Protein Kinase A–Activated Chloride Channel Is Inhibited by the Ca\(^{2+}\)-Calmodulin Complex in Cardiac Sarcoplasmic Reticulum

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Cardiac sarcoplasmic reticulum (SR) has several chloride (Cl\(^{-}\)) channels, which may neutralize the charge across the SR membrane generated by Ca\(^{2+}\) movement. We recently reported a novel 116-picosiemens Cl\(^{-}\) channel that is activated by protein kinase A–dependent phosphorylation in cardiac SR. This Cl\(^{-}\) channel may serve as a target protein in the receptor-dependent regulation of cardiac excitation-contraction coupling. To understand further regulatory mechanisms, the effects of Ca\(^{2+}\) on the Cl\(^{-}\) channel were studied using the planar lipid bilayer-vesicle fusion technique. In the presence of calmodulin (CaM, 0.1 µmol/L per microgram SR vesicles), Ca\(^{2+}\) (3 µmol/L to 1 mmol/L) added to the cis solution reduced the channel openings in a concentration-dependent fashion, whereas Ca\(^{2+}\) (1 mmol/L to 100 mmol/L) alone or CaM (0.1 to 1 µmol/L per microgram SR vesicles) with 1 mmol/L Ca\(^{2+}\) did not affect the channel activity. This inhibitory effect of Ca\(^{2+}\) in the presence of CaM was prevented by CaM inhibitors N-(6 aminohexyl)-5-chloro-1-naphthalenesulfonamide and calmidazolium but not by CaM kinase II inhibitor KN62. These results suggest that the Ca\(^{2+}\)-CaM complex itself, but not CaM kinase II, is involved in this channel inhibition. Thus, the cardiac SR 116-picosiemens Cl\(^{-}\) channel is regulated not only by protein kinase A–dependent phosphorylation but also by the cytosolic Ca\(^{2+}\)-CaM complex. This is a novel second messenger–mediated regulation of Cl\(^{-}\) channels in cardiac SR membrane. (Circ Res. 1993;73:751-757.)

**Key Words**  • Cl\(^{-}\) channel  • cardiac sarcoplasmic reticulum  • Ca\(^{2+}\) dependence  • calmodulin  • single-channel recording

In cardiac myocytes, sarcoplasmic reticulum (SR) plays a central role in excitation and contraction (E-C) coupling.\(^1\) Calcium ions (Ca\(^{2+}\)) are released through ryanodine receptor Ca\(^{2+}\) release channels and taken up via the Ca\(^{2+}\) pump in SR.\(^2\) Other ion channels such as K\(^{+}\) and Cl\(^{-}\) channels also exist in the SR membrane.\(^3\) They are supposed to maintain the charge neutrality across the SR membrane generated by Ca\(^{2+}\) movement during E-C coupling. Therefore, those channels should play important roles in the regulation of release and uptake of Ca\(^{2+}\) mediated by SR during the contraction and relaxation cycle. Several Cl\(^{-}\) channels on cardiac and skeletal SR membranes have been identified by the lipid-bilayer technique.\(^4\) Rousseau\(^5\) reported a cardiac SR Cl\(^{-}\) channel with the conductance of 55 picosiemens in 260 mmol/L Cl\(^{-}\). Tanifuji et al\(^6\) reported a skeletal SR Cl\(^{-}\) channel with the conductance of 200 picosiemens in 100 mmol/L Cl\(^{-}\). The openings of these Cl\(^{-}\) channels increase with membrane depolarization and are blocked by cytosolic Ca\(^{2+}\). These channels seem not to be regulated by intracellular substances, such as protein kinase A (PKA).

Recently, we found a Cl\(^{-}\) channel in the cardiac SR membrane whose conductance was 116 picosiemens with 500 mmol/L Cl\(^{-}\).\(^7\) The channel opening was voltage independent between −40 and +100 mV and activated by the cytosolic catalytic subunit of PKA. Thus, this cardiac SR Cl\(^{-}\) channel is different from those reported by Rousseau\(^5\) and Tanifuji et al\(^6\) and may serve as a novel intracellular target protein of cytosolic PKA. Being actively regulated by such hormones as β-adrenergic agonists and histamine through the cAMP-PKA pathway, this 116-picosiemens Cl\(^{-}\) channel may play an important role in the receptor-dependent regulation of cardiac E-C coupling. However, it is not known whether other intracellular second messengers can regulate this SR Cl\(^{-}\) channel.

In the present study, we examined the effects of Ca\(^{2+}\) on this PKA-activated SR Cl\(^{-}\) channel. We found that Ca\(^{2+}\) added to the cis side solution inhibited the Cl\(^{-}\) channel activity only in the presence of calmodulin (CaM). Our results suggest that the inhibitory effect of Ca\(^{2+}\) with CaM on this Cl\(^{-}\) channel is mediated by the Ca\(^{2+}\)-CaM complex itself but not by activation of CaM kinase II. This is a novel finding of the SR Cl\(^{-}\) channel regulated by intracellular second messengers.

**Materials and Methods**

**Preparation**

Porcine cardiac heavy SR was isolated by discontinuous sucrose gradient centrifugation by a modified
method as described by Meissner and Henderson. Porcine ventricular muscles were minced in a food processor and homogenized for 60 seconds in buffer containing 0.3 mol/L sucrose, 20 mmol/L HEPES, and protease inhibitors (1 μmol/L pepstatin, 1 mmol/L iodoacetamide, 0.1 mmol/L phenylmethylsulfonyl fluoride, 1 μmol/L leupeptin, and 1 mmol/L benzamidine). The homogenate was centrifuged for 30 minutes at 2600g. The supernatant was centrifuged for 30 minutes at 10 400g. After centrifuging, the supernatant was discarded. The pellet was resuspended in 0.6 mol/L KCl and 5 mmol/L PIPES. This material was incubated for 60 minutes at 0°C and centrifuged for 60 minutes at 80 000g. The pellet was resuspended in 0.3 mol/L sucrose, 0.4 mol/L KCl, and 5 mmol/L Na-PIPES. Samples were placed on the top of a discontinuous sucrose gradient consisting of 20%, 30%, and 40% (wt/vol) sucrose. Gradients were centrifuged for 16 hours at 95 000g. Heavy SR was recovered from the interphase between the 30% and 40% sucrose layers and stored frozen at -80°C.

Solution and Drugs

The cis bath solution contained (mmol/L) CsCl, 500; EGTA, 1; HEPES, 10; and Mg-ATP, 5; along with different concentrations of free Ca\(^{2+}\) (1 mmol/L to 1 mmol/L). The trans solution contained 50 mmol/L CsCl, 1 μmol/L free Ca\(^{2+}\), 1 mmol/L EGTA, and 10 mmol/L HEPES. The pH of these solutions was adjusted to 7.4 by adding CsOH. Mg-ATP (5 mmol/L) was added to the cis solution. Free Ca\(^{2+}\) in solution was adjusted by adding different amounts of CaCl\(_2\) as calculated with the computer program described by Fabiato. The phosphodiesterase 3':5'-cyclic nucleotide activator CaM (lot 71H9725) and compound R24571 (calmidazolium) were purchased from Sigma Chemical Co, St Louis, Mo. Concentrated stock solutions of calmidazolium were dissolved in dimethyl sulfoxide (3.44 mg/mL). The stock solution was kept at 5°C and diluted with the cis solution just before use. The final concentration of dimethyl sulfoxide was less than 0.1%. A CaM antagonist, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W7), and a protein kinase inhibitor specific to Ca\(^{2+}\)-CaM–dependent protein kinase II, KN-62, were purchased from Seikagaku Kogyo Co, Tokyo, Japan. These drugs were dialyzed against the bath solution at 0°C for 30 minutes before use.

Electrophysiological Methods and Data Analysis

Currents flowing through the ion channels reconstituted into the planar bilayer were measured by using the voltage-clamp technique. The planar lipid bilayer was composed of brain phosphatidylethanolamine and brain phosphatidylserine (Avanti Polar Lipids, Alabaster, Ala), at a ratio of 1:1, dissolved in decane (20 mg/mL). Purified cardiac heavy SR vesicles were added to the cis chamber and fused into the lipid bilayer formed in the hole (0.3 mm in diameter) in a Lexan polycarbonate partition. In the present experiments, the cis chamber was defined as the side to which SR vesicles were added.
reproduced and low-pass-filtered at 1 kHz or 500 Hz by a filter with Bessel characteristics (octave attenuation, 48 dB), sampled at 5 kHz, and analyzed off-line on a computer (Compaq 386, Compaq Computer Corp, Reston, Va). For single-channel analysis, the threshold used to judge the open state was set at half of the single-channel current.13

Results

PKA Activation of Cardiac SR Cl⁻ Channel

Purified cardiac heavy SR vesicles were incorporated into planar lipid bilayers. By using 500 mmol/L CsCl in the cis and 50 mmol/L CsCl in the trans chamber solutions, several kinds of ion channels were identified in the bilayers as reported previously.6 At 0 mV, Ca²⁺ current through the ryanodine-receptor channel flowed in the outward direction, and Cl⁻ channel current flowed in the inward direction. When sarcotremal vesicles, instead of heavy SR vesicles, were incorporated into the bilayer, we could detect neither the ryanodine-receptor channel currents nor the Cl⁻ channel currents (not shown).

The 116-picosiemens Cl⁻ channel current ran down spontaneously within 2 minutes after incorporation in the absence of Mg-ATP as reported previously (Fig 1). After rundown, either 5 mmol/L Mg-ATP or 148 ng/mL PKA with 0.05 mmol/L Mg-ATP added to the cis solution restored the channel activity, both of which were prevented by the protein kinase inhibitor specific to PKA.6 The channel opening was unaffected by changing the membrane potential between −40 and +100 mV and was inhibited by 4,4'-dinitrostilbene-2,2'-disulfonic acid (DNDS, 0.1 mmol/L).6 The slope conductance of the Cl⁻ channel measured in the presence of 5 mmol/L Mg-ATP was 113±15 picoSiemens with 500 mmol/L Cl⁻ (Fig 1C), and the open-time and closed-time histograms could be fit by the sum of two exponentials (Fig 1A). The time constants of the exponentials for the open-time histogram were 1.5±0.1 (mean±SD) milliseconds (n=5) and 40±5 milliseconds (n=5). Those for the closed-time histogram were 0.7±0.1 milliseconds (n=5) and 6.0±0.5 milliseconds (n=5). These time constants were identical to those reported previously.6 From these results, this channel was confirmed as the PKA-acti-

FIG 2. Cl⁻ channel activities at different Ca²⁺ concentrations. Continuous recordings are shown of Cl⁻ channel openings at 1 mmol/L Ca²⁺ (A), 0.5 μmol/L Ca²⁺ (B), 3 μmol/L Ca²⁺ (C), and 1 mmol/L free Ca²⁺ (D) in the cis solution. C indicates closed levels of the channel; O, open levels. These cis solutions contained 5 mmol/L Mg-ATP to prevent channel rundown.6 Voltage was held at 0 mV.

and the opposite side was referred to as the trans chamber. The cis chamber was equivalent to the cytoplasmic side of the incorporated channel, and the trans chamber was equivalent to the lumen of the SR as determined previously.1,1 The applied voltages were defined with respect to the trans chamber held at ground. Channel activities were recorded at room temperature (22±1°C), amplified by a patch-clamp amplifier (Axopatch 1C, Axon Instruments, Inc, Foster City, Calif), and stored on a videocassette tape recorder through a PCM converter system (RP-880, NF Instruments, Yokohama, Japan) digitized at 10 kHz. Data were

FIG 3. Calmodulin (CaM) effects on the Cl⁻ channel. Continuous recordings are shown of Cl⁻ channel openings before and after the application of CaM into the cis solution. C indicates closed levels of the channel; O, open levels. The cis bath solutions contained 5 mmol/L Mg-ATP and 1 mmol/L free Ca²⁺. Voltage was held at 0 mV. A, The application of less than 0.05 μmol/L CaM per microgram sarcotremal reticulum (SR) vesicles did not affect the Cl⁻ channel openings until the break of bilayer. B, CaM (more than 0.1 μmol/L per microgram SR vesicles) reduced the open probability and finally completely inhibited Cl⁻ channel openings.
vated Cl− channel.6 As reported previously,6 this Cl− channel activity could be restored in the presence of more than 2 mmol/L Mg-ATP or PKA with 0.05 mmol/L ATP and 2 mmol/L MgCl2. Therefore, the following studies were done in the presence of 5 mmol/L Mg-ATP to prevent a rapid rundown.

**Ca2+ and CaM Effects on the PKA-Dependent SR Cl− Channel**

We examined the effects of Ca2+ on the Cl− channel. Fig 2 shows the Cl− channel activities at different Ca2+ concentrations in the cis solutions. The membrane potential was held at 0 mV. Ca2+ at concentrations between 1 nmol/L and 1 mmol/L did not affect the channel opening significantly (see also Fig 4E). Thus, the opening of the PKA-activated Cl− channel is not Ca2+ dependent.

It is well known that CaM is an intracellular Ca2+ binding protein and abundant in cardiac cytosol.15,16 It is also reported that ryanodine receptor Ca2+ release channels in cardiac and skeletal SR are inhibited by CaM.17 Therefore, we examined the effects of Ca2+ on this Cl− channel in the presence of CaM in cis solution (Fig 3). The application of less than 0.05 μmol/L CaM per microgram SR vesicles into the cis solution in the presence of 1 mmol/L free Ca2+ did not alter channel activities (10 of 11 experiments) until the break of the bilayer (8.7±4.3 [mean±SD] minutes, n=10) (Fig 3A). When more than 0.1 μmol/L CaM per microgram SR vesicles was added to the cis solution in the presence of 1 mmol/L free Ca2+, channel openings were remarkably reduced and completely blocked in 7 of 12 experiments within 2 minutes (1.3±0.5 [mean±SD] minutes) (Figs 3B and 4E). These observations suggest that Ca2+ and CaM may affect the channel gating. Therefore, we examined the blocking effect of CaM on the Cl− channel at different Ca2+ concentrations between 1 nmol/L and 1 mmol/L. When free Ca2+ concentration in the cis solution was less than 1 μmol/L, CaM (0.1 μmol/L per 1 μg SR vesicles) had no effect on Cl− channel openings (n=5) (Fig 4A, 4B, and 4E). When free Ca2+ concentration was increased to 3 μmol/L or more in the presence of CaM, the channel activity was reduced in a concentration-dependent fashion (Fig 4C and 4E). In the cis solution, the channel open probability was suppressed to 76±10% of the control value (n=11) at 3
μmol/L Ca\(^{2+}\), to 71±20% (n=9) at 40 μmol/L Ca\(^{2+}\), and to 31±11% (n=12) at 1 mmol/L Ca\(^{2+}\) (Fig 2B and 2C). From the above results, it is concluded that Ca\(^{2+}\) inhibits the Cl\(^{-}\) channel only in the presence of CaM.

The blocking properties of the Cl\(^{-}\) channel by CaM were examined in the presence of 0.5 and 40 μmol/L free Ca\(^{2+}\) with 5 mmol/L Mg-ATP in the cis solution. When the cis bath solution contained less than 0.5 μmol/L Ca\(^{2+}\), CaM did not affect the kinetics of the Cl\(^{-}\) channel (Figs 4A, 4B, and 5B). The channel open-time and closed-time histograms could be fit with two exponentials (Fig 5B), whose time constants were almost identical to those in the control condition (Fig 5A). When the concentration of Ca\(^{2+}\) in the cis solution was increased to 40 μmol/L with CaM, the mean open time seemed to become shorter and the long closed time appeared (Fig 4C). By analyzing those histograms, the open-time histograms could be fit by two exponentials with time constants of 1.0±0.5 and 12±2.6 milliseconds (n=4), in which the slow component became shorter compared with those in the control condition or in the presence of 0.5 μmol/L Ca\(^{2+}\) with CaM (Fig 5C, left). The closed-time histogram analyzed with a single active channel could be fit by three exponentials with time constants of 0.7±0.7, 3.0±1.3, and 30±15 milliseconds (n=4) (Fig 5C, right). Thus, the slowest component of the time constant appeared after application of CaM, indicating the blocked component by Ca\(^{2+}\)-CaM, but the faster two components had time constants not different from those in the control condition or in 0.5 μmol/L Ca\(^{2+}\) with CaM (Fig 5B).

**Mechanism of CaM Action**

To elucidate the mechanism of blocking the channel activity by CaM, we tested the effects of Ca\(^{2+}\)-CaM blockers in the following experiments. After the blocking of the Cl\(^{-}\) channel by Ca\(^{2+}\)-CaM, the further application of calmidazolium (1 μmol/L) in the cis solution could reactivate this channel (Fig 6A). Also, in the presence of W7 (100 μmol/L) or calmidazolium (100 nmol/L) in the cis solution, CaM (greater than 0.1 μmol/L per microgram SR vesicles) with 1 mmol/L free Ca\(^{2+}\) and 5 mmol/L Mg-ATP could not block channel openings (Fig 6B) (n=4 in W7 and n=8 in calmidazolium). W7 or calmidazolium themselves in the absence of CaM did not alter channel activities (data were not shown). Thus, blocking effects of exogenous CaM on this Cl\(^{-}\) channel was reversed by Ca\(^{2+}\)-CaM blockers. When SR vesicles were preincubated with KN62 (10 μmol/L), which is a blocker of CaM kinase II, and 5 mmol/L Mg-ATP for 15 minutes, the application of CaM into the cis solution still inhibited channel openings (Fig 6C) (n=4), indicating that the blocking effect of CaM on the Cl\(^{-}\) channel is not mediated by CaM kinase II–dependent phosphorylation.

**FIG 5.** Calmodulin (CaM) effects on kinetics of the Cl\(^{-}\) channel. A. Closed-time and open-time histograms are shown at 0-mV holding potential in the presence of 1 μmol/L free Ca\(^{2+}\) and the absence of CaM. The open-time histogram was fit by double-exponential curves with time constants T1 and T2 of 1.5 and 41 milliseconds, respectively. The closed-time histogram was fit by double-exponential curves with T1 and T2 of 0.7 and 6.0 milliseconds, respectively. B. In the presence of CaM (0.1 μmol/L per microgram sarcoplasmic reticulum vesicle) and 0.5 μmol/L Ca\(^{2+}\), open-time and closed-time histograms were fit by double-exponential curves. T1 and T2 were 1.5 and 43 milliseconds, respectively, for the open-time histogram and 0.6 and 5.0 milliseconds, respectively, for the closed-time histogram. Those values were not different from those in the control condition (A). C. In the presence of 40 μmol/L Ca\(^{2+}\) and CaM in cis solution, the open-time histogram was fit by double-exponential curves with T1 and T2 of 1.0 and 12 milliseconds, respectively. The slow component of time constants became shorter compared with that in A and B. The closed-time histogram was fit by triple-exponential curves with time constants T1, T2, and T3 of 0.4, 2.3, and 24 milliseconds, respectively. The longer component appeared, indicating the blocking of this Cl\(^{-}\) channel by CaM. Each histogram was obtained by experiments in which only one channel existed in the bilayer. All cis bath solutions contained 5 mmol/L Mg-ATP. Voltage was held at 0 mV.
FIG 6. Effects of blockers of calmodulin (CaM) on the Cl⁻ channel. A, Continuous recordings are shown of channel activities before and after the application of CaM blockers into the cis solution. C indicates closed levels of the channel; O, open levels. Tracings at the points indicated by arrows a, b, and c are expanded in the lower panels. The cis bath solution contained 5 mmol/L Mg-ATP and 1 mmol/L free Ca²⁺. The holding potential was held at 0 mV. After this channel was blocked by the addition of CaM (0.1 μmol/L per microgram sarcoplasmic reticulum vesicles) into the cis solution, the further application of calmidazolium (1 μmol/L) reactivated this channel. B, In the presence of CaM antagonist W7 (100 μmol/L) in the cis solution, CaM did not block channel openings (four of four). C, In the presence of protein kinase inhibitor KN62 (10 μmol/L), CaM completely blocked channel openings (four of four).

Discussion

The present study reports that Ca²⁺-CaM inhibits the cardiac SR Cl⁻ channel activated by PKA-mediated phosphorylation in a Ca²⁺ concentration-dependent manner. Previous studies have shown that several ion channels can be modulated by Ca²⁺-CaM, such as Ca²⁺-dependent K⁺ channels, Ca²⁺-dependent Na⁺ channels in paramecium, and Ca²⁺ release channels in SR. Because the CaM effect on Ca²⁺ release channels in SR did not require ATP, it was suggested that Ca²⁺-CaM may activate the channel by binding directly to the channel protein or to another membrane component that in turn blocked the channel in the absence of Mg-ATP. Activation by CaM on Ca²⁺-dependent Na⁺ channels in paramecium did not require ATP in cell-free systems; therefore, it was speculated that Ca²⁺ and CaM might activate directly to interact with channel proteins.

The blocking effect of CaM on the Cl⁻ channel could not be examined in the absence of ATP in this study, because Mg-ATP was required to maintain channel activities. From the results showing that CaM was Ca²⁺ dependent and could be reversed by calmidazolium and W7 (Fig 6A and 6B) but not by KN62 (Fig 6C), CaM is speculated to be interacting not through the regulation of protein phosphorylation via calmodulin kinase II. The Cl⁻ channel on cardiac SR may be interacting directly by Ca²⁺-CaM. The blocking kinetics of single ion channels by Ca²⁺-CaM has been reported in cardiac and skeletal ryanodine receptor Ca²⁺ release channels. CaM acts on both the open and the closed states of ryanodine channels by reducing the mean duration of open time and by increasing the closed-time constants without affecting the channel conductance. Our results also showed that Ca²⁺-CaM inhibited the Cl⁻ channel activities by reducing the channel mean open time and by prolonging the channel mean closed time with the appearance of long-lived closed (blocked) time (Fig 5C). Thus, calmodulin appears to act on both the open and the closed states of the Cl⁻ channel. However, it is not clear whether the Ca²⁺-CaM-induced inhibition is the result of an open-channel blockade or binding at some inhibitory site distant from the conduction pathway. During the cis application of CaM, the single-channel conductance of the Cl⁻ channel was not reduced, and the flickerings of channel transitions were not observed (Fig 4). Those findings were similar to those of the slow block of the open channel. Since the molecular size of CaM is 17 kD, the pore of the Cl⁻ channel is apparently not large enough for passing Ca²⁺-CaM during the open state. We hypothesize that Ca²⁺-CaM may bind directly the Cl⁻ channel distant from the conduction pathway to cause the conformational change for blockade.

The blocking mechanisms of the Cl⁻ channel were studied using the anion transport inhibitor DNDS, which is known to block apical membrane Cl⁻ channels from several Cl⁻-secreting epithelia. The inhibitory actions of DNDS on single Cl⁻ channels from rat colonic membranes were characterized by Bridges et al. They showed that DNDS increased the number of current transitions within a burst period, reflecting the
rapid blocking and unblocking of the Cl\textsuperscript{-} channel. From that finding, they concluded that DNDS caused a flicker-type blockade of the Cl\textsuperscript{-} channel. During the inhibitory actions by Ca\textsuperscript{2+}-CaM on the cardiac SR Cl\textsuperscript{-} channel, a flicker-type of blocking was not observed. Therefore, the mechanism of blocking by Ca\textsuperscript{2+}-CaM on the SR Cl\textsuperscript{-} channel seems to be different from that by DNDS on the epithelial Cl\textsuperscript{-} channel.

CaM as an intracellular receptor for Ca\textsuperscript{2+} activates a variety of enzymes, such as myosin light chain kinase, CaM kinases, and cytoskeletons, and thereby regulates various cellular functions including E-C coupling and excitation-contraction coupling. In cardiac SR, CaM inhibits Ca\textsuperscript{2+} release from SR by blocking the ryanodine receptor Ca\textsuperscript{2+} release channel, but CaM also promotes the Ca\textsuperscript{2+} release from SR by CaM kinase-dependent phosphorylation of the ryanodine receptor.\textsuperscript{21} It is likely that, during E-C coupling, the increase or decrease of intracellular Ca\textsuperscript{2+} may indirectly affect the Cl\textsuperscript{-} channel activity via the Ca\textsuperscript{2+}-CaM complex. During the contraction phase, Ca\textsuperscript{2+} is released from the ryanodine receptor Ca\textsuperscript{2+} release channel; therefore, the Cl\textsuperscript{-} channel openings may be inhibited by the Ca\textsuperscript{2+}-CaM complex by increasing intracellular Ca\textsuperscript{2+} (1 to 10 \mu mol/L) (Fig 4E). During the relaxation phase, Ca\textsuperscript{2+} in the cytosol is taken up to SR via the Ca\textsuperscript{2+} pump, and then it is speculated that this Cl\textsuperscript{-} channel can be activated by the relief of Ca\textsuperscript{2+}-CaM blocking due to decreasing intracellular Ca\textsuperscript{2+} (less than 0.5 \mu mol/L) (Fig 2C). Thus, the regulation of the Cl\textsuperscript{-} channel by Ca\textsuperscript{2+}-CaM is expected to play a role in SR function during the contraction and relaxation cycle. Therefore, this Cl\textsuperscript{-} channel modulated by the Ca\textsuperscript{2+}-CaM complex may contribute to the neutralization of the potential across the SR membrane generated by Ca\textsuperscript{2+} uptake through the Ca\textsuperscript{2+} pump during relaxation. This is a novel regulation of the SR Cl\textsuperscript{-} channel mediated by intracellular second messengers. Further studies are needed to elucidate physiological and pathophysiological roles of the Cl\textsuperscript{-} channel in the regulation of E-C coupling and cardiac function.

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