGCG (G) was expressed relative to that in control solution before the application of drug (G_c). Each conductance was determined from the mean of five-consecutive slopes of the plot of current vs. voltage between −20 and −100 mV. The symbols represent the means ± S.E.M. IC_{50}: 142 ± 37 μM and h: 0.5 ± 0.1 (n = 5). (D) EGCG inhibition of K_{ATP} currents in a native β-cell. Inside-out patch at holding potential of −60 mV.

channels (Fig. 2C). To confirm that the inhibitory effect of EGCG on the cloned K_{ATP} channels also occurs in native K_{ATP} channels, EGCG (50 μM) was applied to internal side of plasma membrane in a native β-cell and also found to inhibit native K_{ATP} channels (Fig. 2D).

3.2. Effect of EGCG on Kir6.2ΔC36 channels

We next examined which subunit of the K_{ATP} channel interacts with EGCG by testing its effect on Kir6.2ΔC36 (Fig. 3). We found that EGCG also reduced the SUR-free Kir6.2 current, indicating that EGCG preferentially interacts with Kir6.2 to inhibit the channels. IC_{50} for the Kir6.2ΔC36 channel was 19.9 ± 1.7 μM, and h was 0.8 ± 0.1 (n = 4; Fig. 3B), which were higher than for the Kir6.2/SUR1 channel. When Kir6.2ΔC36 was co-expressed with SUR1, the inhibitory potency of EGCG became equivalent to that for the Kir6.2/SUR1 channel (Fig. 3C). This implies that the last 36 amino acids are not critical for the inhibitory effect of EGCG on the wild-type Kir6.2/SUR1 channel.

3.3. The block by EGCG of K_{ATP} channels is not voltage-dependent

Representative current traces before and after addition of EGCG for cloned Kir6.2/SUR1 and Kir6.2ΔC36 channels were shown in Fig. 4A and B. When current inhibition was expressed as a function of membrane potential (Fig. 4C), EGCG-induced inhibition of K_{ATP} currents was not significantly voltage-sensitive across the tested voltage range (from −80 to +60 mV).

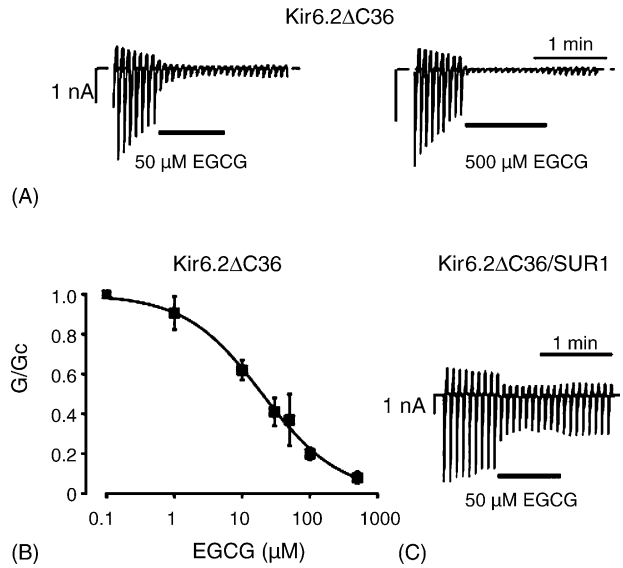

Fig. 3. Effect of EGCG on Kir6.2ΔC36 and Kir6.2ΔC36/SUR1 currents. Macroscopic currents recorded from inside-out patches in response to a series of 3-s voltage ramps from −110 to +100 mV from oocytes expressing Kir6.2ΔC36 alone (A) or in combination with SUR1 (C). The dashed line indicates the zero current level. Holding potential is 0 mV. Downward deflections were inward currents. EGCG was added as indicated by the bars. (B) Concentration–inhibition relationship for blockage of Kir6.2ΔC36 currents by EGCG. The conductance in the presence of EGCG (G) was expressed relative to that in control solution before the application of drug (G_c). Each conductance was determined from the mean of five-consecutive slopes of the plot of current vs. voltage between −20 and −100 mV. The symbols represent the means ± S.E.M. IC_{50}: 19.9 ± 1.7 μM; h: 0.8 ± 0.1 (n = 4).

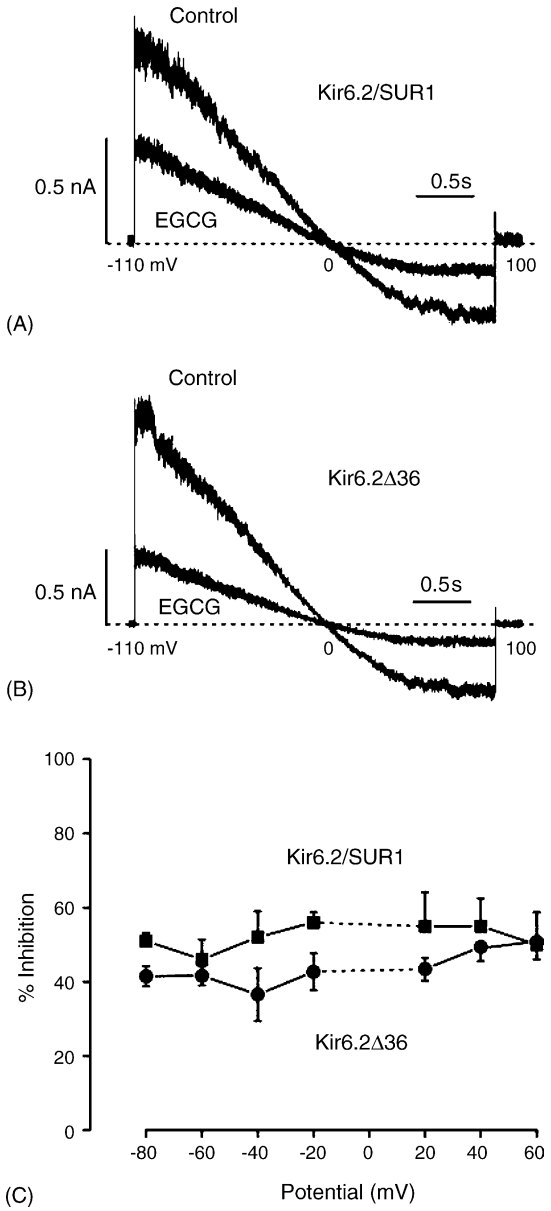

Fig. 4. Voltage independence in inhibition of cloned Kir6.2/SUR1 and Kir6.2ΔC36 currents by EGCG. The original current traces before and after the addition of 100 or 30 μM EGCG for Kir6.2/SUR1 (A) and Kir6.2ΔC36 (B) channels, respectively. Macroscopic currents were recorded from inside-out patches in response to a series of voltage ramps from −110 to +100 mV at holding potential of 0 mV. The dashed lines are zero current level. (C) Percentage inhibition expressed as a function of voltage by 100 or 30 μM EGCG for Kir6.2/SUR1 ($n = 4$) and Kir6.2ΔC36 ($n = 6$) currents, respectively. The symbols represent the means ± S.E.M.

3.4. The cytosolic regions of Kir6.2 may not be critical for EGCG inhibitory effect on K_{ATP} channels

To determine interaction sites on the cytosolic regions of Kir6.2 with EGCG, we used mutant Kir6.2 channels that have an amino acid substitution within the C- or N-terminus, thus having reduced response to ATP, which closes the channel through interaction with the cytosolic region of Kir6.2 [28]. Cloned channels that are composed of SUR1 and a mutant Kir6.2 were expressed in oocyte membranes. With a similar potency to the wild-type Kir6.2/SUR1 channels, intracellular application of EGCG (50 μM) inhibited the currents recorded from Kir6.2-E23K/SUR1, Kir6.2-R50G/SUR1, or Kir6.2-K185Q/SUR1 channels, but not Kir6.2-C166S/SUR1 and Kir6.2-T171A/SUR1 channels (Fig. 5A). Because the last two mutant channels themselves are known to have higher open probability than the wild-type and the other mutant channels [28], we tested much higher concentration of EGCG on those channels. Expectedly, Kir6.2-C166S/SUR1 and Kir6.2-T171A/SUR1 currents were inhibited by 500 μM of EGCG as potently as the wild-type and the other mutant channels at 50 μM of EGCG (Fig. 5B). These data suggest that EGCG does not interact with the cytoplasmic regions of Kir6.2 unlikely to ATP, which exclusively interacts with the C- and N-termini for the channel closure.

3.5. Gallic acid-ester moiety of EGCG is the most important for inhibition of K_{ATP} channels

In addition to EGCG, we tested the effects of other *cis*-catechins found in green tea on K_{ATP} channels (Fig. 6). At 50 μM, EC and EGC, which do not have the gallic acid-ester moiety at the 3-position of the C-ring, exhibited very weak inhibition of the Kir6.2/SUR1 channel. G/G_c ratio, which is the ratio of the conductance of the test condition to that of the control, was 1.0 ± 0.02 ($n = 5$) and 0.9 ± 0.02 ($n = 6$) for EC and EGC, respectively. However, ECG and EGCG, which all have the gallic acid-ester moiety, had G/G_c ratios of 0.2 ± 0.03 ($n = 6$) and 0.6 ± 0.03 ($n = 5$), respectively, suggesting that the gallic acid-ester moiety in EGCG structure is critical for the channel inhibition.

4. Discussion

In the present study, we showed that EGCG, a major component of green tea, inhibited the K_{ATP} channel widely present in human tissues. Interestingly, inhibition of EGCG was observed in all the three types of K_{ATP} channels. The IC_{50} for Kir6.2/SUR1 was 142 μM, which is close to the value observed for rat brain Kv1.5 channels (101 μM), the only previous finding on the effects of EGCG on ion channels [27]. Notably, inhibition of K_{ATP} channel by EGCG was more potent when the K_{ATP} channel was expressed in oocyte membranes without the SUR subunit (IC_{50} for Kir6.2ΔC36 ≈20 μM), thus indicating that EGCG acts on the pore-forming subunit Kir6.2, while SUR, the regulatory subunit, hampers the interaction.

Given that EGCG exerts its blocking action on Kir6.2 via intracellular side of the membrane, some residues accessible to the cytosolic solution are likely to be involved in the process. The H5 loop and part of transmembrane domain (TM) 2 may be expected to line the pore of Kir6.2

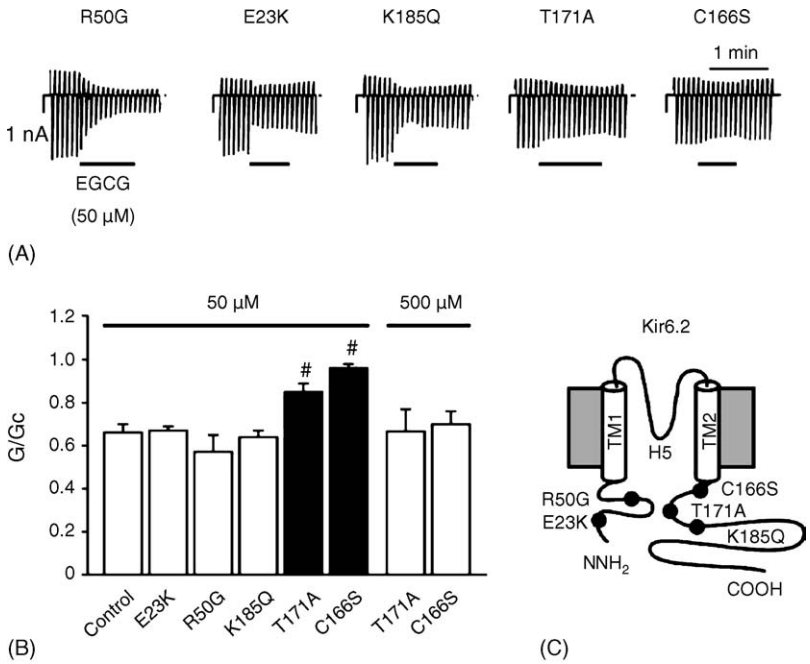

Fig. 5. Effect of EGCG on mutant Kir6.2/SUR1 currents. (A) Macroscopic currents recorded from inside-out patches in response to a series of 3-s voltage ramps from −110 to +100 mV from oocytes co-expressing SUR1 and a Kir6.2 mutant. The dashed line indicates the zero current level. Holding potential is 0 mV. Downward deflections were inward currents. EGCG was added as indicated by the bars. (B) Mean conductance of the mutant channel currents recorded in response to EGCG (50 or 500 μM). The conductance in the presence of EGCG (G) was expressed relative to that in control solution before the application of drug (G_c). Each conductance was determined from the mean of five-consecutive slopes of the plot of current vs. voltage between −20 and −100 mV. $^{\#}p < 0.05$ compared to the value of control. The symbols represent the means ± S.E.M. Numbers on each bar indicate the number of experiments. (C) Schematic diagram of Kir6.2 representing the sites mutated. TM: transmembrane domain.

[29]. Because they lie within the membrane voltage field and the block by EGCG is not voltage-sensitive (Fig. 4), it is doubtful that residues within these regions are related with the EGCG inhibition. We have also observed that among the channels with a mutation on the C- or N-terminus of Kir6.2 tested, only K_{ATP} channels containing either T171A or C166S mutation on the C-terminus significantly show reduced EGCG sensitivity compared with the wild-type channel. It is thought that both mutated channels exhibit spontaneously increased open probability, thereby attenuating the channel ATP sensitivity [28]. The channel sensitivity to ATP or sulfonylureas can also be reduced by membrane phospholipids, such as phosphatidylinositol bisphophate (PIP2), which increase the channel open probability [30]. From these points of view, it is hard to speculate that the two termini of Kir6.2 are critical for the EGCG inhibition.

This study rather showed that the order of inhibitory potency of the various epicatechins toward the K_{ATP} channel is ECG > EGCG ≫ EGC ≥ EC. It has been reported that the catechins directly interact with lipid bilayers and the affinity of tea catechins for lipid bilayers is mainly determined by the number of OH-group on the B-ring, the presence of a gallate-ester moiety on the D-ring, and the stereochemical structure of catechin molecules [31]. This is in agreement with our present finding showing that ECG is more potent than EGCG because the former has fewer B-ring OH-group than EGCG, which thus may support the idea that EGCG interacts preferentially with membrane lipids. This in turn may affect gating kinetics of the K_{ATP} channel embedded in the membrane, thereby reducing the channel open probability. Choi et al. also suggests that EGCG inhibits rKv1.5 channels through an interaction with lipid membrane, acting on the channel closing state and thereby reducing open probability [27]. Taken together, it implies that the gallate-ester moiety of EGCG may modulate various potassium channels through a common mechanism via lipid bilayer. A recent report has suggested that antifungal activity is stronger for pyrogallol catechins, such as EGCG and EGC, than for catechol catechins, such as ECG and EC [32]. According to this report, the antifungal effect was mediated via an amphotericin B-like action of pyrogallol moiety on the B-ring in damaging the cell membrane irrespective of the compound's lipophilicity. In addition, there is substantial evidence that catechins not only bind to the surface of membrane lipid bilayers but that they also perturb the membrane structure [31]. Therefore, we postulate that, albeit small, the pyrogallol moiety may also play a role in modulating channel activity. This may explain the slight but difference in potency between EGC and EC to modulate channel activity.

There are some discrepancies in the levels of human plasma EGCG reported in different studies. When green tea (20 mg/kg) was ingested orally in human volunteers, the maximal plasma concentration of EGCG was 0.17 μM

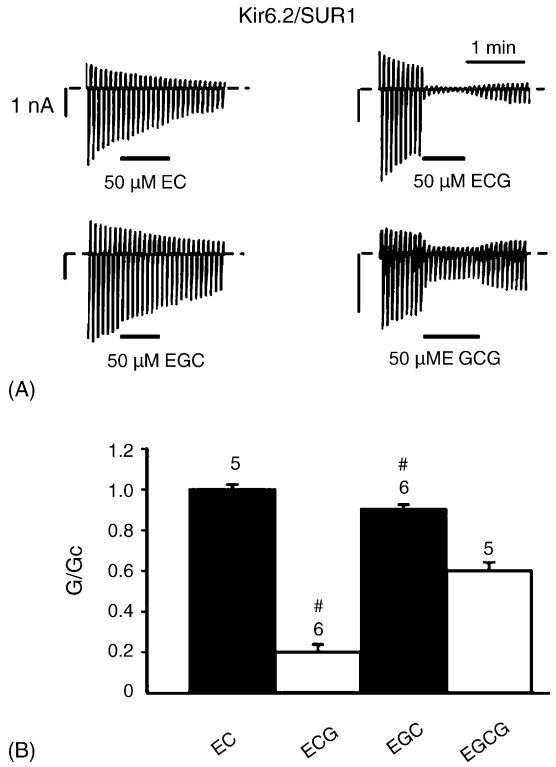

Fig. 6. Effects of various epicatechins on Kir6.2/SUR1 currents. (A) Macroscopic currents recorded from inside-out patches in response to a series of 3-s voltage ramps from −110 to +100 mV from oocytes co-expressing Kir6.2 and SUR1. The dashed line indicates the zero current level. Holding potential is 0 mV. Downward deflections were inward currents. EGCG was added as indicated by the bars. (B) Mean conductance of Kir6.2/SUR1 currents recorded in response to each epicatechin (50 μM). The conductance in the presence of each drug (G) was expressed relative to that in control solution before the application of drug (G_c). Each conductance was determined from the mean of five-consecutive slopes of the plot of current vs. voltage between −20 and −100 mV. $^{\#}p < 0.05$ compared to the value of epicatechin (EC). The symbols represent the means ± S.E.M. Numbers on each bar indicate the number of experiments.

[14]. This is equivalent to consuming up to about two cups of green tea or a single dose of approximately 200 mg of pure EGCG. Even considering variation between individuals, the plasma EGCG levels did not exceed 1 μM. In some cases, the plasma EGCG level could reach approximately 1 μM with a single oral dose of 800 mg of pure EGCG [33]. However, another group reported that oral intake of 525 mg EGCG in humans could attain plasma levels of EGCG up to 4.4 μM [15,34]. This discrepancy suggests that further studies are needed because the biological activities of EGCG depend considerably on the dose.

Given the low bioavailability of green tea catechins in humans [13], if the former finding is true, the modulation of K_{ATP} function by EGCG may not be expected during oral intake of green tea. However, if the latter finding is true or there is a parenteral trial to elevate the plasma EGCG level, there could be unexpected side-effects, including impaired GSIS, vascular dysfunction, cardiac conduction abnormalities and neuropsychiatric disturbances [35], considering that Kir6.2 subunit on which EGCG acts is widely expressed in human tissues. Thus, though plasma EGCG at nanomolar concentrations may not affect normal K_{ATP} channel function, however, it is likely that there may be inhibition of the K_{ATP} channel activity by EGCG at high concentrations as shown herein.

Acknowledgement

This work was supported by Grant No. R13-2002-028-01003-0 from the Basic Research Program of KOSEF.

References

[1] Tapiero H, Tew KD, Ba GN, Mathe G. Polyphenols: do they play a role in the prevention of human pathologies? Biomed Pharmacother 2002; 56:200–7.
[2] Graham HN. Green tea composition, consumption, and polyphenol chemistry. Prev Med 1992;21:334–50.
[3] Lambert JD, Yang CS. Mechanisms of cancer prevention by tea constituents. J Nutr 2003;133:S3262–7.
[4] Hirasawa M, Takada K. Multiple effects of green tea catechin on the antifungal activity of antimycotics against *Candida albicans*. J Antimicrob Chemother 2004;53:225–9.
[5] Yang CS, Maliakal P, Meng X. Inhibition of carcinogenesis by tea. Annu Rev Pharmacol Toxicol 2002;42:25–54.
[6] Setiawan VW, Zhang ZF, Yu GP, Lu QY, Li YL, Lu ML, et al. Protective effect of green tea on the risks of chronic gastritis and stomach cancer. Int J Cancer 2001;92:600–4.
[7] Miura Y, Chiba T, Tomita I, Koizumi H, Miura S, Umegaki K, et al. Tea catechins prevent the development of atherosclerosis in apoprotein E-deficient mice. J Nutr 2001;131:27–32.
[8] Wang X, Tian W. Green tea epigallocatechin gallate: a natural inhibitor of fatty acid synthase. Biochem Biophys Res Commun 2001;288:1200–6.
[9] Broadhurst CL, Polansky MM, Anderson RA. Insulin-like biological activity of culinary and medicinal plant aqueous extracts in vitro. J Agric Food Chem 2000;48:849–52.
[10] Han MK. Epigallocatechin gallate, a constituent of green tea, suppresses cytokine-induced pancreatic beta-cell damage. Exp Mol Med 2003;35:136–9.
[11] Amarowicz R, Pegg RB, Bautista DA. Antibacterial activity of green tea polyphenols against *Escherichia coli* K 12. Nahrung 2000;44:60–2.
[12] Arts IC, Hollman PC. Polyphenols and disease risk in epidemiologic studies. Am J Clin Nutr 2005;81:S317–25.
[13] Yang CS, Chen L, Lee MJ, Balentine D, Kuo MC, Schantz SP. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. Cancer Epidemiol Biomarkers Prev 1998;7:351–4.
[14] Lee MJ, Maliakal P, Chen L, Meng X, Bondoc FY, Prabhu S, et al. Pharmacokinetics of tea catechins after ingestion of green tea and (−)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidemiol Biomarkers Prev 2002;11:125–32.
[15] Miyazawa T. Absorption, metabolism and antioxidative effects of tea catechin in humans. Biofactors 2000;13:55–9.
[16] Mennen LI, Walker R, Bennetau-Pelissero C, Scalbert A. Risks and safety of polyphenol consumption. Am J Clin Nutr 2005;81:S326–9.
[17] Hirose M, Hoshiya T, Mizoguchi Y, Nakamura A, Akagi K, Shirai T. Green tea catechins enhance tumor development in the colon without effects in the lung or thyroid after pretreatment with 1,2-dimethylhydrazine or 2,2′-dihydroxy-di-*n*-propylnitrosamine in male F344 rats. Cancer Lett 2001;168:23–9.

[18] Temme EH, Van Hoydonck PG. Tea consumption and iron status. Eur J Clin Nutr 2002;56:379–86.
[19] Zhou Q, Kwan HY, Chan HC, Jiang JL, Tam SC, Yao X. Blockage of voltage-gated K^+ channels inhibits adhesion and proliferation of hepatocarcinoma cells. Int J Mol Med 2003;11:261–6.
[20] Klimatcheva E, Wonderlin WF. An ATP-sensitive K^+ current that regulates progression through early G1 phase of the cell cycle in MCF-7 human breast cancer cells. J Membr Biol 1999;171:35–46.
[21] Conti M. Targeting K^+ channels for cancer therapy. J Exp Ther Oncol 2004;4:161–6.
[22] Inagaki N, Gonoi T, Clement JP 4th, Namba N, Inazawa J, Gonzalez G, et al. Reconstitution of IK_{ATP}: an inward rectifier subunit plus the sulfonylurea receptor. Science 1995;270:1166–70.
[23] Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP 4th, Boyd AE 3rd, Gonzalez G, et al. Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science 1995; 268:423–6.
[24] Inagaki N, Gonoi T, Clement JP, Wang CZ, Aguilar-Bryan L, Bryan J, et al. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. Neuron 1996;16:1011–7.
[25] Isomoto S, Kondo C, Yamada M, Matsumoto S, Higashiguchi O, Horio Y, et al. A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. J Biol Chem 1996; 271:24321–4.
[26] Gribble FM, Ashfield R, Ämmälä C, Ashcroft FM. Properties of cloned ATP-sensitive K^+ currents expressed in *Xenopus* oocytes. J Physiol 1997;498:87–98.
[27] Choi BH, Choi JS, Min DS, Yoon SH, Rhie DJ, Jo YH, et al. Effects of (−)-epigallocatechin-3-gallate, the main component of green tea, on the cloned rat brain Kv1.5 potassium channels. Biochem Pharmacol 2001;62:527–35.
[28] Tucker SJ, Gribble FM, Proks P, Trapp S, Ryder TJ, Haug T, et al. Molecular determinants of K_{ATP} channel inhibition by ATP. EMBO J 1998;17:3290–6.
[29] Doyle DA, Cabral JM, Pfuetzner RA, Kua A, Gulbis JM, Cohen SL, et al. The structure of the potassium channel: molecular basis of K conduction and selectivity. Science 1998;280:69–77.
[30] Proks P, Reimann F, Green N, Gribble F, Ashcroft F. Sulfonylurea stimulation of insulin secretion. Diabetes 2002;51: S368–S376.
[31] Kajiya K, Kumazawa S, Nakayama T. Steric effects on interaction of tea catechins with lipid bilayers. Biosci Biotechnol Biochem 2001; 65:2638–43.
[32] Hirasawa M, Takada K. Multiple effects of green tea catechin on the antifungal activity of antimycotics against *Candida albicans*. J Antimicrob Chemother 2004;53:225–9.
[33] Chow HH, Cai Y, Alberts DS, Hakim I, Dorr R, Shahi F, et al. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. Cancer Epidemiol Biomarkers Prev 2001;10:53–8.
[34] Nakagawa K, Okuda S, Miyazawa T. Dose-dependent incorporation of tea catechins, (−)-epigallocatechin-3-gallate and (−)-epigallocatechin, into human plasma. Biosci Biotechnol Biochem 1997; 61:1981–5.
[35] Gribble FM, Davis TM, Higham CE, Clark A, Ashcroft FM. The antimalarial agent mefloquine inhibits ATP-sensitive K-channels. Br J Pharmacol 2000;131:756–60.