Multiple levels of the single L-type Ca\(^{2+}\) channel conductance in adult mammalian ventricular myocytes

Ye Chen-Izu *

Department of Pharmacology, University of California, Davis, CA, USA
Department of Biomedical Engineering, University of California, Davis, CA, USA
Department of Internal Medicine, University of California, Davis, CA, USA

Abstract

In cardiac muscle, Ca\(^{2+}\) entry through the L-type Ca\(^{2+}\) channel during excitation triggers Ca\(^{2+}\) release from sarcoplasmic reticulum (SR) via the ryanodine receptor, causing muscle contraction. This Ca\(^{2+}\) induced Ca\(^{2+}\) release (CICR) is locally controlled by openings of the single L-type Ca\(^{2+}\) channel that triggers the opening of ryanodine receptors located within the dyadic junction. The unitary current through the single Ca\(^{2+}\) channel determines the open probability of the ryanodine receptors, and hence the efficacy of CICR.

Here, we report findings of multiple conductance levels of L-type Ca\(^{2+}\) channels in freshly isolated rat ventricular myocytes. When 10 mM Ca\(^{2+}\) was used in the pipette solution as the charge carrier in the cell-attached configuration, the most frequently occurring conductance levels are 6.9 pS and 2.9 pS. Three distinct conductance levels were also observed, although infrequently, in the same cell, corresponding to unitary currents of 0.33 pA, 0.24 pA, and 0.17 pA upon depolarization to −10 mV. In conclusion, our data demonstrate the existence of multiple L-type Ca\(^{2+}\) channel conductance levels in the adult mammalian ventricular myocytes with Ca\(^{2+}\) as the charge carrier. The multiple conductance levels present heterogeneity in the Ca\(^{2+}\) trigger signal strength at local dyadic junctions. The physiological significance of this heterogeneity on affecting the efficacy of CICR in the cardiac muscle is discussed.

Introduction

In cardiac muscle, Ca\(^{2+}\) entry through L-type Ca\(^{2+}\) channels during action potential triggers Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR) through ryanodine receptors, leading to elevation of the intracellular Ca\(^{2+}\) concentration that causes muscle contraction. This Ca\(^{2+}\) induced Ca\(^{2+}\) release (CICR) is locally controlled by the opening of single L-type Ca\(^{2+}\) channel that triggers the opening of ryanodine receptors located within 20 nm distance in the dyadic junction. The unitary current through the single L-type Ca\(^{2+}\) channel determines the open probability of the ryanodine receptors. Hence, the single channel conductance of the L-type Ca\(^{2+}\) channel significantly affects the efficacy of CICR in cardiac muscle.

Single L-type Ca\(^{2+}\) channel conductance has been measured in various preparations. Historically, most single channel studies used Ba\(^{2+}\) as the charge carrier, because channel conductance is much larger with Ba\(^{2+}\) than Ca\(^{2+}\), making it easier to resolve the single channel events from the background noise. For example, a pioneering study by Hess et al. [1] reported that the L-type Ca\(^{2+}\) channel in the ventricular myocyte had a single channel conductance of 25 pS with 50 mM Ba\(^{2+}\), but only of 8 pS with 50 mM Ca\(^{2+}\) as the charge carrier. Intriguingly, multiple conductance levels were reported when Ba\(^{2+}\), instead of Ca\(^{2+}\), was used as the charge carrier. For example, Gondo et al. [2] found that cloned rabbit cardiac L-type Ca\(^{2+}\) channel expressed in Chinese hamster fibroblast displayed four sub-conductance levels of 23 pS, 14 pS, 6 pS, and 3 pS with 110 mM Ba\(^{2+}\). Moreover, the expression of \(\alpha_1\) subunit alone was sufficient to exhibit these sub-conductance levels, and coexpression of \(\beta_2\) subunit significantly increased the number of openings in all four levels without changing the conductance values. Multiple unitary current amplitude with Ba\(^{2+}\) was also observed in the cloned channels (cardiac \(\alpha_1\) with skeletal \(\beta_2\delta\)) expressed in baby hamster kidney cells [3]. Lacerda and Brown [4] further noted that the L-type Ca\(^{2+}\) channel in neonatal rat and guinea pig also displayed four sub-conductance levels of 23 pS, 14 pS, 6 pS, and 3 pS with 110 mM Ba\(^{2+}\). Moreover, the expression of \(\alpha_1\) subunit alone was sufficient to exhibit these sub-conductance levels, and coexpression of \(\beta_2\) subunit significantly increased the number of openings in all four levels without changing the conductance values. Multiple unitary current amplitude with Ba\(^{2+}\) was also observed in the cloned channels (cardiac \(\alpha_1\) with skeletal \(\beta_2\delta\)) expressed in baby hamster kidney cells [3]. Lacerda and Brown [4] further noted that the L-type Ca\(^{2+}\) channel in neonatal rat and guinea pig also displayed at least three unitary current levels in 100 mM Ba\(^{2+}\). However, two questions remained to be answered. One was whether the multiple conductance levels do exist in the adult mammalian cardiac myocytes rather than recombinant channels expressed in cell lines or neonatal cells. Another was whether the multiple conductance levels exist also when Ba\(^{2+}\), instead of Ca\(^{2+}\), is the charge carrier.
Given the physiological importance of the single channel conductance in affecting the Ca\(^{2+}\) trigger signal strength and the efficacy of CICR in cardiac muscle, we focused this project on investigating the sub-conductance levels of L-type Ca\(^{2+}\) channels in adult mammalian ventricular myocyte using Ca\(^{2+}\) as the charge carrier. Here we report that multiple conductance levels do exist when Ca\(^{2+}\) enters the L-type Ca\(^{2+}\) channel in freshly isolated adult rat ventricular myocytes, and we characterize two most frequently observed conductance levels.

**Methods and materials**

**Cell preparation.** Ventricle myocytes were isolated from 2- to 4-month-old Wistar rats using a standard enzymatic technique. Briefly, the rat was anesthetized with pentobarbital (100 mg/kg injected IP). After testing for the suppression of reflexes, the heart was explanted via midline thoracotomy, mounted on a Langendorff system and perfused with a modified Tyrode solution. Collagenase B (\(\sim1\) mg/ml, F. Hoffmann-La Roche Ltd., Switzerland).

![Image](image_url)

**Fig. 1.** Two frequently occurring single L-type Ca\(^{2+}\) channel conductance levels. The single channel activity was recorded using the cell-attached configuration under voltage-clamp. The membrane potential was held at \(-60\) mV to inactivate the T-type Ca\(^{2+}\) channels, and then depolarized to various voltages for 200 ms to record the L-type Ca\(^{2+}\) channel activity. Representative traces of single L-type Ca\(^{2+}\) channel recordings show the unitary current flowing through the single channel opening at 0 mV. Two distinct levels of the unitary currents are shown in A and B, denoted as O\(_1\) and O\(_2\), respectively. The corresponding all-points amplitude histograms and the fitting results using the Gaussian function are shown in C and D. E shows the \(I-V\) relationship of the unitary currents (plotted are mean ± SEM, \(n = 13\) for O\(_1\) group, \(n = 11\) for O\(_2\) group). The unitary current was obtained from the Gaussian fitting results for the voltages between \(-30\) mV and +30 mV. For voltages lower than \(-30\) mV, the unitary current data was obtained from tail openings. The \(I-V\) curves were fitted with a linear function to obtain the single channel conductance levels of 6.9 pS (\(R^2 = 0.970\)) and 2.9 pS (\(R^2 = 0.910\)).
protease type XIV (~0.1 mg/ml), 0.1% BSA and 20 μM Ca\(^{2+}\) were added into the perfusion to digest the tissue. The ventricular tissue was then cut down, minced and further incubated in the enzyme solution. The dissociated cells were collected and stored in the Tyrode solution containing 1 mM Ca\(^{2+}\) at room temperature, and were used for experiments within 10 h after isolation. All the chemicals were purchased from Sigma–Aldrich if not noted otherwise.

Animal handling and all procedures were performed strictly in accordance to the National Institutes of Health (NIH) guidelines and our Institutional Animal Care and Use Committee approved protocols.

**Single channel recording.** We used cell-attached patch-clamp technique to record single L-type Ca\(^{2+}\) channel activity in the ventricular myocyte. The bath solution contained (in mM) K-aspartate 110, KCl 30, MgCl\(_2\) 3.8, Hepes 5, EGTA 5, glucose 10, Mg-ATP 2, and CaCl\(_2\) 1.2, pH 7.3. High K\(^+\) concentration was used in bath to collapse the resting membrane potential to assist the determination of transmembrane voltage. The pipette solution contained (in mM) CaCl\(_2\) 10, TEA 120, Hepes 10, pH 7.4. FPL64176 10 μM was added into the pipette solution to prolong the channel open duration [6] to aid determination of the unitary current. The patch pipette was fabricated from borosilicate glass capillary and the tip diameter was made sub-μm to facilitate capturing a single channel in the patch. After compensating for the junction potential, a tight seal (>10 GM) was made between the pipette tip and the cell membrane to form the cell-attached configuration. Voltage-clamp protocols were generated and currents recorded using an Axopatch 200B amplifier and a DigiData 1200B A/D converter (Molecular Dynamics Inc.) controlled by a computer. The current signal was filtered through a 4-pole lowpass Bessel filter at a cut off frequency of 1 kHz, digitized at a sampling rate of 10 kHz. The membrane potential was held at –60 mV to inactivate the T-type Ca\(^{2+}\) channels, and then depolarized to various potentials for 200 ms to record the L-type Ca\(^{2+}\) channel activity.

**Results**

We recorded the single L-type Ca\(^{2+}\) channel activity in the freshly isolated rat ventricular myocytes with Ca\(^{2+}\) as the charge carrier. The voltage was held at –60 mV for 800 ms to inactivate the T-type Ca\(^{2+}\) channel, and then depolarized to various test potentials for recording of the unitary current through L-type Ca\(^{2+}\) channel. In order to better resolve the unitary current (which ranged from 0.1 to 1.0 pA with 10 mM Ca\(^{2+}\)) from background noise (~0.2 pA peak-to-peak) we used a known Ca\(^{2+}\) channel agonist, FPL64176, to promote the mode-2 opening of the L-type Ca\(^{2+}\) channel. Fig. 1 demonstrates two sets of most frequently recorded single channel activities. Panel A shows one set of representative traces of the channel activity at 0 mV depolarization, denoted O1. Notice that at the end of the test voltage pulse, some traces show the tail opening (pointed to by arrows) which is produced by continued channel opening under a sudden increase of the driving force upon repolarization. Panel B shows another set of representative traces of the channel activity, denoted O2. Importantly, the current amplitudes in the two sets of recordings are distinctively different. We plotted the amplitude histograms and best-fitted the data using the Gaussian function (Panels C and D). We then constructed the current–voltage (I–V) relationship using unitary currents calculated from the amplitude histograms (Panel E). To expand the range for I–V, we also included measurements from the tail openings obtained by stepping the membrane potential to –60 mV, –80 mV, and –120 mV. We used the I–V curve to calculate the single channel conductance, and obtained two clearly distinct conductance levels in the ventricular myocytes: 6.9 pS for O1 and 2.9 pS for O2.

The O1 group with 6.9 pS conductance was recorded in 13 out of 25 cells; the O2 group with 2.9 pS conductance was recorded in 11 out of 25 cells, demonstrate their frequent occurrences. The reversal potential for both conductance levels occurs near +50 mV, consistent to that reported by Hess et al. [1]. We did not see transition between the two conductance levels within a single open event, nor in any of the patches for recordings as long as 20–30 min.

We also used a ramp protocol to verify the above conductance measurements. Fig. 2A shows representative traces of the single channel opening during the voltage ramp. The slope of the open events is a direct measure of the single channel conductance. This measurement is facilitated by using FPL 64176 to promote the mode-2 events with long open durations. Notice the difference in the slopes of the currents. Panel B shows the I–V relations of the single channel openings. These openings can be clearly grouped into two clusters: O1 group and O2 group. The average conductance value of the O1 group is 6.5 ± 0.5 pS and of the O2 group is 2.9 ± 1.0 pS. These two conductance levels are in close agreement with those measured in Fig. 1.

On rare occasions we also observed three different conductance levels in a single patch. Fig. 3 depicts sample traces and the amplitude histogram of such a recording. The histogram can be best-fitted with the sum of four Gaussian functions: one for the closed

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**Fig. 2.** Conductance levels confirmed using the ramp protocol. We used a ramp protocol to measure channel conductance during a single open event (A). The I–V relationship shows two distinctive groups of conductance (B). The open events in the O1 group have a conductance of 6.5 ± 0.49 pS (mean ± SD, n = 10), and those in the O2 group have a conductance of 2.9 ± 1.00 pS (mean ± SD, n = 10).
The existence of multiple levels of the single L-type Ca\(^{2+}\) channel conductance in ventricular myocytes has significant physiological implications to the cardiac excitation–contraction coupling. During the action potential, L-type Ca\(^{2+}\) channels open to allow Ca\(^{2+}\) entry into the cell. The Ca\(^{2+}\) entering through a single channel open event can quickly reach the ryanodine receptors clusters located about 20 nm away in the dyadic junction, and trigger the opening of the ryanodine receptors to release larger amount of Ca\(^{2+}\) from the intracellular Ca\(^{2+}\) stores in SR; the resulting intracellular Ca\(^{2+}\) transient then causes the muscle contraction. Importantly, the open probability of ryanodine receptors is dependent on the amount of Ca\(^{2+}\) entry through a single L-type Ca\(^{2+}\) channel opening. Hence, different single channel conductance levels give rise to heterogeneity of the Ca\(^{2+}\) trigger signals; for example, the different unitary currents of 0.33 pA, 0.24 pA, and 0.17 pA in our observation would give rise to different amounts of Ca\(^{2+}\) entry during a single channel open event. The different Ca\(^{2+}\) trigger signal strength is expected to affect the efficacy of CICR at local dyadic junctions and thereby affecting the excitation–contraction coupling in cardiac muscle.

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References


