Title

Protocol to assess two distinct components of the nonlinear capacitance in mouse cardiomyocytes

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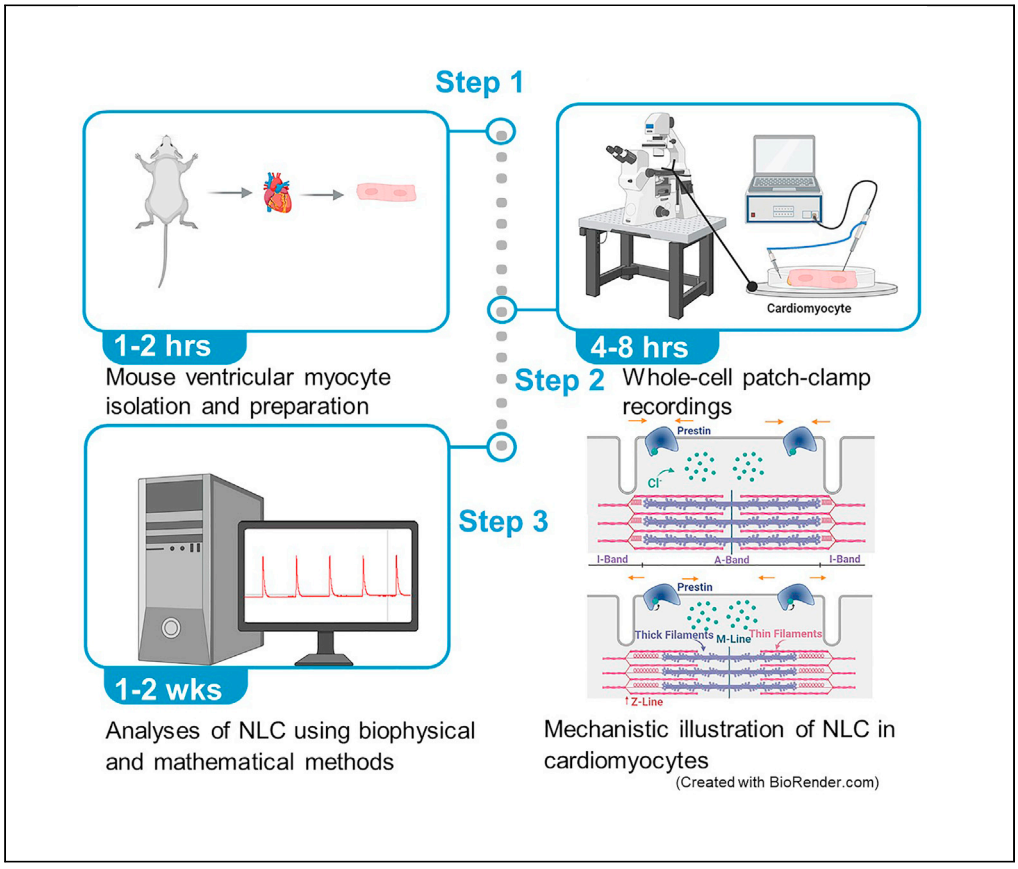

Prestin (Slc26a5) is a motor protein previously considered to be expressed exclusively in outer hair cells (OHCs) of the inner ear. However, we recently identified the functional expression of prestin in the heart. Nonlinear capacitance (NLC) measurement in OHCs is used to evaluate the signature function of prestin, which exhibits membrane potential-dependent conformational changes. Here, we describe detailed recording techniques and quantification methods for NLC to evaluate the prestin function in mouse ventricular myocytes.

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Highlights

Prestin is functionally characterized by nonlinear capacitance (NLC) in the cell

Prestin is expressed in cardiomyocytes

Prestin accounts partly for the NLC of cardiomyocytes

We describe recording techniques and quantification methods for NLC in cardiomyocytes

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Protocol to assess two distinct components of the nonlinear capacitance in mouse cardiomyocytes

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SUMMARY

Prestin (Slc26a5) is a motor protein previously considered to be expressed exclusively in outer hair cells (OHCs) of the inner ear. However, we recently identified the functional expression of prestin in the heart. Nonlinear capacitance (NLC) measurement in OHCs is used to evaluate the signature function of prestin, which exhibits membrane potential-dependent conformational changes. Here, we describe detailed recording techniques and quantification methods for NLC to evaluate the prestin function in mouse ventricular myocytes.

For complete details on the use and execution of this protocol, please refer to Zhang et al. (2021).

BEFORE YOU BEGIN

The protocol below describes the specific steps for nonlinear capacitance (NLC) recordings in mouse ventricular myocytes. However, the NLC recording protocol can also be applied to cardiomyocytes from other species, as well as other cell types including Chinese hamster ovary (CHO) cells with heteroexpression of prestin (Slc26a5). Additionally, the protocol can be used to study other determinants of membrane capacitance.

Prestin was previously considered to be exclusively expressed in the outer hair cells of inner ear, functioning as a cochlear motor protein and cochlear amplifier, characterized by NLC. However, prestin was recently identified to be functionally expressed in cardiomyocytes to amplify actin-myosin force generation accounting partly for the NLC of cardiomyocytes. Therefore, prestin serves a broader cellular motor function (Zhang et al., 2021). The function of prestin can be directly assessed by NLC recordings and analyses.

All animal care and procedures were performed in accordance with the protocols approved by Institutional Animal Care and Use Committee of the University of California, Davis and in accordance with National Institutes of Health guidelines. The experiments described in the protocol were conducted in a blinded fashion. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO), except specifically indicated. Ventricular myocytes were isolated from wild-type (WT) and *Slc26a5*$^{-/-}$ (*Prestin* knockout) mice.

Genotyping

Timing: 1–2 days

The *Slc26a5*$^{-/-}$ mouse model was generated previously (Liberman et al., 2002; Yamashita et al., 2015) and maintained in C57Bl/6J and SV-129 mixed background. Genotyping analyses were performed using RT-PCR of genomic DNA from WT and *Slc26a5*$^{-/-}$ mice. The primers used are as follow:

WT (Forward): 5′- GCTTGATGATTGGAGGTGTG-3′;

WT (Reverse): 5′-CTGAATGATTCCTGAAAGTAAGG-3′;

Slc26a5$^{-/-}$ (Forward): 5′-CTGTTGTCCAAGTGCTTGCC-3′

Slc26a5$^{-/-}$ (Reverse): 5′-GATCGCTATCAGGACATAGCG-3′

WT band is 175 bp, and Slc26a5$^{-/-}$ band is 500 bp.

PCR cycling conditions			
Steps	Temperature	Time	Cycles
Initial Denaturation	95°C	10 min	1
Denaturation	95°C	30 s	30–40 cycles
Annealing	55°C	30 s	
Extension	72°C	30 s	
Final extension	72°C	10 min	1
Hold	4°C	Forever	

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
HEPES	Sigma-Aldrich, USA	H4034
K-Glutamate	Sigma-Aldrich, USA	G1501
EGTA	Sigma-Aldrich, USA	E4378
TEA-Cl	Sigma-Aldrich, USA	T2265
TEA-OH	Sigma-Aldrich, USA	331635
SYLGARD™ 184	Dow Corning	025052
2,3-Butanedione monoxime (BDM)	Sigma-Aldrich, USA	B0753
Blebbistatin	Abcam, USA.	Ab120425
Salicylic acid	Sigma-Aldrich, USA	W398500
Collagenase type II	Worthington, USA	LS004176
Ketamine hydrochloride injection (100 mg/mL)	Vedco, Inc., USA	NDC 50989-161-06
Xylazine Sterile Solution (20 mg/mL)	Akorn Inc., USA	NDC59399-110-20
Buprenorphine hydrochloride injection (0.3 mg/mL)	Par Pharmaceutical, USA	NDC42023-179-05
Heparin (1000 USP units/mL)	Fresenius Kabi USA, LLC	NDC63323-540-57
Software and algorithms		
GraphPad Prism	GraphPad Software	https://www.graphpad.com
Origin 6.1 software	OriginLab Corp., Northampton, MA	https://originlab.com
BioRender	BioRender	https://biorender.com
pClamp10 software	Molecular Devices, LLC, Sunnyvale, CA	https://mdc.custhelp.com/app/

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
Slc26a5$^{-/-}$ knockout mouse model	Previously generated (Liberman et al., 2002)	N/A
C57Bl/6J mice	The Jackson Laboratory	https://www.jax.org/
SV-129 mice	Taconic Biosciences	https://www.taconic.com/
Oligonucleotides		
WT (forward)	(Liberman et al., 2002)	5′-GCTTGATGATTGGAGGTGTG-3′
WT (reverse)	(Liberman et al., 2002)	5′-CTGAATGATTCCTGAAAGTAAGG-3′
Slc26a5$^{-/-}$ (forward)	(Liberman et al., 2002)	5′-CTGTTGTCCAAGTGCTTGCC-3′
Slc26a5$^{-/-}$ (reverse)	(Liberman et al., 2002)	5′-GATCGCTATCAGGACATAGCG-3′
Other		
Axopatch 200A amplifier	Molecular Devices, LLC., Sunnyvale, CA	N/A
Digidata 1440 digitizer	Molecular Devices, LLC., Sunnyvale, CA	N/A
P-97 micropipette puller	Sutter Instruments, Novato, CA	N/A
Inverted microscope	Olympus, Tokyo, Japan	IX70
Microforge	NARISHIGE, Tokyo, Japan	MF-830
Osmometer	Wescor, Inc. USA	Vapro 5600
pH meter	Thermo Scientific, USA	Orion Star A111
Perfusion pump	Cole-Parmer Instrument Co., USA	MasterFlex
Pressure monitor	World Precision Instruments, USA	BP-1
Water bath temperature controller	Fisher Scientific, USA	Model 9105
ELGA Veolia water purification system	Veolia Water Systems Ltd, USA	Purelab Chorus PC1LSCXM2
0.22 μm Filter	EMD Millipore Corporation, USA	SCGP00525
Langendorff apparatus	Harvard Apparatus	EasyCell System for Cell Isolation, 73–4430
Nylon mesh cell strainer	Corning	431752
Glass capillaries	World Precision Instruments	TW150F-4
Silver/silver chloride ground electrode	World Precision Instruments	EP1

MATERIALS AND EQUIPMENT

Timing: 2–4 h

Solution preparations:

Note: Prepare all solutions using the water from ELGA Veolia water purification system (Purelab Chorus PC1LSCXM2) with a resistivity of 18.2 MΩ.cm.

Note: Prepare at room temperature (21°C–22°C).

Note: Filtration with a 0.22 μm filter.

Note: Store solutions at 4°C, and maximum time for storage is 7 days.

Note: Before use, warm the solution to room temperature.

- Blebbistatin stock solution: 100 mM blebbistatin stock solution is made by adding 855 μL of dimethyl sulfoxide (DMSO) to 25 mg of blebbistatin.

Refer to the key resources table and materials and equipment sections for the list of materials and equipment.

- Ca^{2+}-free Tyrode's solution (osmolarity: 292 ± 6 mOsmol/kg, n=7, i.e., seven independent measurements)

Reagent	Final concentration (mM)	Amount / 1 L
NaCl	140	8.18 g
KCl	5.4	0.40 g
$MgCl_2 \cdot 6H_2O$	1	0.20 g
D-Glucose	10	1.80 g
HEPES	10	2.38 g
pH 7.4 with NaOH		

- Ca^{2+}-free Tyrode's solution containing collagenase type II:

Add 40 mg collagenase type II to 50 mL Ca^{2+}-free Tyrode's solution to a final concentration of 0.8 mg/mL. The collagenase activity for digestion solution is ~224 U/mL based on the collagenase activity of ~280 U/mg.

- High potassium solution (osmolarity: 278 ± 7 mOsmol/kg, n=6)

Reagent	Final concentration (mM)	Amount / 1 L
K-Glutamate	120	24.38 g
KCl	20	1.49 g
$MgCl_2 \cdot 6H_2O$	1	0.20 g
D-Glucose	10	1.80 g
HEPES	10	2.38 g
EGTA	0.3	0.11 g
pH 7.4 with KOH		

- Bath and pipette solutions for NLC recordings (osmolarity: 265 ± 9 mOsmol/kg, n=6)

  Note: The same bath and pipette solutions will maintain equal osmolarity across the cell membrane, and the TEA-containing solutions on both sides will minimize ionic currents across the cell membrane. This will minimize contaminations by ionic currents, and avoid the asymmetrical osmolarity challenges to the cell membrane during NLC recordings, to improve the quality of NLC recordings.

Reagent	Final concentration (mM)	Amount / 1 L
TEA-Cl	145	24.03 g
$MgCl_2 \cdot 6H_2O$	2	0.41 g
D-Glucose	5	0.90 g
HEPES	10	2.38 g
EGTA	1	0.38 g
pH 7.4 with TEA-OH		

- Bath solutions containing 10 mM salicylate for NLC recordings (osmolarity: 277 ± 7 mOsmol/kg, n=6)

Reagent	Final concentration (mM)	Amount / 1 L
TEA-Cl	145	24.03 g
$MgCl_2 \cdot 6H_2O$	2	0.41 g
D-Glucose	5	0.90 g
HEPES	10	2.38 g
EGTA	1	0.38 g
Salicylic acid	10	1.38 g
pH 7.4 with TEA-OH		

- Bath solutions containing 10 mM BDM for NLC recordings (osmolarity: 270 ± 6 mOsmol/kg, n=6)

Reagent	Final concentration (mM)	Amount / 1 L
TEA-Cl	145	24.03 g
$MgCl_2 \cdot 6H_2O$	2	0.41 g
D-Glucose	5	0.90 g
HEPES	10	2.38 g
EGTA	1	0.38 g
2,3-Butanedione Monoxime	10	1.01 g
pH 7.4 with TEA-OH		

- Bath solutions containing 25 μM blebbistatin for NLC recordings (osmolarity: 265 ± 8 mOsmol/kg, n=6)

Reagent	Final concentration (mM)	Amount / 1 L
TEA-Cl	145	24.03 g
$MgCl_2 \cdot 6H_2O$	2	0.41 g
D-Glucose	5	0.90 g
HEPES	10	2.38 g
EGTA	1	0.38 g
Blebbistatin	0.025	0.25 mL of 100 mM stock solution
pH 7.4 with TEA-OH		

STEP-BY-STEP METHOD DETAILS

Mouse ventricular myocyte isolation and preparation

Timing: 1–2 h

Note: Preheat the water bath (Model 9105, Fisher Scientific, USA) of the Langendorff apparatus to 37°C.

1. Mouse anesthesia. Inject 80 mg/kg of ketamine and 5 mg/kg of xylazine to adult mice (both male and female, 12–16 weeks) intraperitoneally. Assess level of anesthesia by firm toe pinch.
2. Inject 300 USP unit sodium heparin intraperitoneally.
3. Perform a midline thoracotomy and heart excision.
4. Transfer the heart to a petri dish containing ice-cold Ca^{2+}-free Tyrode's solution.
5. Trim the connective tissues using a fine scissor.
6. Cannulate the heart through aorta onto a Langendorff apparatus prefilled with Ca^{2+}-free Tyrode's solution, gassed with 100% O_2. Retrogradely perfuse the heart with the Ca^{2+}-free

Tyrode's solution at 37°C, circulated by a perfusion pump (MasterFlex, Cole Parmer Instrument Co., USA).

7. Washout the blood by perfusion for 3 min, and adjust the perfusion speed at a flow rate of 4–6 mL/min and control the initial pressure at ≈75 mmHg using a pressure monitor (BP-1, World Precision Instruments, USA) connected to the Langendorff apparatus. Switched the perfusion solution to Ca^{2+}-free Tyrode's solution containing collagenase type II (0.8 mg/mL) for digestion of the tissue.
8. Continuous monitoring of the declining perfusion pressure for 15–20 min during tissue digestion by collagenase type II, until the pressure is below 30 mmHg. Check the color of the heart surface, and the softness of heart tissue to determine the digestion level.
9. Perfuse the heart with high potassium solution for 3 min. The high potassium solution depolarizes the membrane potentials of cardiomyocytes to allow cardiomyocytes and cardiac tissues to become less excitable.
10. Remove the heart from the perfusion apparatus and transfer to a petri dish containing high potassium solution. Mince the left ventricle and mechanically dissociate the tissue to obtain the cardiomyocytes by pipetting.
11. Harvest single isolated cardiomyocytes by filtering through nylon mesh cell strainer (100 μm) and store the cell in high potassium solution at room temperature for recording. The cardiomyocytes in high potassium solution will be quiescent and can be stored for 6–8 h.

Whole-cell patch-clamp recordings

Timing: 4–8 h

12. Recording glass patch pipette preparation. Pull borosilicate glass capillaries (World Precision Instruments, TW150F-4) to make glass patch pipette with a P-97 micropipette puller (Sutter Instruments, Novato, CA), and polish the pipette tip with a microforge (Narishige MF-830, Tokyo, Japan). The resistance of the electrodes is 1.5–3.0 MΩ when filled with the pipette solution.

⚠ CRITICAL: To minimize pipette capacitance, pipettes should be filled with a small amount of internal solution and relatively thicker wall glass capillaries should be used. Coating the pipette tip with a layer of SYLARD™ 184 (Dow Corning) will also reduce the pipette capacitance.

13. Set the voltage-clamp protocol. In Clampex 10 software, set the stair voltage protocol ranging from −140 to +100 mV with 10-mV increment as shown in Figure 1A.

14. Patch-clamp recordings. Seed cardiomyocytes in a recording chamber with a volume size of 0.5 mL connected to a gravity driven solution perfusion system. The perfusion speed is kept at 1–2 mL/min.

⚠ CRITICAL: To minimize pipette capacitance, the solution level is kept low to reduce the depth of the pipette immersion.

a. Connect the recording chamber with the silver/silver chloride ground electrode by a 3 M KCl agar bridge.
b. Five minutes after seeding the cells, healthy cardiomyocytes will attach to the cover glass of the recording chamber, and the perfusion of the solution will wash away the unhealthy and dead cells.
c. Use a square 5 mV test pulse with a holding potential of 0 mV for seal test, and form a seal with a resistance larger than ten giga-ohm.

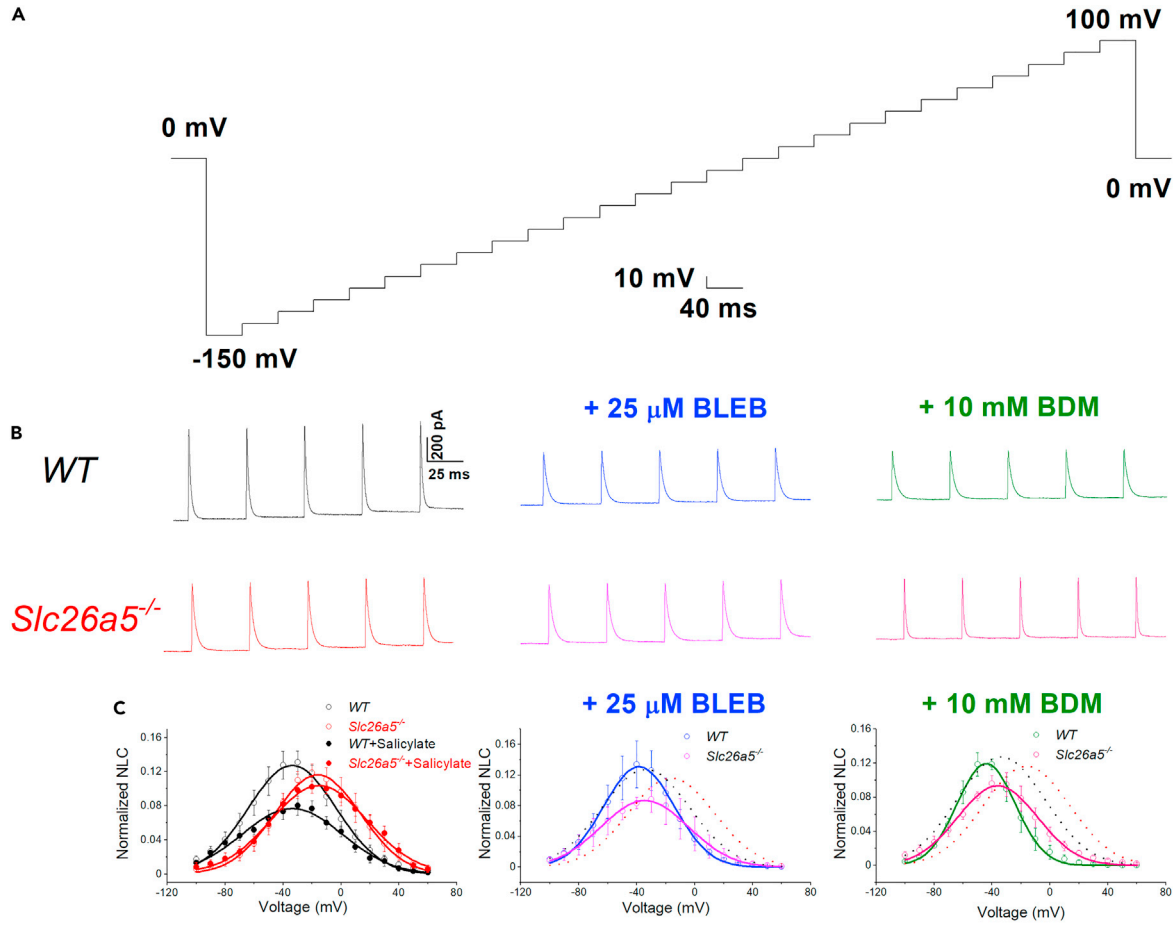

Figure 1. Nonlinear capacitance (NLC) in ventricular myocytes from WT and *Slc26a5*$^{-/-}$ mice

(A) The voltage stair protocol used for NLC recordings.

(B) Representative traces of capacitive currents recorded from WT and *Slc26a5*$^{-/-}$ cardiomyocytes at baseline (left panels), after application of 25 μM blebbistatin (BLEB, middle panels) and 10 mM BDM (right panels). Reprinted with permission from (Zhang et al., 2021).

(C) Normalized NLC as a function of voltage. The data is fitted with the first derivative of Boltzmann function. 10 mM salicylate is applied to inhibit the NLC as shown in the left panel (n=6 for WT, and n=5 for *Slc26a5*$^{-/-}$). In the presence of salicylate, Q_{max} /C_{ln} for WT cardiomyocytes is significantly reduced, while Q_{max} /C_{ln} for *Slc26a5*$^{-/-}$ cardiomyocytes is not significantly altered. NLC for WT and *Slc25a5*$^{-/-}$ at baseline are shown in dotted lines in the middle and right panels. The NLC of *Slc26a5*$^{-/-}$ myocytes at -20, -10, 0, 10, 20, 30, and 40 mV in the presence of BLEB or BDM is significantly smaller than that in the absence of BLEB and BDM ($p<0.05$). Reprinted with permission from (Zhang et al., 2021). Data represent mean ± SEM.

d. After giga-ohm seal formation, compensate the pipette capacitance in cell-attached mode by adjusting "Fast" and "Slow" pipette capacitance compensation knobs of the amplifier to minimize the pipette capacitive currents.
e. After rupturing the membrane in whole-cell configuration with a seal resistance of at least one giga-ohm, hold the membrane potential at 0 mV.
f. With continuous perfusion, perform whole-cell recordings at room temperature (21°C–22°C) to record the capacitive currents using the voltage stair protocol and an Axopatch 200A amplifier, Digidata 1440 digitizer, and pClamp10 software (Molecular Devices, LLC., Sunnyvale, CA). The recordings are digitally filtered at 1 kHz and digitized at 2 kHz.

15. Pharmacological interventions. To separate the NLC component resulting from prestin function, use bath solution containing 10 mM salicylate to perfuse the cells for 3 min, and then record the salicylate-sensitive capacitive currents during perfusion.

Note: 10 mM salicylate is used for effective NLC inhibition in outer hair cells with a half-maximal concentration of 1.6 mM (Kakehata and Santos-Sacchi, 1996; Zheng et al., 2000). The salicylate inhibitory effect on NLC is shown in Figure 1C, left panel.

Note: During the capacitive current recordings, we also add two different excitation-contraction (EC) uncouplers, 25 μM blebbistatin (BLEB) or 10 mM 2,3-butanedione monoxime (BDM) to the bath solution for 3 min to inhibit the myosin ATPase to disengage cellular excitation from contraction (Bond et al., 2013), and then record the capacitive currents during perfusion.

Analyses of NLC using biophysical and mathematical methods

Timing: 1–2 weeks

16. Use pClamp 10, Microsoft Excel, Origin 6.1, and GraphPad Prism software to analyze the capacity currents.
17. Determine total membrane capacitance (C_m) by analyzing the capacitive currents and measuring the input resistance (R_{in}) at each step voltage (V_c). $C_m=(R_{in}/R_m)^2Q/V_c$, where R_{in} ($R_{in} = R_m+R_s$) is the input resistance which can be determined by the steady state current and V_c; R_m is membrane resistance, R_s is the series resistance which can be calculated by the time constant (τ) of the capacitor discharge current, the charge movement (Q), step voltage and R_{in} ($R_s= \tau V_c R_{in}/(QR_{in}+\tau V_c)$).

Note: As part of R_s, the pipette resistance has been considered and accounted for in determining the C_m from the experimental data, and will not affect the quantification of NLC due to pipette resistance changes in different recordings.

18. Determine the NLC. $C_m= C_{ln} + C_v$, where C_{ln} is the linear capacitance, and C_v is the nonlinear capacitance. Normalized NLC can be expressed as $[C_m- C_{ln}]/ C_{ln}$. C_v is described as a function of voltage and fitted with the first derivative of a Boltzmann function describing the nonlinear charge's movement(Santos-Sacchi, 1991). Specifically, we plot C_v as a function of voltage and fitted with the first derivative of Boltzmann function: $C_v= (Q_{max}ze/kT)\exp(-ze(V-V_h)/kT)/(1+\exp(-ze(V-V_h)/kT))^2$, where V is the membrane potential, V_h is the voltage at half-maximal nonlinear charge transfer, e is the electron charge, k is Boltzmann's constant, T is the absolute temperature, z is the valence, and Q_{max} is maximum nonlinear charge transfer. The original recording traces of the total capacity currents are shown in Figure 1B. The normalized NLC is shown in Figure 1C. The total NLC has two components, one is generated from the voltage-gated ion channel gating charge movement, and another one results from prestin activities.

EXPECTED OUTCOMES

We record and compare the NLC of cardiomyocytes from WT and *Slc26a5*$^{-/-}$ mice (Zhang et al., 2021). Ablation of Slc26a5 results in a significant reduction and depolarization shift of NLC (V_h = −37 ± 2 mV, n = 11 and −18 ± 3 mV, n = 14 for WT and *Slc26a5*$^{-/-}$, respectively; $p<0.05$) as shown in Figure 1C, left panel; there are no significant changes of C_{ln} (167 ± 15 pF for WT, and 166 ± 12 pF for *Slc26a5*$^{-/-}$, n=14); Q_{max} /C_{ln} for WT is significant larger than that of *Slc26a5*$^{-/-}$ (10.81 ± 0.99 v.s. 7.74 ± 1.03 nC/μF, $p<0.05$, n=11 for WT and n=14 for *Slc26a5*$^{-/-}$). In the presence of 10 mM salicylate, Q_{max} /C_{ln} for WT cardiomyocytes is significantly reduced (7.21 ± 1.33 nC/μF, n=6, $p<0.05$), while Q_{max} /C_{ln} for *Slc26a5*$^{-/-}$ cardiomyocytes is not significantly altered (7.56 ± 1.41 nC/μF, n=5). The voltage sensitivity, K (kT/ze), can be calculated based on the z values with K = 17.16 ± 1.15 mV (n=11 for WT) and 15.20 ± 1.31 mV (n=14 for *Slc26a5*$^{-/-}$), p=NS.

The excitation-contraction uncouplers, BLEB and BDM significantly restrict the dynamic range of NLC, and the amplitude is markedly reduced in *Slc26a5*$^{-/-}$ cardiomyocytes as shown in Figure 1C,

middle and right panels. V_h for *Slc26a5*$^{-/-}$ ventricular myocytes showed a depolarization shift in the presence of BLEB and BDM, similar to that in the absence of BLEB and BDM. V_h for WT and *Slc26a5*$^{-/-}$ ventricular myocytes after BLEB are -37 ± 5 (n = 4) and -25 ± 3 (n = 5) mV, respectively. V_h for *WT* and *Slc26a5*$^{-/-}$ ventricular myocytes after BDM are -43 ± 4 (n = 3) and -32 ± 3 (n = 7) mV, respectively. NLC for WT and *Slc25a5*$^{-/-}$ at baseline are shown in dotted lines in the middle and right panels. The NLC of *Slc26a5*$^{-/-}$ myocytes at −20, −10, 0, 10, 20, 30, and 40 mV in the presence of BLEB or BDM is significantly smaller than that in the absence of the myosin inhibitors ($p<0.05$). The effects of BLEB and BDM on NLC suggest the functional coupling of myofilaments with prestin, and possibly the regulation of ion channel gating properties by prestin in cardiomyocytes.

QUANTIFICATION AND STATISTICAL ANALYSIS

We estimate a sample size of 4 per experiment to detect at least 15% difference between WT and *Slc26a5*$^{-/-}$ groups with alpha = 0.05 for a two-tailed test to achieve a power of 95%, assuming the standard deviation of the differences to be 5% (SigmaStat, Systat Software Inc.). No data were excluded. Data are presented as mean ± SEM. Statistical comparisons were analyzed by t test (two-tailed). Statistical significance was considered to be achieved when $p < 0.05$.

LIMITATIONS

NLC recorded by the voltage protocol includes not only the component from prestin activities but also NLC component resulting from voltage-gated ion channel gating charge movement. This is a limitation for the quantification of NLC in WT mouse cardiomyocytes. However, we took advantage of the *Slc26a5*$^{-/-}$ cardiomyocytes and specific prestin inhibitor to dissect the NLC component resulting from prestin.

TROUBLESHOOTING

Problem 1

Cardiomyocyte quality control is critical for the patch-clamp recordings. The isolation procedures of mouse cardiomyocytes need to be optimized during the experiments. (Steps 1–11)

Potential solution

Heart excision, trimming and cannulation need to be rapid and precise. Heart excision should be properly performed to ensure the remaining ascending aorta section is long enough for cannulation. The aorta should be securely cannulated to ensure that there is no leak for the maintenance of proper retrograde perfusion. The concentrations of collagenase type II may need to be adjusted and optimized with each new batch of collagenase.

Problem 2

Cardiomyocytes may not seed well on the cover glass of the chamber depending on the cell quality, glass type and surface quality. The perfusion may wash away the cells during recordings. (Step 14)

Potential solution

Choose cover glass from different manufacturers (for example Corning, VWR) may help. Another solution is to coat the cover glass with 0.1% (w/v) Poly-L-Lysine for 30 min before seeding the cells.

Problem 3

Leak currents can affect the capacitive current recordings and make the data analysis difficult and generate errors in NLC analyses. Therefore, it is paramount to obtain a very high and consistent giga-ohm seal throughout the recordings to minimize the leak current in the capacitive current recordings. (Step 14)

Potential solution

A very high and consistent giga-ohm seal (at least greater than 1 giga-ohm for the whole-cell configuration) needs to be achieved and maintained during the recordings by altering the pipette-tip

shape and polishing, optimizing the cardiomyocyte isolation protocol through setting the collagenase digestion time, perfusion pressure, and improving the recording techniques. Obtaining healthy and high-quality ventricular myocytes is also essential for successful recordings.

Problem 4

Ventricular myocytes are relatively large with electromotility and multiple volage-gated ion channels embedded in the cell membrane, and the total NLC represents only ~10% of the total linear capacitance. Furthermore, the NLC from prestin activities represents a very small component of the total NLC. (Steps 15, 17, and 18)

Potential solution

The capacity current recordings on cardiomyocytes need to be of very high quality in order to differentiate between these two distinct NLC components, especially the one originated from prestin.

Problem 5

NLC analysis is time-consuming that includes curve fitting for each step voltage and the fitting of NLC data by the first derivative of a Boltzmann function to extract the related parameters. The errors introduced during this step will significantly change the results. (Steps 17 and 18)

Potential solution

High-quality capacitive current recordings are critical for precise NLC analysis. At the same time, each curve fitting needs to be examined to ensure good fitting, even performed by automatic computer analysis. Finally, we need to choose the appropriate software tools for the Boltzmann function fitting.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Xiao-Dong Zhang (xdzhang@ucdavis.edu).

Materials availability

This study did not generate new unique reagents or animal models.

Data and code availability

The data supporting the current study have not been deposited in a public repository because they are only used for the publication of this manuscript but are available from the corresponding author on request.

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AUTHOR CONTRIBUTIONS

Conceptualization, X.-D.Z., E.N.Y., and N.C.; experiments and data analyses, X.-D.Z., M.C.P.F., and V.T.; resources, E.N.Y., N.C., and X.-D.Z.; funding acquisition, X.-D.Z., E.N.Y., and N.C.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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