In Vitro Electrocardiographic and Cardiac Ion Channel Effects of (−)-Epigallocatechin-3-Gallate, the Main Catechin of Green Tea

Jiesheng Kang, Hsien Cheng, Junzhi Ji, Josephine Incardona, and David Rampe

Disposition, Safety, and Animal Research, Sanofi-Aventis US Inc., Bridgewater, New Jersey

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ABSTRACT

Epigallocatechin-3-gallate (EGCG) is the major catechin found in green tea. EGCG is also available for consumption in the form of concentrated over-the-counter nutritional supplements. This compound is currently undergoing clinical trials for the treatment of a number of diseases including multiple sclerosis, and a variety of cancers. To date, few data exist regarding the effects of EGCG on the electrophysiology of the heart. Therefore, we examined the effects of EGCG on the electrocardiogram recorded from Langendorff-perfused guinea pig hearts. EGCG had no significant effects on the electrocardiogram but prolonged PR and QRS intervals, slightly shortened the QT interval, and altered the shape of the ST-T-wave segment. The ST segment merged with the upstroke of the T wave, and we noted a prolongation in the time from the peak of the T wave until the end. Patch-clamp studies identified the KvLQT1/minK K+ channel as a target for EGCG (IC_{50} = 30.1 μM). In addition, EGCG inhibited the cloned human cardiac Na+ channel Na_{1.5} in a voltage-dependent fashion. The L-type Ca^{2+} channel was inhibited by 20.8% at 30 μM, whereas the human ether-a-go-go-related gene and Kv4.3 cardiac K+ channels were less sensitive to inhibition by EGCG. EGCG has a number of electrophysiological effects in the heart, and these effects may have clinical significance when multigram doses of this compound are used in human clinical trials or through self-ingestion of large amounts of over-the-counter products enriched in EGCG.

Green tea, prepared from the leaves of *Camellia sinensis*, is a popular beverage that is purported to have a number of beneficial health effects including antithrombotic, anti-inflammatory, and anticancer activities (Higdon and Frei, 2003; Wolfram, 2007; Clement, 2009). Green tea is rich in polyphenolic compounds known as catechins, and these catechins are believed to be responsible for the physiological activity of green tea and its extracts. The major catechin found in green tea is epigallocatechin-3-gallate (EGCG) (Fig. 1), which constitutes approximately 65% of the total catechins found in green tea (Balentine et al., 1997). On average, brewed green tea provides 78 mg of EGCG per cup (U.S. Department of Agriculture database for the flavonoid content of selected foods, release 2.1, http://www.nal.usda.gov/fnic/foodcomp/data/flav/flav.html). EGCG is also available for consumption in the form of concentrated extracts of green tea sold as over-the-counter nutritional supplements containing up to 200 to 400 mg of EGCG per dose. These concentrated preparations are used both as dietary supplements and in controlled human clinical trials (see http://www.clinicaltrials.gov).

A number of studies have examined the effects of EGCG on various biochemical pathways (Beltz et al., 2006; Chen et al., 2008; Tachibana, 2009). However, relatively few studies have been conducted to assess its effects on voltage-dependent ion channels. In one study performed in hippocampal neurons, it was shown that voltage-dependent Na+ channels were only weakly inhibited by EGCG with an IC_{50} value of approximately 200 μM (Deng et al., 2008). In contrast, another study in dorsal root ganglion cells found that the voltage-dependent Na+ current in this preparation was much more sensitive to EGCG, being inhibited by submicromolar concentrations (Kim et al., 2009). In vascular smooth muscle cells, EGCG produced a complex biphasic effect (both activation and inhibition) on the L-type Ca^{2+} channel (Campos-Toimil and Orallo, 2007), whereas Ca^{2+} channel currents in bovine chromaffin cells were unaffected by the compound (Pan et al., 2002). Finally, the human ether-a-go-go-related gene (HERG) cardiac K+ channel was shown to be inhibited by EGCG with...
Materials and Methods

Experimental procedures and protocols were approved by the sanofi-aventis Institutional Animal Care and Use Committee (Bridgewater, NJ) and conform to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Institute of Laboratory Animal Resources, 1996).

Langendorff Heart Preparation. The method used has been described previously (Cheng et al., 2006). Male guinea pigs, weighing 830 to 850 g, were used for this investigation. Under isoflurane anesthesia (4.5%), the heart was removed rapidly from the animal after isolation. The heart was perfused with vehicle (Locke's solution) and then with EGCG treatment data. A probability of

Cell Culture. Chinese hamster ovary cells (CHO) (American Type Culture Collection, Manassas, VA) were stably transfected with the cDNA encoding the human cardiac K+ channels HERG or KV10.1/minK as described previously (Rampe et al., 1997; Kang et al., 2000). CHO cells were stably transfected with cDNAs encoding the human cardiac Kv4.3 channel and Kv channel-interacting protein 2 with resistance to 4G18 and Zocerin (Invitrogen, Carlsbad, CA). CHO cells expressing these cardiac K+ channels were grown in Ham's F-12 media supplemented with 10% fetal bovine serum in an atmosphere of 95% air/5% carbon dioxide. The cDNA encoding SCN5A, the human cardiac Na+ channel (Na1.5), was stably transfected into human embryonic kidney cells (American Type Culture Collection) as described previously (Kurysh et al., 2000). Single ventricular myocytes were isolated from guinea pigs and used to record Ca2+ channel currents. Single ventricular myocytes were isolated from guinea pigs using a method modified from that described by Salata et al. (1995). In brief, male Hartley guinea pigs were anesthetized with 5% isoflurane (Baxter Healthcare, Deerfield, IL) in a mixture of nitrous oxide and oxygen (7:3). A thoracotomy was performed, and the heart was removed and immediately transferred to oxygenated cold saline. The heart was perfused retrogradely at 10 ml/min through the aorta with an oxygenated (100% oxygen) Ca2+-free saline at 36°C in three stages: first with standard Ca2+-free saline for 5 min, then with the same solution containing 143 U/ml type II collagenase (Worthington Biochemicals, Freehold, NJ) plus 0.6 U/ml type XIV protease (Sigma-Aldrich, St. Louis, MO) for 8 min, and finally with saline containing 0.2 mM CaCl2 for an additional 7 min. The left ventricle was cut into small pieces and was gently shaken at room temperature for approximately 2 min to disperse single myocytes. The isolated myocytes were then maintained at room temperature for electrophysiological recording within 4 to 5 h after isolation.

Patch-Clamp Recording. Unless stated otherwise, all ionic currents were recorded at 35 ± 1°C using the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981). Cells were exposed to EGCG until any observed effect on the currents came to equilibrium, generally about 3 to 4 min. Electrodes (1–3 MΩ resistance) were made from TW150F glass capillary tubes (WPI, Sarasota, FL). For HERG and Kv4.3 K+ channel recordings, electrodes were filled with the following solution: 120 mM potassium aspartate, 20 mM KC1, 4 mM sodium adenosine triphosphate, 5 mM HEPES, and 1 mM MgCl2 (pH 7.2 with KOH), and for KvLQT1/minK channel recordings, electrodes were filled with internal solution containing 120 mM potassium aspartate, 20 mM KC1, 4 mM Na2ATP, 5 mM HEPES, 1 mM MgCl2, 14 mM sodium phosphocreatine, 0.3 mM sodium GTP, 50 U/ml creatine phosphokinase (pH 7.2 with KOH). The external solution contained 130 mM NaCl, 4 mM KC1, 2.8 mM sodium acetate, 1 mM MgCl2, 10 mM HEPES, 10 mM glucose, and 1 mM CaCl2 (pH 7.4 with NaOH). The internal and external solutions for Na+ and Ca2+ channel recordings have been described previously (Kang et al., 2004). All ionic currents were recorded using an Axopatch 200B amplifier (Danaher Corporation, Sunnyvale, CA) and were analyzed using the Clampfit program within the pCLAMP suite of software (Danaher Corporation). IC50 values and 95% confidence limits were obtained by nonlinear least-squares fit of the data (GraphPad Software, Inc., San Diego, CA). All other data are expressed as the mean ± S.E.M.

Chemicals. EGCG was purchased from Sigma-Aldrich and was dissolved directly into the external solutions. EGCG was prepared fresh from powder just before all experiments because prolonged storage or freezing/thawing stock solutions appeared to cause degradation (e.g., brown discoloration of solutions). All other reagents were obtained from commercial sources.

Results

To establish whether EGCG has effects on cardiac electrophysiology, we first examined its activity on ECG waveforms.
measured in guinea pig hearts using the Langendorff method. At concentrations of 3 and 10 μM, no statistically significant changes were apparent in any of the ECG values measured (PR, QRS, QT, and Tp-Te intervals) (Table 1). At a concentration of 30 μM, the ECG waveform was clearly altered, and all of the measured parameters were significantly different from their corresponding control values. The most obvious difference noted in the ECG was a change in the ST-T-wave segment (Fig. 2). This change was characterized by an elevation in the area that approximates the J point and a merging of the ST segment with the upslope of the T wave (observed in three of five hearts). We also noted a slowing of some of the major voltage-dependent ion channels found in the myocardium. Figure 4 illustrates the activity of EGCG on HERG, the K+ channel that underlies the rapid delayed rectifier current, Ik, in the heart (Sanguinetti et al., 1995). Chinese hamster ovary cells stably expressing HERG were held at −90 mV, depolarized to +10 mV for 300 ms and then repolarized via a −0.5 V/s ramp back to −90 mV (stimulation frequency, 0.2 Hz). Peak outward currents during the repolarizing ramps were recorded in the absence and presence of EGCG (Fig. 4A). HERG was only weakly affected by EGCG. At a concentration of 30 μM, HERG currents were inhibited by 1.3 ± 2.4%, whereas at 100 μM, the highest concentration tested, inhibition measured 22.7 ± 6.6% (Fig. 4B). We also tested the effects of EGCG using a step-step protocol at room temperature identical to what we have described previously (Kang et al., 2004). Under these experimental conditions, 10 μM EGCG produced no inhibition of HERG current, whereas 30 μM produced a modest 13 ± 5% reduction (n = 5). Likewise, Kv4.3, the K+ channel that carries the transient out-

<table>
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<tr>
<th>Parameter</th>
<th>Control</th>
<th>3 μM</th>
<th>10 μM</th>
<th>30 μM</th>
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<tbody>
<tr>
<td>PR (ms)</td>
<td>61 ± 2</td>
<td>61 ± 2</td>
<td>60 ± 2</td>
<td>82 ± 6*</td>
</tr>
<tr>
<td>QRS (ms)</td>
<td>22 ± 1</td>
<td>22 ± 1</td>
<td>23 ± 1</td>
<td>27 ± 2*</td>
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<tr>
<td>QT (ms)</td>
<td>168 ± 2</td>
<td>168 ± 2</td>
<td>169 ± 2</td>
<td>158 ± 4*</td>
</tr>
<tr>
<td>Tp-Te (ms)</td>
<td>15 ± 3</td>
<td>14 ± 2</td>
<td>19 ± 4</td>
<td>39 ± 10*</td>
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* p < 0.01 vs. control.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>EGCG (30 μM)</th>
</tr>
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<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>177 ± 6</td>
<td>175 ± 7*</td>
</tr>
<tr>
<td>PR (ms)</td>
<td>67 ± 1</td>
<td>80 ± 1*</td>
</tr>
<tr>
<td>QRS (ms)</td>
<td>18 ± 2</td>
<td>21 ± 1*</td>
</tr>
<tr>
<td>QT (ms)</td>
<td>174 ± 2</td>
<td>168 ± 4</td>
</tr>
<tr>
<td>Tp-Te (ms)</td>
<td>16 ± 2</td>
<td>35 ± 4*</td>
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* p < 0.01 vs. control.
ward current in the human heart (Dixon et al., 1996), was little affected by EGCG. Kv4.3 currents were elicited by 300-ms depolarizing pulses to $-1100\text{mV}$ from a holding potential of $-90\text{mV}$ at a frequency of 0.2 Hz. Maximal inhibition of Kv4.3 measured 9.8 $\pm$ 5.0% at a concentration of $100\text{M}$ EGCG (Fig. 4, C and D).

Figure 5 shows the effects of EGCG on KvLQT1/minK, the $K^+$ channel that underlies the slow delayed rectifier current, $I_{Ks}$, in the heart. KvLQT1/minK was expressed in Chinese hamster ovary cells, and currents were measured at the end of a 300-ms test pulse to +10 mV from a holding potential of $-90\text{mV}$ at a frequency of 0.2 Hz. Maximal inhibition of Kv4.3 measured 9.8 $\pm$ 5.0% at a concentration of $100\text{M}$ EGCG (Fig. 4, C and D).

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Figure 6 illustrates the effects of EGCG on KvLQT1/minK currents recorded over a wide range of test potentials. Representative currents in the absence and presence of $30\text{M}$ EGCG are shown in Fig. 6, A and B, respectively. The resulting current-voltage (I-V) relationships, averaged from six cells, are presented in Fig. 6C. The percent inhibition of KvLQT1/minK by EGCG is plotted as a function of voltage in Fig. 6D. No correlation between voltage and drug effect was observed with inhibition ranging from 45.5 $\pm$ 7.3% at $30\text{mV}$ to 50.7 $\pm$ 5.3% at +30 mV ($p = 0.345$, one-way ANOVA).

The effects of EGCG on the cloned human cardiac Na$^+$ channel, Na$\text{a}_{1.5}$, are shown in Fig. 7. Currents were generated by 50-ms step depolarization to $-20\text{mV}$ from a holding potential of either $-90\text{mV}$ (Fig. 7A) or $-70\text{mV}$ (Fig. 7B) at a frequency of 0.2 Hz. Inhibition of Na$\text{a}_{1.5}$ by EGCG was voltage-dependent. The IC$_{50}$ for the EGCG block of Na$\text{a}_{1.5}$ was 24.6 $\mu$M (17.4–34.7 $\mu$M, 95% confidence limits) when measured from the $-70\text{mV}$ holding potential and $>100\text{M}$ (45.7% inhibition at $100\text{M}$) when measured at the $-90\text{mV}$
**Fig. 5.** EGCG inhibition of KvLQT1/minK K⁺ channel currents. A, KvLQT1/minK channel currents were recorded at a frequency of 0.2 Hz at 35°C using the indicated pulse protocol. The effects of 10, 30, and 100 μM EGCG are shown. B, dose-response relationship for EGCG inhibition of peak KvLQT1/minK channel currents. EGCG inhibited KvLQT1/minK with an IC₅₀ value of 30.1 μM (18.4–49.1 μM, 95% confidence limits). Error bars indicate S.E.M. (n = 6).

**Fig. 6.** Effects of membrane potential on EGCG inhibition of KvLQT1/minK channel currents. Cells were held at −90 mV and depolarized for 2 s to potentials ranging from −60 to +30 mV in 10-mV increments. Traces in the absence and presence of 30 μM EGCG are shown in A and B, respectively. C, peak KvLQT1/minK currents were normalized to those obtained after the +30 mV pulse in the absence of drug. The normalized current amplitudes are plotted as a function of test potential. Data in the absence (●) and after the addition of 30 μM EGCG (○) are shown. Error bars indicate S.E.M. (n = 6). D, inhibition of KvLQT1/minK currents is plotted as a function of test potential. No relationship between test potential and inhibition was observed. Error bars denote S.E.M. (n = 6).
holding potential. We also noted a slight slowing of inactivation, which was noted after exposure to 30 or 100 μM EGCG (Fig. 7, A and B). As was the case for KvLQT1/minK, the inhibitory effects of EGCG on Nav1.5 were not reversible on washout. Figure 7D illustrates the effects of EGCG on the voltage-dependence of Na⁺ channel inactivation. Cells were held at -90 mV, and conditioning prepulses (1-s duration) were applied from -130 to 30 mV in 10-mV increments; these were followed by a second pulse to -20 mV. Peak currents in the absence and presence of EGCG were recorded during the -20 mV pulses. The currents were normalized to the first pulse obtained in the absence of EGCG (i.e., after the -130 mV prepulse), and the mid-potentials (V₀.5) and slopes (k₀) of the curves were fit to a Boltzmann equation (I = Iₘₐₓ/[1 + exp((V₀.5 - Vₘ)/k₀)]). In the absence of EGCG, the V₀.5 value measured -64.7 ± 0.5 mV (n = 6). In the presence of EGCG, this value was significantly (p < 0.01 paired t test) different and measured -70.6 ± 1.8 mV (n = 6). The slope of the inactivation curve was not affected by EGCG measuring -4.9 in both the presence and absence of the drug.

Figure 8 shows the effects of EGCG on the L-type Ca²⁺ channel recorded from guinea pig myocytes. Currents were elicited by 300-ms step depolarizations to 0 mV from a holding potential of -40 mV at a frequency of 0.2 Hz. EGCG produced a dose-dependent inhibition of Ca²⁺ channel currents that reached a maximum of 37.1 ± 4.2% at a concentration of 100 μM (Fig. 8, A and B).

**Discussion**

The present study demonstrates that EGCG can interact with certain cardiac ion channels and can alter the ECG waveform measured in vitro. These activities occur at concentrations of approximately 10 to 30 μM. An important question is whether these levels of EGCG are observed clinically. Several clinical trials, in which EGCG is administered in gram quantities per day are ongoing (see http://www.clinicaltrials.gov). For example, in a recently published study conducted in patients with chronic lymphocytic leukemia, EGCG was orally administered at doses ranging from 400 to 2000 mg twice daily (Shanafelt et al., 2009). After 1 month of therapy, trough plasma levels (obtained 12 h after dosing) were measured and showed that some patients had EGCG plasma levels of approximately 10 μM (no electrocardiographic data were reported in this study). The plasma half-life of EGCG is approximately 3 h and approximately 80% of
EGCG exists in the plasma in the unbound form (Chow et al., 2001; Lee et al., 2002). Therefore, peak free plasma levels of at least 10 to 30 μM and possibly higher are likely to be obtained, especially when daily multigram doses of EGCG are used. These plasma levels of EGCG may not only be encountered in controlled clinical trials but could presumably be reached with the use, or misuse, of over-the-counter dietary supplements containing high levels of EGCG.

EGCG displayed a number of electrocardiographic effects in the Langendorff perfused heart. Although no QT prolongation was observed, there was a pronounced alteration of the ST-T-wave segment. This activity was characterized by a merging of the ST segment with the upstroke of the T wave as well as a prolongation of the Tp-Te interval. We presumed that these effects may be mediated, at least in part, by an interaction of EGCG with cardiac K+ channels, and so we first examined the effects of the drug on the HERG K+ channel. A previous study has reported that HERG is inhibited by EGCG with an IC50 value of 6 μM (Kelemen et al., 2007). Using either near-physiological temperatures and a sensitive step-ramp protocol (Kirsch et al., 2004) or a step-step protocol at room temperature, we were unable to confirm these results and indeed found that EGCG was only a weak inhibitor of HERG. Likewise, Kv4.3 channel currents were little changed by concentrations of EGCG up to 100 μM. Instead, we found EGCG to be an inhibitor of KvLQT1/minK channel currents. A previous study has reported that EGCG inhibited Nav1.5 in a dose-dependent manner and that this inhibition was enhanced at more depolarized holding potentials. Furthermore, EGCG shifted the mid-potential of the Nav1.5 steady-state inactivation curve to a more hyperpolarized potential. These data suggest that EGCG may preferentially interact with an inactivated state of the Na+ channel and that inhibition of cardiac Na+ channel currents underlies the observed prolongation of the QRS interval on the ECG. The effects of EGCG on L-type Ca2+ channel currents have been studied in vascular smooth muscle cells, in which a biphasic effect (initial stimulation followed by inhibition) was observed (Campos-Toimil and Orallo, 2007). We observed no stimulatory effect on the L-type Ca2+ channel measured in guinea pig heart cells. Instead, EGCG produced a modest dose-dependent inhibition of the L-type channel current that measured 21 and 37% at concentrations of 30 and 100 μM, respectively. We believe that this inhibitory effect on the L-type channel probably underlies the small prolongation in PR interval that we observed in the Langendorff heart preparation. Furthermore, the inhibitory effects of EGCG on Na+ and Ca2+ channels may serve to dampen any QT interval prolongation that might be expected to occur from the block of KvLQT1/minK. Finally, inhibition of these inward currents may lead to early repolarization and contribute to elevations in the J point and ST segment, leading to what has been described as J-wave syndrome (Antzelevitch and Yan, 2010).

In summary, this study has examined the effects of EGCG on the ECG waveform and its effects on the main voltage-dependent ion channels found in the myocardium. EGCG has numerous electrocardiographic effects that include changes in the ST-T-wave segment as well as widening of the QRS and PR intervals. These effects are probably due to blockade of several cardiac ion channels including KvLQT1/minK K+ channels as well as Na+ and Ca2+ channels. Although differences exist in the electrophysiology of the guinea pig heart and the human heart, the guinea pig Langendorff preparation has been widely used to accurately predict drug-induced ECG changes in humans (Cheng et al., 2006). Thus, the effects on cardiac ion channels and the resultant changes in
the ECG waveform observed in this study suggest some possible proarrhythmic potential for EGCG. In addition to direct cardiac effects, these findings also indicate the potential for EGCG to interact with other cardiovascular drugs including antiarrhythmic drugs, Ca\(^{2+}\) channel blockers, and \(\beta\)-receptor antagonists. We believe caution should be exercised when large, multigram quantities of EGCG are ingested, especially in the setting of underlying cardiovascular disease or when given concurrently with other cardiovascular drugs. These dose levels may be reached in clinical trials, or through the excessive use/abuse of over-the-counter products containing high levels of EGCG.

References