Ginsenoside Re suppresses electromechanical alternans in cat and human cardiomyocytes


Departments of Physiology, Stritch School of Medicine, Loyola University Chicago, Maywood, Illinois

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Wang YG, Zima AV, Ji X, Pabbidi R, Blatter LA, Lipsius SL. Ginsenoside Re suppresses electromechanical alternans in cat and human cardiomyocytes. Am J Physiol Heart Circ Physiol 295: H851–H859, 2008. First published June 20, 2008; doi:10.1152/ajpheart.01242.2007.— Ginseng botanicals are increasingly used as complementary or alternative medicines for a variety of cardiovascular diseases, yet little is known about their cellular actions in cardiac muscle. Electromechanical alternans (EMA) is a proarrhythmic cardiac abnormality that results from disturbances of intracellular Ca\(^{2+}\) homeostasis. This study sought to determine whether a purified ginsenoside extract of ginseng, Re, exerts effects to suppress EMA and to gain insight into its mechanism of action. Alternans was induced by electrically pacing cardiomyocytes at room temperature. Re (≥10 nM) reversibly suppressed EMA recorded from cat ventricular and atrial myocytes and Langendorff-perfused cat hearts. In cat ventricular myocytes, Re reversibly suppressed intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)\(_i\)]) transient alternans. Re exerted no significant effects on baseline action potential configuration or sarclemmal L-type Ca\(^{2+}\) current (I\(_{Ca,L}\)).

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K⁺ conductances. A single suction pipette recorded either voltage (bridge mode) or ionic currents (discontinuous voltage-clamp mode) using an Axoclamp 2A amplifier (Axon Instruments/Molecular Devices, Sunnyvale, CA). Computer software (pCLAMP, Axon Instruments/Molecular Devices) was used to deliver voltage protocols and to acquire and analyze data. L-type Ca²⁺ current (I_{Ca,L}) was activated by depolarizing pulses from a holding potential of −40 to 0 mV for 200 ms every 5 s. The peak I_{Ca,L} amplitude was measured in relation to steady-state current. Total K⁺ conductance was measured by voltage-clamp ramps from −130 to +30 mV, as previously described (25). Na⁺ current (I_{Na}) was recorded by the ruptured patch whole cell method, as previously described (22). I_{Ca,L} was measured at room temperature and with extracellular [Na⁺] reduced to one-third normal by substitution with equimolar tetraethylammonium (TEA)-Cl. The external recording solution contained (in mM) 50 NaCl, 67 TEA-Cl, 5.4 KCl, 1 MgCl₂, 2 CaCl₂, 20 CsCl, 5 HEPES, and 11 glucose and titrated with CsOH to pH 7.35. In addition, I_{Ca,L} was isolated by blocking I_{Ca,L} with 5 μM verapamil and blocking transient outward current with 2 mM 4-aminopyridine. I_{Ca,L} was activated by depolarizing voltage steps from a holding potential of −80 mV for 80 ms and measured with respect to steady-state current. In the ruptured patch method, the junction potential (10 mV) measured between the internal pipette and bath solutions was subtracted from all voltage measurements.

**Measurements of alternans.** Alternans was elicited by field stimulation of cardiomyocytes at room temperature. Microscopic visual inspection was used to select cells that displayed alternating mechanical activity. At a stimulation frequency of 1 Hz at room temperature, ~10% of cat myocytes that responded to stimulation displayed alternans. Because alternans is frequency dependent, higher frequencies of stimulation significantly increase the number of cells displaying alternans. Cell shortening (contraction) was measured with a video edge detector (Crescent Electronics) using a raster line placed on one edge of the cell. Mechanical (or Ca²⁺ transient) alternans was quantified as the alternans ratio (AR), which was defined as follows: AR = 1 − S/I, where S/I is the ratio of the small (S) to large (I) contraction (or [Ca²⁺]), transient) amplitude. AR = 0 indicates no alternans, and AR = 1 indicates the maximum degree of alternans.

**Integral and Ca²⁺ measurements.** Fast one-dimensional (line scan) imaging was performed using a confocal scanning unit (Bio-Rad Radiance 2100 and Bio-Rad 2000 MP) equipped with an argon-ion laser and attached to an inverted microscope. Single cells were loaded with the fluorescent indicator fluo-4 (fluor-4 AM). Fluorescence was excited at 488 nm and simultaneously recorded at wavelengths > 515 nm. The line scan was positioned along the longitudinal axis of the cell, avoiding the nucleus. Fluo-4 fluorescence emission (F) was normalized to baseline fluorescence emission (F₀) to correct for loading differences. Changes in [Ca²⁺], are presented as changes of F/F₀.

**Measurements of Ca²⁺ uptake by SR microsomes.** SR microsomes were prepared from cat ventricular tissue as previously described (30). SR vesicles (50 μg) were added to a cuvette containing 1 ml of buffered phosphate medium, which contained (in mM) 100 KH₂PO₄, 3 MgCl₂, 2 ATP, 0.01 ruthenium red, and 0.2 antipyrlyrazil II (APIII; Sigma); pH 7.0. Changes of [Ca²⁺] were measured as changes in absorbance between 710 and 790 nm of the Ca²⁺-sensitive dye APIII with the use of an ultraviolet-visible diode array spectrophotometer (Cary 50, Varian). Energized Ca²⁺ uptake was initiated by the addition of Ca²⁺ aliquots (10 μM CaCl₂) to the cuvette. The rapid rise in Ca²⁺-dependent APIIII absorbance was followed by a slower absorbance decrease due to ATP-dependent Ca²⁺ uptake by SR vesicles. The rate of Ca²⁺ loading of the vesicles, i.e., the net Ca²⁺ uptake, equaled the SR pumping rate minus the Ca²⁺ leak rate. The latter reflects the activity ofryanodine receptors (RyRs), which were blocked by ruthenium red. Thus, under our experimental conditions, net Ca²⁺ uptake by the vesicles is equal to the SR Ca²⁺-ATPase pumping rate.

**Single-channel records of RyRs.** RyR (type 2) single-channel recordings were performed as previously described (30). Planar lipid bilayers were formed from a lipid mixture containing phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine (ratio: 5:4:1) dissolved in n-decane at a final lipid concentration of 45 mg/ml. SR vesicles were added to the cis chamber, which corresponded to the cytosolic side of the RyR channel. The trans chamber (luminal side of the RyR) was connected to the virtual ground of the amplifier. During fusion, the cis and trans chambers contained solutions of the following composition (in mM): 400 (cis) and 40 (trans) CsCH₂SO₄, 0.1 CaCl₂, and 20 HEPES; pH 7.3 (CsOH). After channel incorporation, the concentration of CsCH₂SO₄ in the trans chamber was increased to 400 mM and free [Ca²⁺] in the cis chamber was adjusted to 3 μM by the addition of EGTA. Free [Ca²⁺] in the experimental solutions was verified with a Ca²⁺-sensitive mini-electrode (2). Single-channel currents were recorded using an Axopatch 200B amplifier (Axon Instruments/Molecular Devices). All recordings were obtained at a holding potential of −20 mV. Currents were filtered at 1 kHz and digitized at 5 kHz.

**Langendorff-perfused hearts.** Cat hearts were mounted on a Langendorff perfusion apparatus via cannulation of the aorta and perfused at a constant pressure of 45–50 mmHg at room temperature. The pulmonary artery was cut to decompress the right ventricle, and the left ventricle was vented through an apical stab wound. A latex fluid-filled balloon on the end of a polyethylene-100 catheter was inserted into the left ventricle through the mitral orifice and inflated using a 100-μl syringe to obtain an end-diastolic pressure (EDP) of 10 mmHg. The size of the balloon was selected (from balloon pressure-volume curves) to ensure that the EDP reflected left ventricular wall stiffness rather than balloon wall stiffness. To electrically pace the ventricles, the atrioventricular (AV) nodal region was ligated to produce complete AV block. Left ventricular balloon pressure was measured with a fluid-filled pressure transducer. Stainless steel hook electrodes on the right atrium and left ventricle were used to record bipolar electrograms, which represented a pseudo-ECG recording. The heart was electrically paced with paired bipolar hook electrodes attached to the ventricular wall. Data were recorded on a Grass polygraph chart recorder.

**Human atrial myocytes.** Discarded human left atrial muscle was obtained from patients at the time of surgery. Approval from the Loyola University Medical Center Internal Review Board was determined to be exempt. Tissue was transported to the laboratory in Ca²⁺-free Tyrode solution containing 50 mM tauro. In the laboratory, the tissue was bubbled with 100% O₂, minced into small pieces (1 mm²), and washed three times in Ca²⁺-free solution. Tissues were exposed to type I collagenase (350 U/ml) plus 4 U/ml protease in Ca²⁺-free solution for 30 min and then collagenase solution containing 30 μM Ca²⁺ for an additional 30 min at 37°C in a shaking water bath. The supernatant was collected, and the remaining tissue pieces were incubated in fresh collagenase solution containing 60 μM Ca²⁺. This last step was repeated every 10 min until no further cells were obtained. Supernatants containing cells were centrifuged at 1,000 rpm at 4°C for 2 min. The supernatant was removed, and the pellet was resuspended in 100 μM Ca²⁺. Extracellular [Ca²⁺] was slowly increased to 1 mM. Only quiescent rod-shaped cells showing regular striated patterns were used in these experiments.

**Ginseng and ginsenosides.** Ginsenosides Re and Rb1 were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC indicated that the purity of Re was 98%.

**Statistics.** Data are presented as means ± SE. Measurements were analyzed using either paired or unpaired Student’s t-tests for significance at P < 0.05.
RESULTS

Figure 1 shows EMA recorded from a ventricular myocyte field stimulated (2 Hz) at room temperature. The longer action potentials triggered the larger contractions, and the shorter action potentials triggered the smaller contractions. Within ~2 min of exposure, 10 nM Re markedly increased the smaller contraction (+249 ± 96%, n = 4, P < 0.05), whereas the larger contraction was decreased slightly (−23 ± 4%, n = 4, P < 0.05) in amplitude. At the same time, the longer action potentials shortened and the shorter action potentials lengthened. Figure 1B shows the steady-state (~5 min) effect of Re to suppress EMA and normalize both electrical and mechanical activities. Figure 1C shows that the effects of Re reversed upon washout (~5 min). EMA was quantified by measurements of contraction amplitudes and APDs at 90% repolarization (APD90) to determine the AR (see METHODS). Control contraction AR = 0.87 ± 0.07 versus Re AR = 0.01 ± 0.01 (n = 4, P < 0.001; see Fig. 5), and control APD90 AR = 0.25 ± 0.02 versus Re AR = 0.03 ± 0.02 (n = 4, P < 0.01). In addition, Re significantly decreased the cycle length at which EMA could be elicited (control: 543 ± 157 ms vs. Re: 440 ± 124 ms, −19%, P < 0.05). In other words, Re raised the pacing threshold at which EMA could be induced. As shown in Fig. 5, Re also exerted similar effects in cat atrial myocytes (control contraction AR = 0.74 ± 0.09 vs. Re AR = 0.05 ± 0.03, n = 3, P < 0.001). These findings indicate that nanomolar concentrations of Re suppress EMA in both atrial and ventricular myocytes and raise the threshold for the induction of EMA.

To determine whether the effects of Re are shared by other ginsenosides, we tested the effects of Rb1, a biologically active, major constituent of panax ginseng belonging to the protopanaxadiol group (16). Rb1 (10 μM) failed to exert any effects to suppress EMA (data not shown; n = 3). These findings suggest that the effects of Re are not shared by Rb1, another major ginsenoside component of ginseng.

EMA is thought to result from disturbances in the gain of SR Ca2+ release (4). We therefore used confocal laser scanning microscopy to record [Ca2+]i release, i.e., the [Ca2+]i transient, during EMA. Figure 2 shows line scan profiles of [Ca2+]i transients (top) and corresponding line scan images of [Ca2+]i (bottom) obtained from a ventricular myocyte paced at room temperature. As shown in Fig. 2A, the cell exhibited alternations in [Ca2+]i transient amplitudes that were consistent with mechanical alternans. Exposure to Re markedly increased the amplitude of the smaller (+147 ± 21%, n = 7, P < 0.05) [Ca2+]i transient and slightly decreased the amplitude of the larger (−9 ± 1%, n = 7, P < 0.05) [Ca2+]i transient, resulting in normalization of [Ca2+]i transient amplitudes (Fig. 2B). Washout of Re restored [Ca2+]i transient alternans (Fig. 2C). These findings are consistent with the effects of Re to suppress EMA, as shown in Fig. 1. As shown in Fig. 5, control [Ca2+]i transient AR = 0.49 ± 0.04 vs. Re AR = 0.02 ± 0.01 (n = 7, P < 0.001). The fact that Re primarily increases the amplitudes of the smaller contractions and [Ca2+]i transients during alternans supports the idea that Re suppresses EMA by enhancing SR Ca2+ release.

An important question is whether Re exerts similar actions in an intact heart preparation. We therefore determined the effects of Re in a Langendorff-perfused cat heart. The records shown in Fig. 3 show isovolumic pressure changes (top trace) and electrograms (bottom trace) recorded from a Langendorff-perfused heart electrically paced (2 Hz) at room temperature. As shown in Fig. 3A, electrical and mechanical activities displayed typical EMA. Note that in the electrogram tracings the repolarization waveform exhibits alternating amplitudes. Within minutes of exposure, Re (100 nM) markedly increased the smaller pressure pressure change and slightly decreased the larger pressure change. Figure 3B shows the steady-state effects of Re to suppress EMA and normalize both the electrical and mechanical activities. Washout of Re restored EMA (Fig. 3C). Results are shown in Fig. 5 (control pressure AR = 0.45 ± 0.02 vs. Re AR = 0.07 ± 0.02, n = 2). These findings were similar to those recorded from isolated ventricular and atrial myocytes and indicated that Re suppresses EMA in an intact heart preparation.

The records shown in Fig. 4 address the question of whether Re suppresses EMA in human cardiomyocytes. The traces show cell shortenings recorded from a single atrial myocyte paced from a single atrial myocyte of isolated from human left atrial tissue. The cell was electrically paced (0.75 Hz) at room temperature. Figure 4A shows typical mechanical alternans. Figure 4B shows steady-state effects of Re (1 μM) to suppress mechanical alternans and normalize contraction amplitudes. The effects of Re were reversed upon washout (Fig. 4C). As shown in Fig. 5, control contraction AR = 0.51 ± 0.03 vs. Re AR = 0.03 ± 0.02 (n = 3, P <

![Fig. 1. Re suppresses electromechanical alternans (EMA) in a cat ventricular myocyte (VM). A: action potentials (top) and cell shortenings (bottom) recorded during EMA induced by electrical pacing (2 Hz) at room temperature. B: steady-state effects of 10 nM Re to suppress EMA and normalize both electrical and mechanical activities. C: washout of Re restored EMA.](image-url)
0.01. These findings indicate that Re suppresses EMA in human cardiomyocytes, similar to its actions in cat heart.

To gain insight into the potential mechanism of Re action, we determined the effects of Re on \( I_{\text{Ca,L}} \), fast \( I_{\text{Na}} \), and total K\(^+\) conductances as well as baseline APD\(_{90}\). In paced (1 Hz) cells that did not display EMA, APD\(_{90}\) was unchanged by 10 nM Re (−0.8 ± 2.9%, \( n = 5 \)) and slightly shortened by 10 \( \mu \)M Re (−15 ± 4%, \( n = 7 \)). Figure 6A shows original recordings of \( I_{\text{Ca,L}} \) from a single ventricular myocyte in the absence (control) and presence of Re. Compared with control, 10 \( \mu \)M Re had no effect on the holding current or \( I_{\text{Ca,L}} \) (traces superimposed). The results shown in Fig. 6B indicate that Re had no significant effect on peak \( I_{\text{Ca,L}} \) (control: 685 ± 110 pA vs. Re: 649 ± 117 pA, \( n = 5 \)). Importantly, Re also had no significant effects on \( I_{\text{Ca,L}} \) recorded from human atrial myocytes (control: 876 ± 218 pA vs. Re: 847 ± 193 pA, \( n = 3 \)). The fact that Re had no significant effect on peak \( I_{\text{Ca,L}} \) suggests that Re does not exert significant effects on second messenger signaling pathways that regulate \( I_{\text{Ca,L}} \). As shown in Fig. 6C, we also analyzed the effects of Re on total K\(^+\) conductance recorded from cat ventricular myocytes using voltage ramps from −130 to +30 mV (40 mV/s), as previously described (24, 25). Compared with control, 10 \( \mu \)M Re had no effect on total K\(^+\) conductance throughout the voltage range (traces superimposed). The results shown in Fig. 6D indicate that Re had no effect on \( K^+ \) conductance measured at −130 mV (control: 396 ± 84 pA vs. Re: 392 ± 82 pA) and +30 mV (control: 188 ± 56 pA vs. Re: 182 ± 53 pA, \( n = 6 \)). Similarly, lower (10 nM) concentrations of Re exerted no effect on \( I_{\text{Ca,L}} \) (\( n = 3 \)) or total K\(^+\) conductances (\( n = 3 \)). Additional experiments used ruptured whole cell patch recordings in low (50 mM) extracellular [Na\(^+\)] at room temperature to study \( I_{\text{Na}} \), as previously described (22). Re (10 \( \mu \)M) had no effect on peak \( I_{\text{Na}} \) (control: 2,815 ± 643 pA vs. Re: 2,768 ± 617 pA, \( n = 5 \); data not shown). Together, these results indicate that nanomolar concentrations of Re that suppressed EMA failed to affect baseline APD\(_{90}\), consistent with their lack of effect on \( I_{\text{Ca,L}} \) or total K\(^+\) conductance. Even micromolar concentrations of Re had no significant effects on surface membrane ion channels. The fact that nanomolar concentrations of Re had no effect on action potential configuration, \( I_{\text{Ca,L}} \), or total K\(^+\) conductance and yet suppressed EMA supports the idea that the effects of Re to suppress EMA are not mediated via changes in sarcolemmal ion channels or second messenger signaling pathways that regulate these channels.

As shown in Fig. 7, we determined the effects of Re on SR Ca\(^{2+}\) release (i.e., \([Ca^{2+}]_i\), transients) and SR Ca\(^{2+}\) content (load). \([Ca^{2+}]_i\), transients were elicited by field stimulation (1 Hz), and SR Ca\(^{2+}\) content was assessed by brief, rapid exposure to 10 mM caffeine. Figure 7A shows original recordings of stimulated \([Ca^{2+}]_i\), transients and caffeine-induced \([Ca^{2+}]_i\), transients from a single ventricular myocyte before (control) and during exposure to 10 nM Re. Within the first 2 min of Re exposure, \([Ca^{2+}]_i\), transient amplitude increased with little change in SR Ca\(^{2+}\) content. At 6 min (steady state),
Re suppresses electromechanical alternans.

**DISCUSSION**

The present study demonstrates that the ginsenoside Re suppresses alternans in cat and human cardiomyocytes as well as in Langendorff-perfused cat hearts. Re exerts no significant effects on the major sarcolemmal ion channel currents or action potential configuration. Instead, Re directly increases the opening probability of RyRs, i.e., increases SR Ca\(^{2+}\) release.

Several of the present findings indicate that Re suppresses EMA by increasing the opening of RyR channels and thereby enhancing SR Ca\(^{2+}\) release. First, Re suppressed alternans in a variety of different preparations by consistently causing a marked increase in the smaller contraction or [Ca\(^{2+}\)] transient amplitude was reduced but still larger than control, and SR Ca\(^{2+}\) content was reduced. Figure 7B shows normalized data and demonstrates that Re elicited a small but significant increase in [Ca\(^{2+}\)] transient amplitude and a small but significant (6 min) decrease in SR Ca\(^{2+}\) content (n = 6). Because Re had no significant effect on peak I\(\text{ca,L}\) (the trigger for SR Ca\(^{2+}\) release), Re significantly increased fractional SR Ca\(^{2+}\) release (the ratio of [Ca\(^{2+}\)] transient/content).

SR Ca\(^{2+}\) content can be affected by either changes in SR Ca\(^{2+}\) release or uptake. To assess the latter, we determined the effects of a relatively high concentration of Re (10 \(\mu\)M) on Ca\(^{2+}\) uptake by SR microsomes isolated from cat ventricles (see METHODS). Figure 8A shows the decrease in extravesicular [Ca\(^{2+}\)] resulting from Ca\(^{2+}\) uptake into SR microsomes after the addition of 10 \(\mu\)M Ca\(^{2+}\). SR Ca\(^{2+}\) uptake in the absence (control) and presence of 10 \(\mu\)M Re were essentially the same. Similar results were obtained with 10 nM Re. In contrast, 0.1 \(\mu\)M thapsigargin, an agent that specifically inhibits SR Ca\(^{2+}\) uptake, abolished Ca\(^{2+}\) uptake. The data were fit by a single exponential and quantified as the time constant of uptake (Fig. 8B). As shown in Fig. 8B, there were no differences among control, 10 nM Re, and 10 \(\mu\)M Re. These findings indicated that Re has no effect of SR Ca\(^{2+}\) uptake (Ca\(^{2+}\)-ATPase) and that the effect of Re to decrease SR Ca\(^{2+}\) content is likely due to an increase in SR Ca\(^{2+}\) release (SR Ca\(^{2+}\) leak).

So far, the results suggested that Re increased the opening of SR Ca\(^{2+}\)-release channels, i.e., RyRs. Therefore, in the next experiments, we determined the direct effects of Re on RyRs isolated from cat ventricles and incorporated into planar lipid bilayers (see METHODS). Figure 9A, top, shows original single RyR channel recordings before (control) and during an exposure to 1 \(\mu\)M Re. The addition of Re to the cis chamber markedly increased the frequency of RyR channel openings without affecting single-channel conductance. The addition of Re to the trans chamber had no effect (data not shown). The graph in Fig. 9B shows the effects of 1 \(\mu\)M Re to significantly increase the open probability of RyRs (n = 6). Fig. 9A, bottom, shows that the addition of 1 \(\mu\)M Re to the cis chamber failed to increase RyR channel openings (n = 3). The effects of Re are shown in the graph of Fig. 9B. Figure 9C, left, shows the dose-dependent increase in open probability of RyRs exerted by three different concentrations of Re (0.1, 1, and 10 \(\mu\)M). Figure 9C, right, shows the same data calculated as percent changes normalized against control (100%). These data show that each concentration of Re caused a significant dose-dependent increase in RyR channel opening. Re concentrations below 0.1 \(\mu\)M were not tested because their effects would be below the resolution of the single-channel recording method. Nevertheless, the present findings support the idea that Re suppresses EMA by directly increasing RyR channel opening. They also indicate that the effects of Re are not shared by Rb1.
amplitude. This is consistent with direct measurements of intra-SR Ca$^{2+}$ content during alternans, which indicated that the smaller Ca$^{2+}$ transient is due to less SR Ca$^{2+}$ release and that SR Ca$^{2+}$ release is enhanced when the smaller Ca$^{2+}$ transient amplitude is increased (14). In the present study, Re also elicited a relatively small decrease in the larger contraction or [Ca$^{2+}$] transient amplitudes, consistent with a decrease in available SR Ca$^{2+}$ that resulted from the enhanced efflux of SR Ca$^{2+}$ induced by Re during the smaller beat. In other words, the total steady-state SR Ca$^{2+}$ content is slightly reduced. This is, in fact, consistent with the slight but significant reduction in steady-state SR Ca$^{2+}$ content as measured by exposure to caffeine (Fig. 7). The fact that Re had little effect on the trigger ($I_{Ca,L}$) for SR Ca$^{2+}$ release and yet increased stimulated Ca$^{2+}$ transient amplitudes and decreased SR Ca$^{2+}$ content indicates that Re increased fractional SR Ca$^{2+}$ release. Finally, direct measurements of RyR channel function showed that Re increased the open probability of RyR channels without affecting

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**Fig. 6. Effects on L-type Ca$^{2+}$ current ($I_{Ca,L}$) or K$^+$ conductance [peak K$^+$ current ($I_K$)].** A: original recordings of $I_{Ca,L}$ obtained from a cat VM. Compared with control, Re (10 μM) had no effect on $I_{Ca,L}$. B: compared with control (solid bar), Re (open bar) had no significant effects on peak $I_{Ca,L}$ in cat VMs. C: original recordings of total K$^+$ conductance as measured by a voltage ramp between −130 and +30 mV obtained from a cat VM. Compared with control (solid trace), Re (dashed trace) had no effect on total K$^+$ conductance. D: total K$^+$ conductance measured at −130 (solid bar) and +30 mV (open bar) showed that Re had no effect on total K$^+$ conductance.

**Fig. 7. Effects of Re on [Ca$^{2+}$] transients and sarcoplasmic reticulum (SR) Ca$^{2+}$ content.** A: original recordings of electrically stimulated [Ca$^{2+}$] transients and caffeine-induced [Ca$^{2+}$] transients recorded from a cat VM. Within 2 min of exposure to 10 nM Re, stimulated [Ca$^{2+}$] transient amplitude increased without significant changes in SR Ca$^{2+}$ content. However, at 6 min of Re exposure (steady state), [Ca$^{2+}$] transient amplitude was reduced but still larger than control, whereas SR Ca$^{2+}$ content was reduced. B: graph summarizing normalized data (dashed line, 100%) measured at 2 min (open bars) and 6 min (solid bars). The data show that Re increased [Ca$^{2+}$] transient amplitudes and decreased SR Ca$^{2+}$ content, resulting in an increase in fractional SR Ca$^{2+}$ release. *P < 0.05.
single-channel conductance. These bilayer experiments were performed on native channels in the absence of ATP or Mg$^{2+}$, indicating that the effects of Re were not dependent on either. Moreover, additional bilayers experiments (unpublished observations) performed in the presence of ATP and Mg$^{2+}$ yielded similar results. It is also important to note that another biologically active ginseng extract, ginsenoside Rb1, failed to suppress EMA and failed to affect RyR channel opening. These findings provide an important negative control that supports a cause-and-effect relationship between Re-induced opening of RyR channels and suppression of EMA. Moreover, they indicate that Re exerts an effect not shared by all ginsenoside compounds. Taken together, the present study suggests that Re suppresses EMA by directly increasing the opening of RyRs.

Fig. 8. Re fails to affect Ca$^{2+}$ uptake into SR microsomes isolated from cat ventricular muscle. A: compared with control, Re (10 μM) had no effect on the rate of decay of extravesicular Ca$^{2+}$ concentration ([Ca$^{2+}]_{\text{ev}}$), i.e., Ca$^{2+}$ uptake into SR microsomes. As a positive control, thapsigargin (0.1 μM) blocked Ca$^{2+}$ uptake. The dashed line represents the signal level before the addition of Ca$^{2+}$. B: bar graph summarizing the lack of effect of 10 nM or 10 μM Re on the rate of [Ca$^{2+}]_{\text{ev}}$ decay ($\tau_{\text{decay}}$; n = 5).

Fig. 9. Re increases single ryanodine receptor (RyR) channel activity. A, top: compared with control, Re (1 μM) increased the opening of RyRs without changing single-channel conductance. Bottom, compared with control, Rb1 (1 μM) failed to affect RyR channel opening. c, Closed; o, open. B: bar graph summarizing the effect of Re to significantly increase RyR open probability ($P_o$) and the lack of effect of Rb1 to affect RyR $P_o$. C: bar graphs summarizing the dose-dependent effects of Re to increase $P_o$ (left) and the percent increase in $P_o$, normalized against the control (right). n = 7 for 0 μM Re (control), 7 for 0.1 μM Re, 5 for 1 μM Re, and 5 for 10 μM Re. *P < 0.05.
and adds further support to the idea that the ultimate cause of EMA is a disturbance in SR Ca\(^{2+}\) release.

In the present study, EMA was elicited by electrical pacing at room temperature (hypothermia). Our previous work has also indicated that disturbances of metabolic activity can precipitate alternans (4, 8). This raises the question of whether the alternans seen here is elicited by a hypothermia-mediated impairment of metabolic activity and whether Re is acting to suppress alternans by somehow rectifying the impaired metabolic activity. The present experiments cannot eliminate this possibility. However, it should be noted that alternans can be precipitated by a wide range of abnormal conditions (6, 20), and it is not clear how these different conditions actually cause alternans. There is, however, compelling evidence that the ultimate cause of alternans is a subcellular disturbance of [Ca\(^{2+}\)]\(_i\) handling (4, 8, 20). More specifically, we reported that in cat cardiomyocytes, inhibition of SR Ca\(^{2+}\) release by ryanodine abolishes electrical alternans (15), indicating that abnormal SR Ca\(^{2+}\) handling is the primarily mechanism responsible for electrical alternans. Moreover, alternans is not associated with alternations of \(I_{\text{Ca,L}}\) or intracellular SR Ca\(^{2+}\) content but rather with alternations in the gain of SR Ca\(^{2+}\) release, i.e., the efficiency of a given trigger to activate SR Ca\(^{2+}\) release (8). This is consistent with direct measurements of intra-SR Ca\(^{2+}\) content, which showed that alternations in SR Ca\(^{2+}\) content are not required for Ca\(^{2+}\) alternans to occur and that RyR channel availability may be of greater importance (14). In light of these considerations, the simplest interpretation of the present experiments is that Re suppresses EMA by directly opening RyR channels.

The effects of Re to increase open probability of RyR channels without changing single-channel conductance appear similar to those of caffeine. However, exposure to caffeine also induces rapid diastolic Ca\(^{2+}\) release that can result in arrhythmic activity (18), effects not shared by Re. In other words, in the present study, Re did not induce Ca\(^{2+}\)-mediated arrhythmic activity. Further experiments will be required to determine whether the molecular mechanism of Re action on RyR channels is similar to that of caffeine.

In rat ventricular myocytes, Re decreased [Ca\(^{2+}\)]\(_i\) transients and contraction strength, and these effects were blocked by inhibition of nitric oxide (NO) synthase activity, leading to the conclusion that the negative inotropic effects of Re were mediated by intracellular NO production (16). In the present study, Re failed to significantly affect \(I_{\text{Ca,L}}\), which is regulated by NO production (23, 26). Moreover, the antiarrhythmic effects of Re to suppress EMA were unaffected by exposure to L-N\(^{3-}\)-(1-iminoethyl)-ornithine, a potent inhibitor of constitutive NO synthase activity (unpublished observations). Therefore, in cat cardiomyocytes, the effects of Re are not mediated via NO production. In fact, in paced ventricular myocytes exhibiting normal excitation-contraction coupling, nanomolar concentrations of Re had no effect on baseline action potential configuration and yet suppressed EMA by normalizing the Ca\(^{2+}\) transient amplitude. It is worth noting that nanomolar concentrations are compatible with those found in human plasma after the oral administration of ginsenoside (21).

The present results also indicate that in cat cardiomyocytes, Re does not affect sarcolemmal \(I_{\text{Ca,L}}\), \(I_{\text{K_a}}\), or K\(^+\) conductances. These findings differ from those reported for guinea pig (1) and rat (9) ventricular myocytes, where Re decreased \(I_{\text{Ca,L}}\) and single-channel recordings of \(I_{\text{Ca,L}}\), respectively. In guinea pig ventricular myocytes, Re also shortened APD and increased the activation of delayed rectifier K\(^+\) current (1). In the present study, we found no evidence that Re significantly affected \(I_{\text{Ca,L}}\) or K\(^+\) conductance. This is consistent with the fact that concentrations of Re (≤1 μM) that suppress EMA had no effect on APD\(_{90}\). The apparent discrepancies between the present findings and published reports could result from several factors, including differences in animal species, the purity of ginsenoside compounds, and/or the recording methods used. It should be noted, however, that in the present study Re failed to affect \(I_{\text{Ca,L}}\) in either cat or human cardiomyocytes and yet suppressed EMA in both cat and human cardiomyocytes. Therefore, any potential effect of Re on \(I_{\text{Ca,L}}\) is unrelated to the ability of Re to suppress EMA. In addition, these findings establish the relevance of the present study in cat to human cardiac physiology.

In summary, this study demonstrates that a main biologically active component of ginseng, ginsenoside Re, acts via a specific subcellular mechanism that targets RyR channels. Further experiments are needed to determine how Re acts to open RyRs and whether Re may serve as a safe, inexpensive, and effective drug therapy to reduce the development of arrhythmic activities arising from EMA. On the other hand, this study also demonstrates that ginseng extracts exert significant cardiac actions that have the potential to interact with other therapeutic and nontherapeutic cardioactive drugs. For example, the addition of ginseng to sports drinks or soft drinks that already contain relatively high concentrations of caffeine may have the potential to significantly alter cardiac Ca\(^{2+}\) homeostasis and adversely affect cardiac function.

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