Cl^-/HCO_3^- Exchanger slc26a6

A pH Regulator Shapes the Cardiac Action Potential

See Article by Sirish et al

Ernesto A. Aiello, PhD*
Joseph R. Casey, PhD
Bernardo V. Alvarez, PhD*

Regulation of intracellular pH (pH_i) is often considered a housekeeping function, contributing little to cardiac contractile activity. With the study published by Sirish et al[1] in this issue of *Circulation: Arrhythmia and Electrophysiology*, a Cl^-/HCO_3^- exchanger is revealed to have a more central role in the heart.

pH_i is an important modulator of cardiac excitation and contraction[2] and can adversely contribute to electric arrhythmia.[3] Correspondingly, cardiac myocytes express a complex apparatus to regulate pH_i. Cardiac muscle cytosolic pH (≈7.2) is maintained by sarcolemmal ion transport proteins that move H^+, OH^-, or HCO_3^- ions across the membrane.[4] Along with the acid extruders, Na^+/H^+ exchanger 1 and Na^+/HCO_3^- cotransporter (NBC, electrogenic NBCe1/e2 and electroneutral NBCn1) myocytes possess Cl^-/HCO_3^- exchangers (SLC4 family members AE1, AE2, and AE3) and Cl^-/OH^- exchanger (with no molecular identity) alkali extruders.[4] *SLC26* gene family members were identified as (mouse slc26a6[5] and its human orthologue SLC26A6,[6] and slc26a3[7]) responsible for Cl^-/HCO_3^- and Cl^-/OH^- exchange at plasma membrane of heart ventricles.[7]

The work of Sirish et al[1] in this issue revealed that ablation of *slc26a6*, encoding a plasma membrane Cl^-/HCO_3^- exchange protein, results in cardiac action potential (AP) shortening, cardiomyocyte Ca^{2+} transient and sarcoplasmic reticulum Ca^{2+} load reduction, cardiomyocyte diminution of sarcomeric shortening, and cardiomyocyte pH_i elevation. In *slc26a6*$^{-/-}$ mice, these factors led to a reduction of cardiac fractional shortening and cardiac contractility responses and altered cardiac conduction system, as seen in sinus bradycardia and fragmentation of the QRS electrocardiographic-recorded complex. Because slc26a6 has stoichiometry of 2 (or more) HCO_3^-: Cl^-, its transport function is electrogenic, with significance to the cardiomyocyte membrane potential.

Sirish et al[1] suggested that slc26a6 may be the predominant acid loader of cardiomyocytes because resting pH_i shifted to a more alkaline steady-state pH_i in isolated myocytes from *slc26a6*$^{-/-}$ mice compared with controls. Furthermore, recovery from acetate-induced alkalinization was severely impaired in *slc26a6*$^{-/-}$ cardiomyocytes. Underscoring the importance of slc26a6 in the heart, at the transcript levels, slc26a6 was the predominant Cl^-/HCO_3^- and Cl^-/OH^- exchanger of mouse myocardium.[7] In this earlier study, slc26a6 displayed comparable HCO_3^- transport activity to the AE3 Cl^-/HCO_3^- exchanger. On balance, it was concluded that cardiac Cl^--dependent acid loading results largely from the activity of slc26a6. In a similar scenario, *ae3* gene ablation in mice resulted in no change in steady-state pH while the rate of recovery of pH_i from imposed alkalosis was significantly slower in *ae3*$^{-/-}$ cardiomyocytes.[8] Discrepancy at steady-state pH_i levels between these findings may arise from changes in the pattern of expression of other transporters on *ae3* gene knockout, with upregulation of the AE1 Cl^-/HCO_3^- exchanger and Na^+/H^+

*These authors are established investigators of CONICET, Argentina.

Correspondence to: Joseph R. Casey, PhD, Department of Biochemistry, University of Alberta, Edmonton, T6G 2H7, Canada. E-mail joe.casey@ualberta.ca

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exchanger 1 at the protein level. Thus, changes in the expression levels of other transporters in the *slc26a6*$^{-/-}$ mice cannot be discarded.

Sirish et al[1] found that *slc26a6*$^{-/-}$ mice presented short cardiac AP, which leads to a decreased Ca^{2+} influx through L-type Ca^{2+} channels, reduced Ca^{2+} transient, and reduced sarcomeric fractional shortening. Changes in pH_i directly affect cardiac contractility, and the cellular acidification reduces Ca^{2+} transient and contraction in cardiomyocytes by potentially decreasing the binding of Ca^{2+} to troponin C and by affecting cross-bridges action leading to maximal force reduction.[2,9] Conversely, intracellular alkalosis increases twitch tension, resting tonic tension, voltage-dependent tonic tension, and after-contraction contractile parameters in sheep cardiac fibers.[10] In this issue of *Circulation: Arrhythmia and Electrophysiology*, Sirish et al[1] demonstrated an opposite effect of alkaline pH_i on the myocardium of *slc26a6*$^{-/-}$ mice with reduced cardiac contractility, linking the higher cardiomyocyte pH_i to a decrease in the sarcoplasmic reticulum store Ca^{2+} loading and to other cellular mechanisms that require future consideration.

The participation of electrogenic pH-regulatory transporters, like slc26a6, in shaping the cardiac AP waveform is an interesting issue, deserving fuller discussion in the literature. Regarding the influence of HCO_3^- transporters on AP, many electrophysiological studies omit HCO_3^- in the composition of extracellular solutions, masking the physiological relevance of these mechanisms. Early experiments performed in canine Purkinje fibers showed that lowering extracellular HCO_3^- at constant extracellular pH produced depolarization of resting membrane potential and AP duration lengthening.[11] These effects, which were suggested to be because of changes in a background HCO_3^- current, are consistent with the stimulation of an inward HCO_3^- current through slc26a6. However, a reduction of an outward HCO_3^- current mediated by NBCe1 (with cotransported stoichiometry Na^+: 2 HCO_3^-) can also account for these changes in AP duration.[12] Thus, there may be HCO_3^- currents in opposite directions in cardiomyocytes: a depolarizing current generated by slc26a6 and a repolarizing one produced by NBCe1. Hence, ablation of *slc26a6* would favor the repolarizing effect mediated by NBCe1, in agreement with the experiments of Shiri et al.[1] It would be interesting to know the sarcolemmal localization of slc26a6 because the potential presence of this transporter in the T-tubules, as is the case for NBCe1,[13] would predict an important role in excitation–contraction coupling. The reduction of cardiac Ca^{2+} transient amplitude in the *slc26a6*$^{-/-}$ mice reported by Sirish et al[1] supports this idea.

The work of Sirish et al[1] raises additional questions about the role of slc26a6 in the cardiac force–frequency response. Because the equilibrium potential for HCO_3^- is ≈+36 mV, the inward HCO_3^- current should be greater at values close to the resting membrane potential than at plateau potentials. This is consistent with the difference observed at AP $duration_{90}$ but not at AP $duration_{50}$ between wild-type and *slc26a6*$^{-/-}$ mice. No differences were observed at resting membrane potential values, a finding that warrants additional study to reach a full explanation. Nevertheless, the electrogenicity and stoichiometry of slc26a6 transport activity suggest that increased contractile frequency would induce decreased slc26a6 activity, increasing cardiomyocyte pH_i. Interestingly, an increase in pH_i was detected with increased pacing rate in heart papillary muscles, only in the presence of HCO_3^- in the extracellular milieu, an effect originally attributed to the activation of NBCe1.[14]

The article by Sirish et al[1] reports cardiac AP shaping arising from slc26a6 activity in mouse heart. Earlier, Clark et al[15] described striking differences between transport activity of mouse slc26a6 and its human orthologue, SLC26A6. Mouse slc26a6 mediates bidirectional electrogenic oxalate/Cl^- exchange while human SLC26A6-mediated oxalate transport was electroneutral.[15] The present article demonstrates electrogenic function by the murine slc26a6 orthologue as reported earlier, which makes this finding interesting for its potential significance to human cardiac physiology. Furthermore, Sirish et al[1] also described the cloning of 2 human SLC26A6 splice forms from human heart. Remarkably, both variants are functional electrogenic Cl^-/oxalate and Cl^-/HCO_3^- exchangers and thus electrophysiologically relevant.

An important in vivo decrease in fractional shortening associated with a detectible in vitro reduction of cell shortening, computed tomographic amplitude, and sarcoplasmic reticulum Ca^{2+} load were observed in the hearts from the *slc26a6*$^{-/-}$ mice.[1] Fragmented QRS was also measured in these transgenic mice. However, these effects, which could resemble the phenotype of cardiomyocytes from failing hearts,[16,17] are not accompanied by changes in cardiac hypertrophy or fibrosis.

Together the work of Sirish et al[1] reveals distinct characteristics of the hearts from *slc26a6*$^{-/-}$ mice, which provide an exciting model to study electrophysiological consequences of proarrhythmogenic threats. Clearly, the slc26a6 Cl^-/HCO_3^- exchanger contributes far more than housekeeping roles to the heart function and needs to be considered for its role in normal cardiac function and disease processes.

AFFILIATIONS

From the Department of Biochemistry, Membrane Protein Disease Research Group, University of Alberta, Edmonton, Canada (J.R.C.); and Centro de Investigaciones Cardiovasculares CIC-CONICET, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Argentina (E.A.A., B.V.A.).

DISCLOSURES

None.

FOOTNOTES

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