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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 (JK, HC, JJ, JI, DR), sanofi-aventis US Inc.,

Route 202-206, Bridgewater, NJ 08807 USA

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Corresponding Author: David Rampe

sanofi-aventis US Inc.

Mailstop JR2-203A

Route 202-206

Bridgewater, NJ 08807 USA

Tel: (908) 231-3078

Fax: (908) 231-2520

Email: david.rampe@sanofi-aventis.com

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Abbreviations: EGCG, (-)-epigallocatechin-3-gallate; ECG, electrocardiogram; CHO, Chinese hamster ovary; HERG, *human ether-a-go-go*-related gene.

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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, little data exists 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, and on cardiac ion channels using patch clamp electrophysiology. EGCG had no significant effects on the electrocardiogram at concentrations of 3 and 10 μM. At 30 μM, EGCG 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 Nav1.5 in a voltage-dependent fashion. The L-type Ca^{++} channel was inhibited by 20.8% at 30 μM while the K^+ channels HERG and Kv4.3 were less sensitive to inhibition by EGCG. ECGC has a number of electrophysiological effects in the heart and these effects may have clinical significance when multi-gram 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.

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Introduction

Green tea, prepared from the leaves of *Camillia sinesis,* 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 it is these catechins that 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, Figure 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 (US Department of Agriculture). 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 200-400 mg of ECGC per dose. These concentrated preparations are used both as dietary supplements as well as in controlled human clinical trials (see www.clinicaltrials.gov).

A number of studies have examined the effects of EGCG on various biochemical pathways (Beltz et al., 2006: Milacic et al., 2008; Tachibana, 2009). However, relatively few studies have been conducted to assess its effects on voltage-dependent ion channels. In one study carried out 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 sub-micromolar concentrations (Kim et al., 2009). In vascular smooth muscle cells, EGCG produced a complex biphasic effect (both activation and

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inhibition) on the L-type Ca^{++} channel (Campos-Toimil and Orallo, 2007) while Ca^{++} 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 an IC_{50} value of 6 μM (Kelemen et al., 2007). These studies suggest that voltage-dependent ion channels could, at least in some tissues, be pharmacological targets for the effects of EGCG. Nevertheless, with the exception of the study on HERG, little if anything is know about the effects of EGCG on voltage-dependent ion channels in the heart. For this reason we decided to examine the activity of EGCG on the electrocardiogram (ECG) waveform recorded from Langendorff-perfused guinea pig hearts. Following this we tested the effects of EGCG on individual voltage-dependent ion channels found in the myocardium.

Methods

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

Langendorff Heart Preparation. The method used has been described previously (Cheng et al., 2006). Male guinea pigs, weighing 830 to 850g, were used for this investigation. Under isofluorane anesthesia (4.5%), the heart was removed rapidly from the animal and was cannulated for perfusion of the coronary artery (70 cm H_2O) with Locke solution according to the Langendorff method. The heart was bathed in a 100 ml tissue chamber containing Locke's solution that was continuously bubbled with O_2 at 35^{o}C and had the following composition (in mM): NaCl, 153.6, KCl, 5.6, $CaCl_2$, 2.0, $NaHCO_3$, 6.0, dextrose, 11.1, Sodium pyruvate, 2.0, and Na_2EDTA, 0.05. *In vitro* equivalent lead II ECG waveforms were recorded via volume-conducted current using a ring electrode assembly (Cheng et al., 2006), and an ECG data acquisition system (Buxco Electronics, Inc., Wilmington, NC).

The heart was paced by using a pair of needle electrodes positioned in the right atrium near the SA node at 1 msec pulse duration, approximately 20% above threshold voltage at a rate of 210 beats/min and was allowed to equilibrate for 2 hours before drug testing. The heart was perfused with vehicle (Locke's solution), and then with 3, 10, and 30 μM of EGCG, for 20 minutes at each concentration. EGCG (30 μM) was also tested in spontaneously beating guinea pig hearts and its effects on ECG parameters were recorded for 20 minutes.

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AcqKnowledge 3.73 software (BIOPAC Inc, Goleta, CA) was used for analyzing ECG waveforms. The following ECG parameters were obtained from an average of 2-second segments of ECG recording (7 waveforms): PR, QRS, and QT intervals, and time of the peak to the end of T-wave (Tp-Te). These ECG parameters were analyzed for the control period (right before drug perfusion), and for EGCG treatment at the end of each 20 minutes perfusion period.

Data were expressed as mean and SEM. A one-way repeated measures ANOVA was used for analyzing ECG parameters, followed by Bonferroni t-test procedure for comparing control and post-EGCG treatment data. A probability of less than 0.05 was considered to be statistically significant.

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 KvLQT1/minK as previously described (Rampe et al., 1997; Kang et al., 2000). CHO cells were stably transfected with cDNAs encoding the human cardiac Kv4.3 channel and KCHIP2 (Kv Channel-Interacting Protein 2) with resistance to G418 and Zeocin (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 (Nav1.5), was stably transfected into human embryonic kidney cells (American Type Culture Collection) as described previously (Kuryshev et al., 2000). Single ventricular myocytes were isolated from guinea pigs and used to record Ca^{++} 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 thoractomy was

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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) Ca^{++}-free saline at 36°C in three stages: first with standard Ca^{++}-free saline for 5 min, then with the same solution containing 143 U/ml type II collegenase (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 $CaCl_2$ 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 3-4 minutes. 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 KCl, 4 mM di-sodium adenosine triphosphate, 5 mM HEPES, 1 mM $MgCl_2$, pH 7.2 with KOH, while for KvLQT1/minK channel recordings, electrodes were filled with internal solution containing 120 mM potassium aspartate, 20 mM KCl, 4 mM Na_2 ATP, 5 mM HEPES; 1 mM $MgCl_2$, 14 mM sodium phosphocreatine, 0.3 mM sodium GTP, 50 units/ml creatine phosphokinase (pH 7.2 with KOH). The external solution contained: 130 mM NaCl, 4 mM KCl, 2.8 mM sodium acetate, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, 1 mM $CaCl_2$, pH 7.4

with NaOH. The internal and external solutions for Na^+ and Ca^{++} channels 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). IC_{50} values and 95% confidence limits (95% C.L.) 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 (St Louis, MO) and was dissolved directly into the external solutions. EGCG was prepared fresh from powder just prior to all experiments since 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 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 (Figure 2). This 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 3/5 hearts). We also noted a slowing of the terminal phase of the T-wave (observed in all hearts tested). Since it was consistently seen in all hearts tested, we quantitated the effects of EGCG on the terminal phase of the T-wave by measuring the Tp-Te interval. The Tp-Te value measured 15 ± 3 ms under control conditions and 39 ± 10 ms following the addition of 30 μM EGCG ($p < 0.01$, ANOVA). Along with the alteration in the ST-T-wave segment, we also observed that PR and QRS interval durations were prolonged, and that the QT interval was slightly shortened (Table 1). In addition to this data, obtained in paced hearts, we also examined the effects of 30 μM EGCG in spontaneously beating hearts, mainly to assess its effects on heart rate. EGCG at 30 μM had no effect on heart rate (177 ± 6 bpm in control versus 175 ± 7 bpm after EGCG; $p>0.05$ ANOVA). All other ECG parameters were changed in a quantitatively similar manner as described for the paced heart. This includes altered ST-T-waves as described for the paced hearts (observed in 4/5 hearts, Figure 3) as well as all other

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measured ECG parameters (Table 2). Although notched P-waves were observed in all hearts treated with EGCG Figure 3), they were also present in 3/5 hearts prior to exposure to the drug.

We next set about to examine what effects EGCG had on 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, I_{Kr}, 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 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 (Figure 4A). HERG was only weakly affected by EGCG. At a concentration of 30 μM, HERG currents were inhibited by 1.3 ± 2.4% while at 100 μM, the highest concentration tested, inhibition measured 22.7 ± 6.6% (Figure 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 while 30 μM produced a modest 13 ± 5% reduction (n=5). Likewise Kv4.3, the K^+ channel that carries the transient outward 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 +10 mV from a holding potential of -90 mV at a frequency of 0.2 Hz. Maximum inhibition of Kv4.3 measured 9.8 ± 5.0% at a concentration of 100 μM EGCG (Figure 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

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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 mV (stimulation frequency, 0.2 Hz). EGCG produced a concentration-dependent inhibition of KvLQT1/minK currents (Figure 5A). The dose-response relationship for EGCG inhibition of KvLQT1/minK is shown in Figure 5B. EGCG inhibited KvLQT1/minK with an IC_{50} value of 30.1 μM (18.4 to 49.1 μM, 95% confidence limits). The effects of EGCG were not reversible upon washing the cells with drug-free solution.

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 μM EGCG are shown in Figures 6A and 6B, respectively. The resulting current-voltage (I-V) relationships, averaged from six cells, are presented in Figure 6C. The percent inhibition of KvLQT1/minK by EGCG is plotted as a function of voltage in Figure 6D. No correlation between voltage and drug effect was observed with inhibition ranging from 45.5 ± 7.3% at -30 mV to 50.7 ± 5.3 % at +30 mV (P=0.345, one-way ANOVA).

The effects of EGCG on the cloned human cardiac Na^+ channel, Nav1.5, are shown in Figure 7. Currents were generated by 50 ms step depolarization to -20 mV from a holding potential of either -90 mV (Figure 7A) or -70 mV (Figure 7B) at a frequency of 0.2 Hz. Inhibition of Nav1.5 by EGCG was voltage-dependent. The IC_{50} for EGCG block of Nav1.5 was 24.6 μM (17.4 to 34.7 μM, 95% C.L.) when measured from the -70 mV holding potential and >100 μM (45.7% inhibition at 100 μM) when measured at the -100 mV holding potential. We also noted a slight slowing of inactivation which was noted after exposure to 30 or 100 μM EGCG (Figure 7A and B). As was the case for

KvLQT1/minK, the inhibitory effects of EGCG on Nav1.5 were not reversible upon wash out. 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 mV to -30 mV in 10 mV increments and these were followed by a second pulse to -20 mV. Peak current 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 ($V_{0.5}$) and slopes (k) of the curves were fit to a Boltzman equation ($I=Imax/[1+exp((V_{0.5}-Vm)/k])$. In the absence of EGCG, the $V_{0.5}$ 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 affect 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 Ca^{++} 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 Ca^{++} channel currents that reached a maximum of 37.1 ± 4.2% at a concentration of 100 μM (Figures 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 10-30 μM. An important question is whether these levels of EGCG are observed clinically. Several clinical trials are ongoing where EGCG is administered in gram quantities per day (see 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 one month of therapy, trough plasma levels (obtained 12 hours after dosing) were measured and showed that some patients had EGCG plasma levels of approximately 10 μM (no electrocardiographic data was reported in this study). The plasma half life of EGCG is about 3 hours 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 10–30 μM, and possibly higher, are likely to be obtained, especially when daily multi-gram 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 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

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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 IC_{50} value of 6 μM (Kelemen et al., 2007). Employing 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. Block of KvLQT1/minK by EGCG was consistent throughout a wide range of voltages and reached approximately 50% at a concentration of 30 μM, the same concentration where the alterations in the ST-T-wave segment were noted. Some of the electrocardiographic changes observed in this study, including merged ST-T-wave segments and broad T-waves, have been documented in patients with congenital long QT syndrome type 1 wherein I_{Ks} is reduced due to mutations in KvLQT1 (Zhang et al., 2000). The present data demonstrate that KvLQT1/minK is a target for inhibition by EGCG and that this interaction may underlie some of the changes observed in the ST-T-wave segment of the ECG.

Other electrocardiographic changes induced by EGCG included prolongations of the QRS-interval and the PR-interval. Since the QRS-wave is dependent upon Na^+ channel activity, we examined the effects of EGCG on the cloned human cardiac Na^+ channel Nav1.5. We found 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 Ca^{++} channel currents have been studied in vascular smooth muscle cells where a biphasic effect (initial stimulation followed by inhibition) was observed (Campos-Toimil and Orallo, 2007). We observed no stimulatory effect on the L-type Ca^{++} 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 likely underlies the small prolongation in PR-interval that we observed in the Langendorff heart preparation. Furthermore, the inhibitory effects of EGCG on Na^+ and Ca^{++} channels may serve to dampen any QT-interval prolongation that might be expected to occur from 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 likely due to blockade of several cardiac ion channels including KvLQT1/minK K^+ channels as well as Na^+ and Ca^{++} 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 anti-arrhythmic drugs, Ca^{++} channel blockers, and β-receptor antagonists. We believe caution should be exercised when large, multi-gram 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.

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Legends for Figures

Figure 1. Chemical structure of EGCG [(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl] 3,4,5-trihydroxybenzoate.

Figure 2. Effects of EGCG on ECG waveforms recorded from paced guinea pig hearts. ECG tracing under control conditions and after 20 min exposure to 30 μM EGCG. Hearts were paced at 210 beats per minute and the stimulation artifact, P wave, QRS complex, and T wave are indicated.

Figure 3. Effects of EGCG on ECG waveforms recorded from spontaneously beating guinea pig hearts. ECG tracings under control conditions and 20 min after exposure to 30 μM EGCG are shown.

Figure 4. Effects of EGCG on HERG and Kv4.3 K^+ channel currents. A, Whole-cell HERG channel currents were elicited at a frequency of 0.2 Hz and were recorded at 35ºC using the step-ramp protocol shown. The effects of 30 and 100 μM EGCG are indicated. B, Dose-response relationship for EGCG inhibition of HERG. EGCG was a weak inhibitor of HERG producing a maximal 22.7 ± 6.6% inhibition at 100 μM. Error bars denote SEM (n=6). C, Kv4.3 channel currents were recorded at a frequency of 0.2 Hz at 35ºC using the pulse protocol shown. The effects of 30 and100 μM EGCG are illustrated.

D, Dose-response relationship for EGCG inhibition of Kv4.3. EGCG produced 9.8 ± 5.0% inhibition at a concentration of 100 μM. Error bars indicate SEM (n=6).

Figure 5. EGCG inhibition of KvLQT1/minK K^+ channel currents. A, KvLQT1/minK channel currents were recorded at a frequency of 0.2 Hz at 35ºC 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 IC_{50} value of 30.1 μM (18.4 to 49.1 μM, 95% confidence limits). Error bars indicate SEM (n=6).

Figure 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 panels 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 (filled circles) and after the addition of 30 μM EGCG (open circles) are shown. Error bars indicate SEM (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 SEM (n=6).

Figure 7. EGCG inhibition of human cardiac Na^+ channel ($Na_v1.5$) currents. $Na_v1.5$ currents were recorded at a frequency of 0.2 Hz at 35ºC using the pulse protocol

indicated in the Figure. The holding potential was set at -90 mV (A) or -70 mV (B) and the effects of 10, 30, and 100 μM are shown. C, dose-response relationships for EGCG inhibition of $Na_v1.5$ channel currents at holding potentials of -70 mV (filled circles) or -90 mV (open circles). The IC_{50} value for EGCG inhibition of $Na_v1.5$ at the -70 mV holding potential measured 24.6 μM (17.4 to 34.7 μM 95% confidence limits). The IC_{50} value of EGCG measured at the -90 mV holding potential approximated 100 μM (45.7 ± 6.9% inhibition at 100 μM). D, voltage-dependent inactivation of $Na_v1.5$ in the absence (filled circles) and presence (open circles) of 30 μM EGCG. Inactivation curves were generated as described in Results and fit to a Boltzman equation. In the absence of EGCG the midpotential ($V_{0.5}$) measured -64.7 mV while in the presence of EGCG $V_{0.5}$ measured -70.6 mV. Error bars indicate SEM (n=6).

Figure 8. Effects of EGCG on L-type Ca^{++} channel currents. A, L-type Ca^{++} channel currents from guinea pig myocytes were recorded at 35ºC at a frequency of 0.2 Hz according to the pulse protocol indicated in the Figure. The effects of 30 and 100 μM EGCG are shown. B, Dose-response relationship for EGCG inhibition of L-type Ca^{++} channel current. EGCG produced 37.1 ± 4.2% inhibition at a concentration of 100 μM. Error bars indicate SEM (n=6).

Tables

Table 1. Effects of EGCG on ECG parameters measured in isolated paced guinea pig hearts perfused according to the Langendorff method.

Parameter	Control	3 µM	10 µM	30 µM
PR (ms)	61 ± 2	61± 2	60 ± 2	82 ± 6**
QRS (ms)	22 ± 1	22 ± 1	23 ± 1	27 ± 2**
QT (ms)	168 ± 2	168 ± 2	169 ± 2	158 ± 4**
Tp-Te (ms)	15 ± 3	14 ± 2	19 ± 4	39 ± 10**

Data are expressed as the mean ± SEM (n=5). ** $p<0.01$ vs control

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Table 2. Effects of EGCG (30 μM) on ECG parameters measured in isolated spontaneously beating guinea pig hearts perfused according to the Langendorff method.

Parameter	Control	EGCG (30 μM)
Heart Rate (beats per min)	177 ± 6	175 ± 7
PR (ms)	67 ± 1	80 ± 1**
QRS (ms)	18 ± 2	21 ± 1**
QT (ms)	174 ± 2	168 ± 4
Tp-Te (ms)	16 ± 2	35 ± 4**

Data are expressed as the mean ± SEM (n=5). ** $p<0.01$ vs control

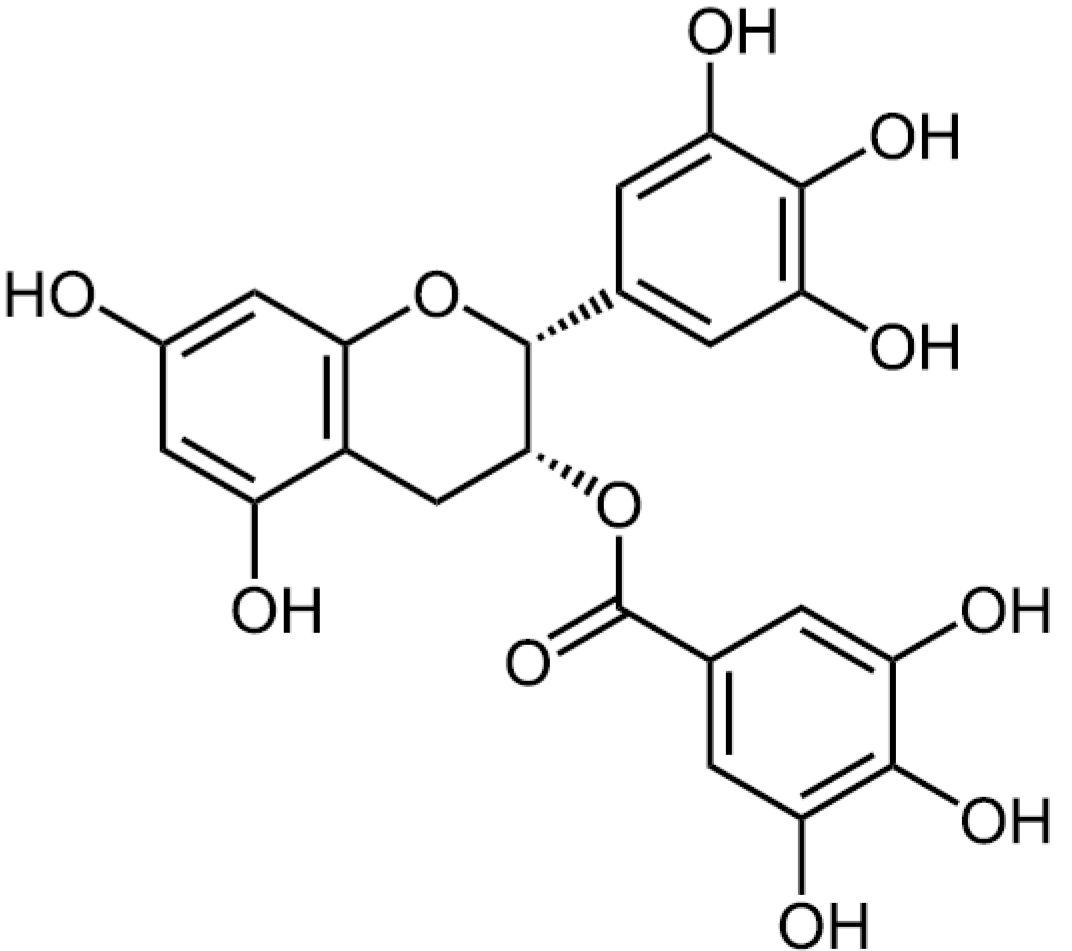
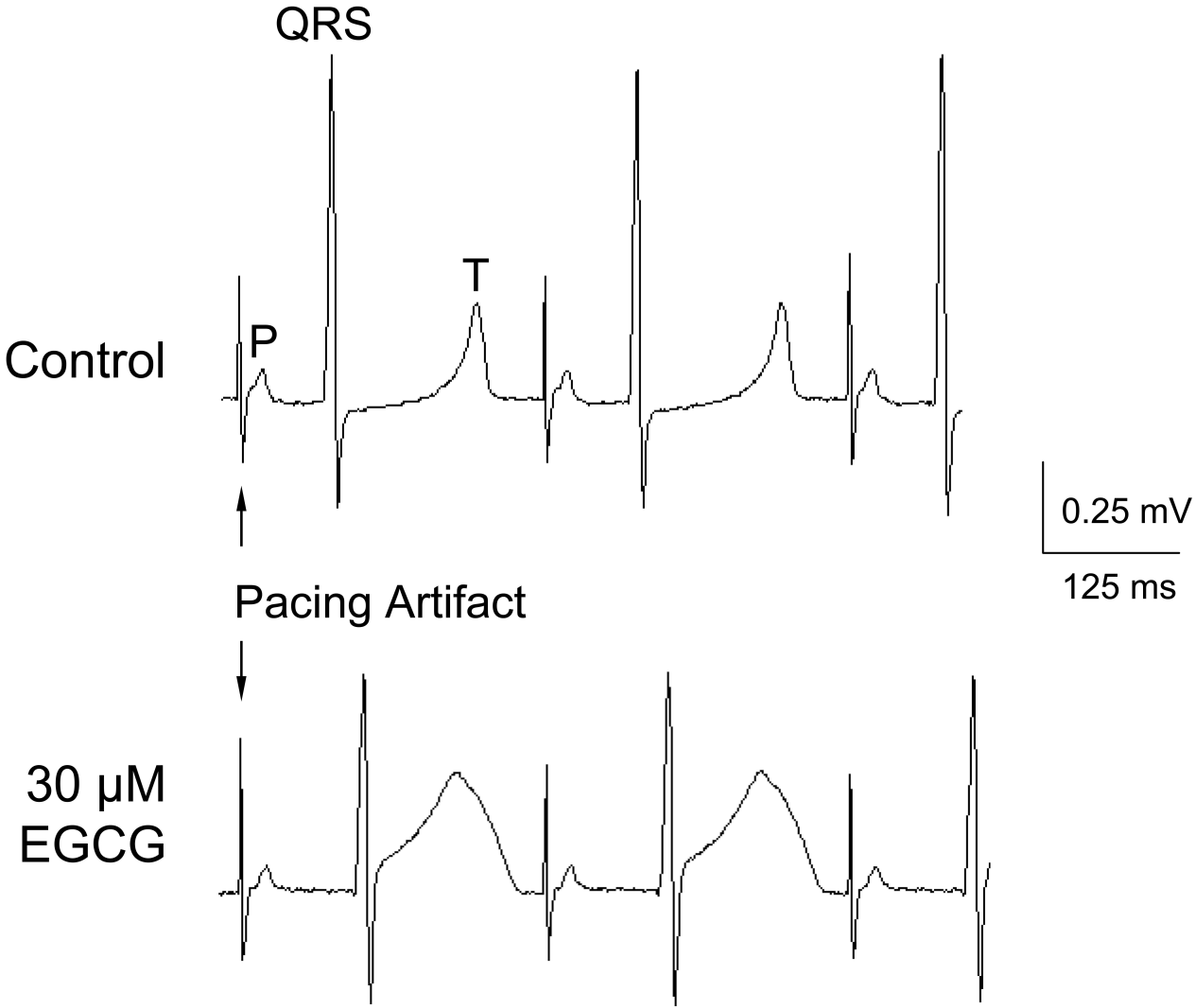


Figure 1



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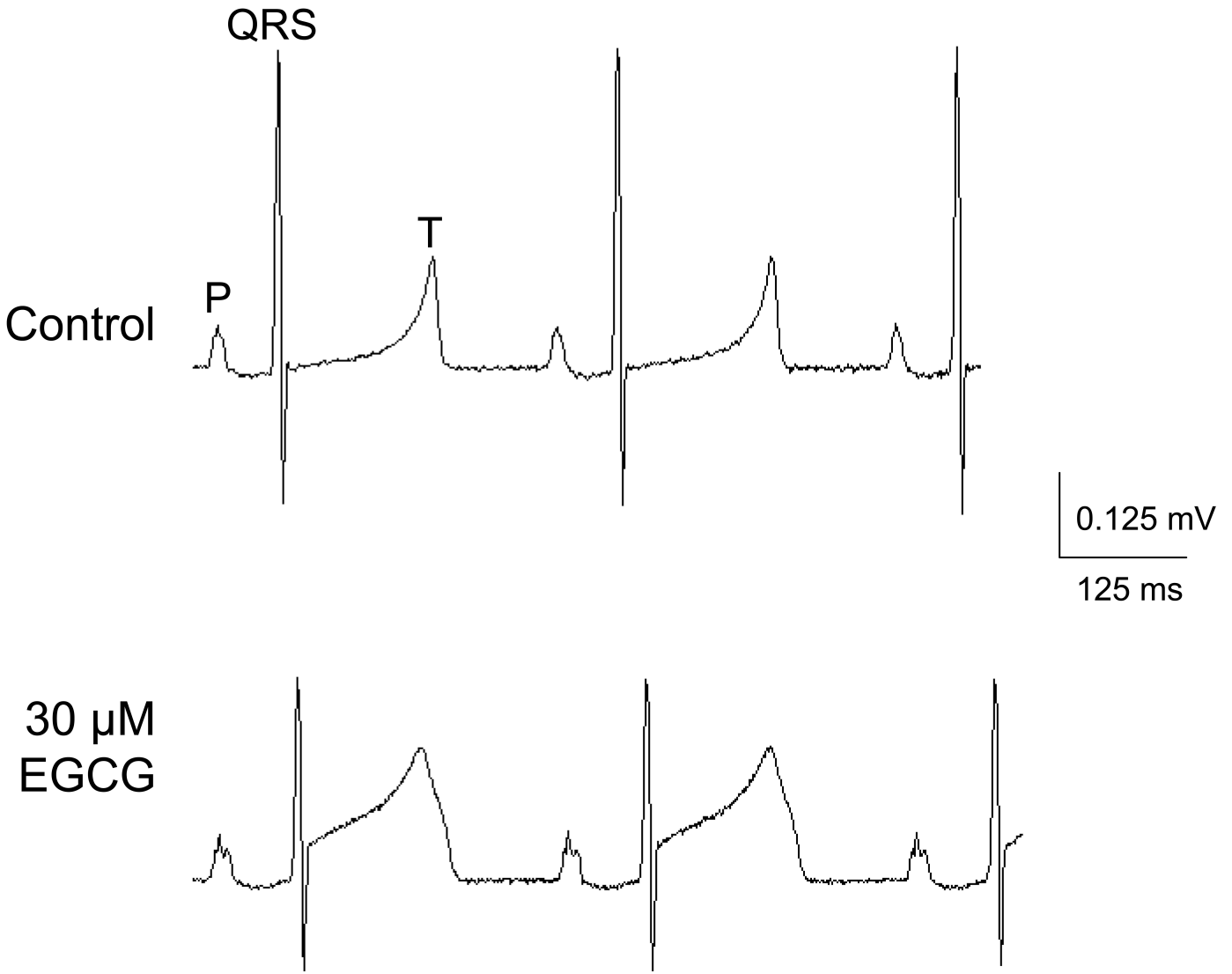

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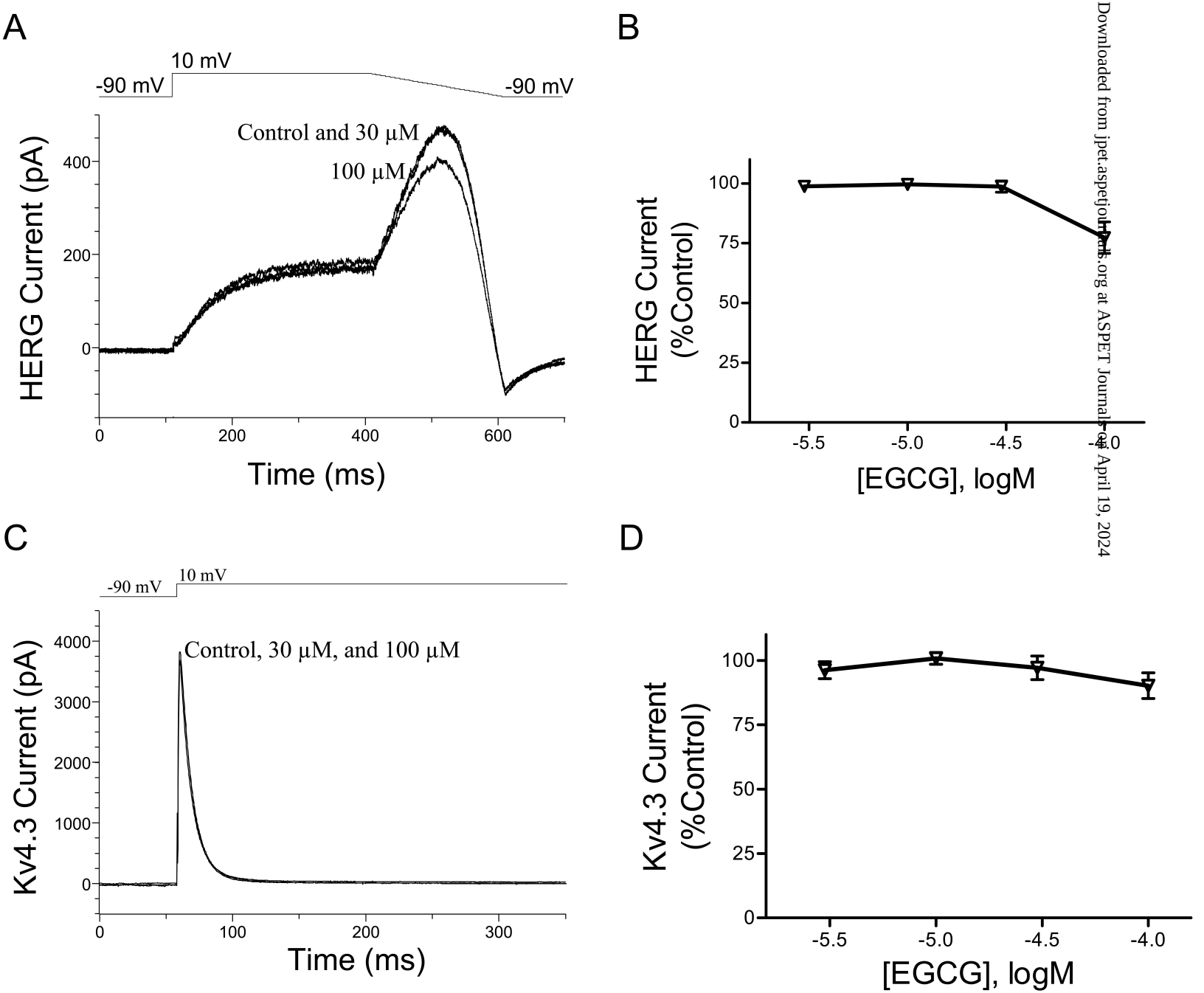

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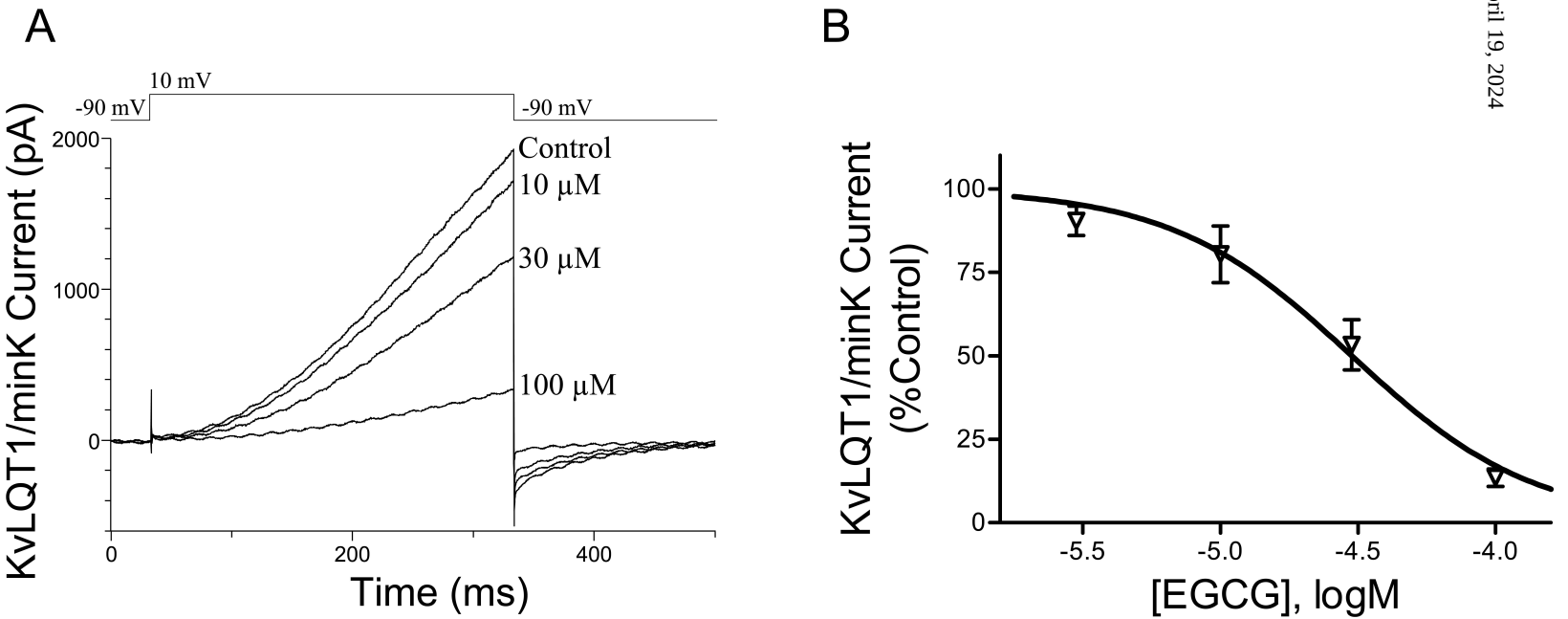

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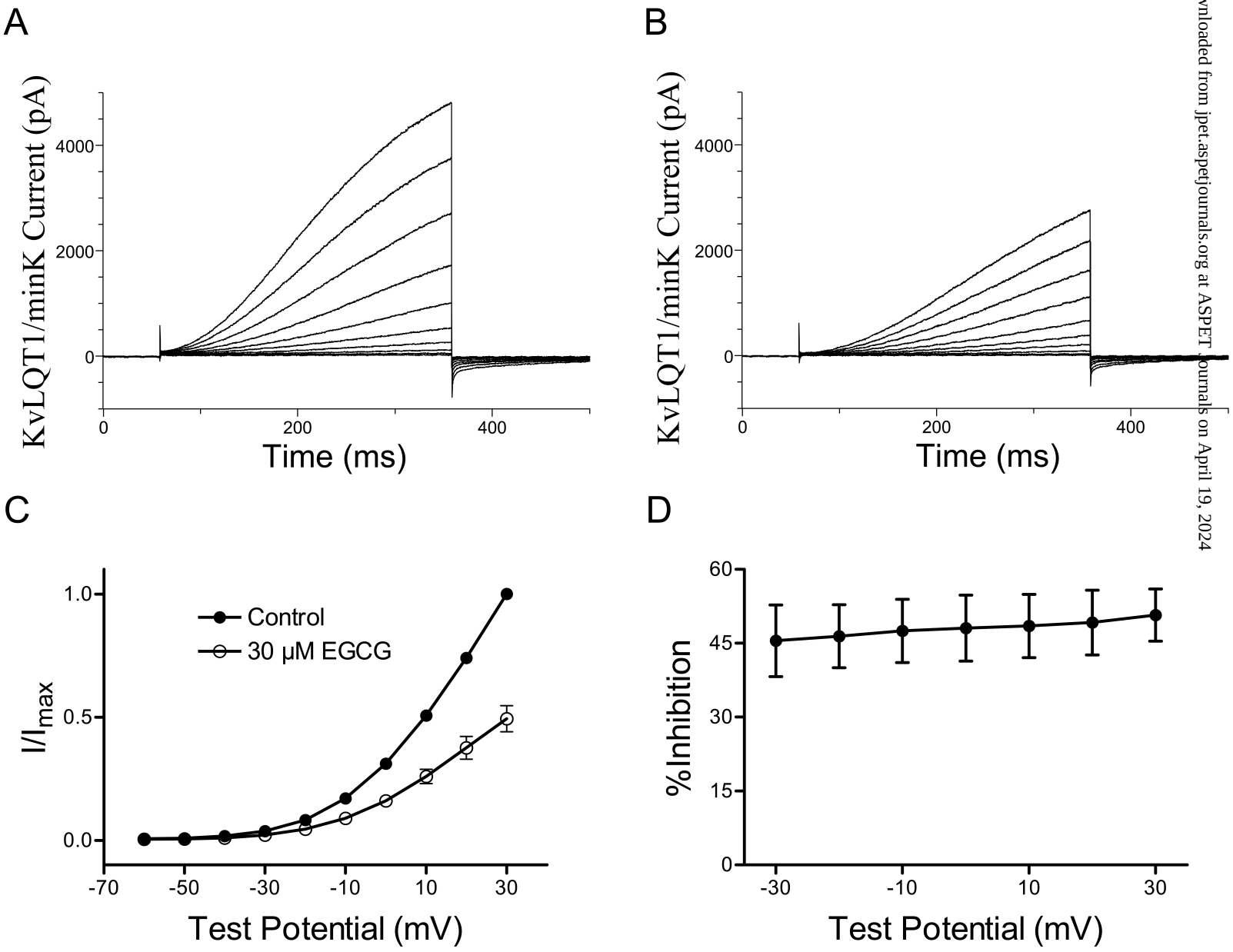

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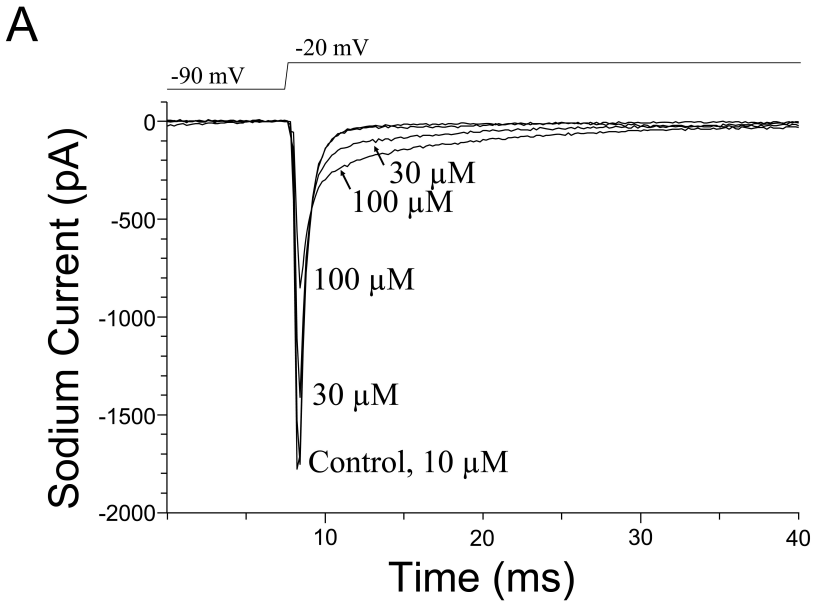
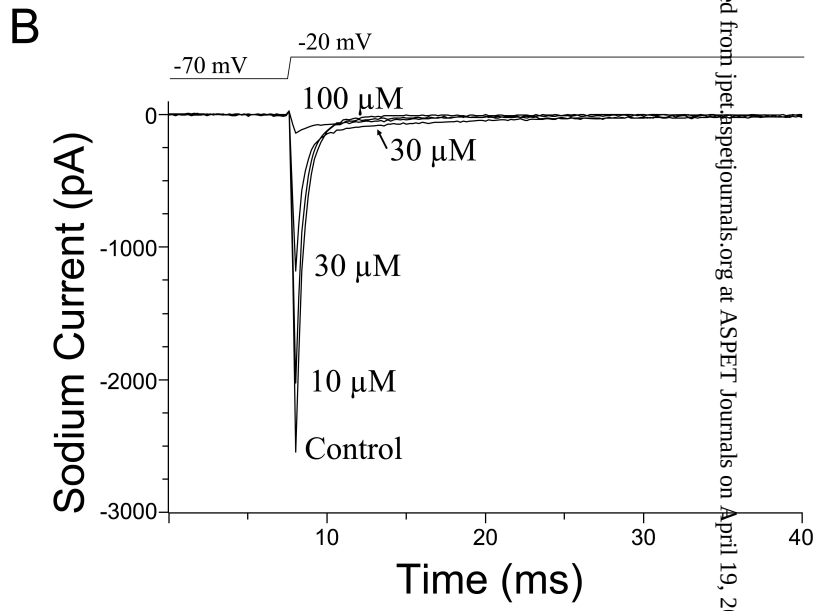
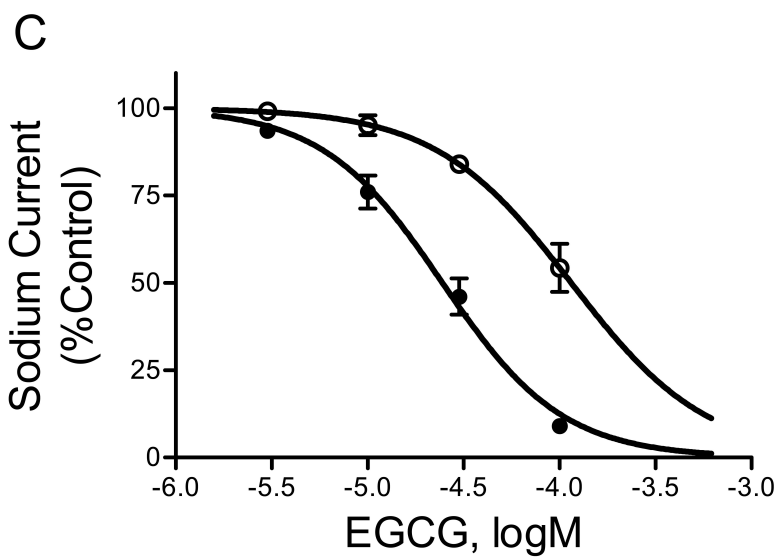






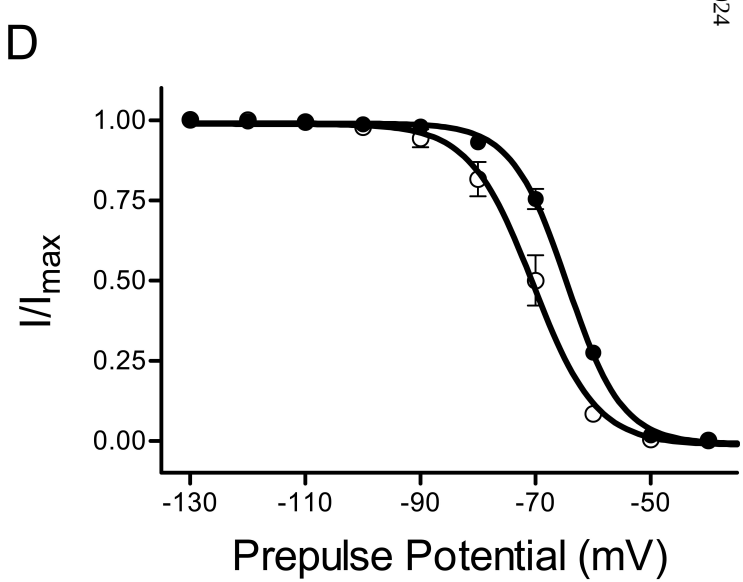
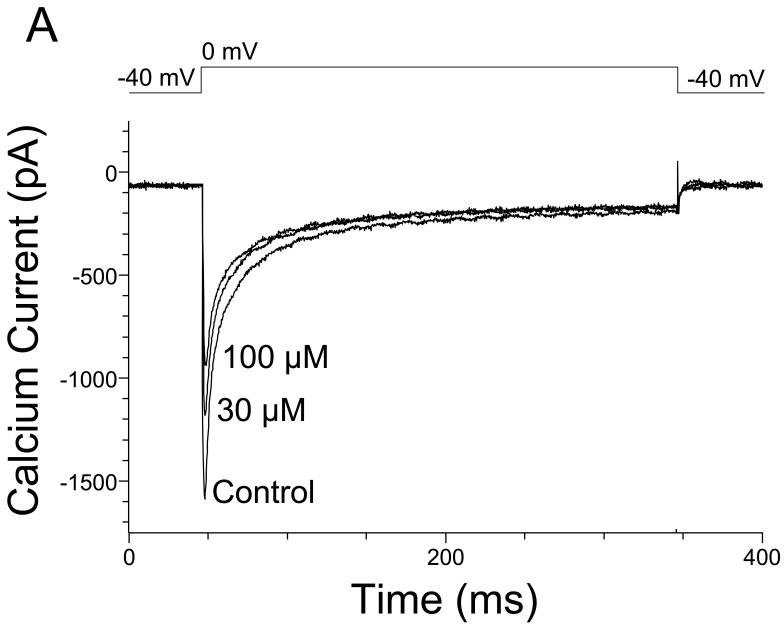


Figure 7



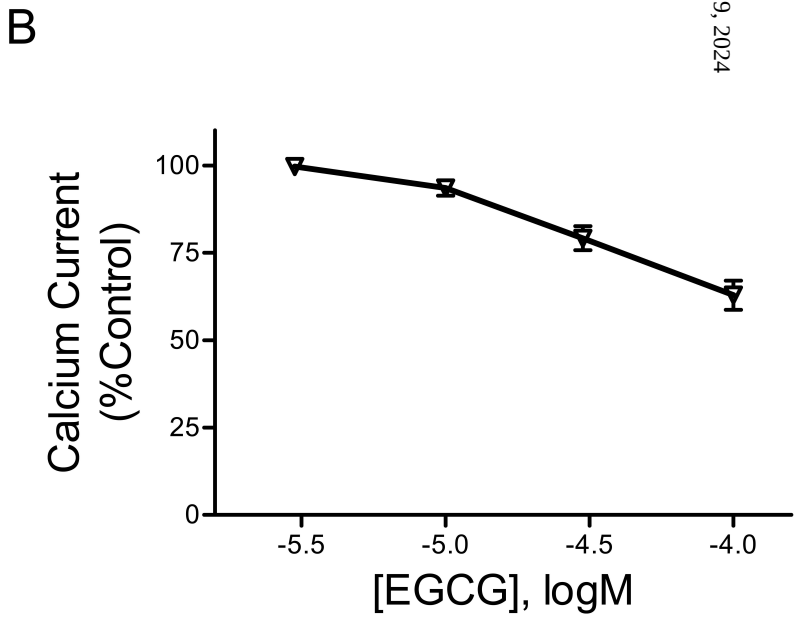


Figure 8