SIII-2

Stoichiometry of Na^+/Ca^{2+} Exchange Quantified with Ion-selective Microelectrodes in Giant Excised Cardiac Membrane Patches

Tong Mook Kang[1] and Donald W. Hilgemann[2]

[1]Department of Physiology, Sungkyunkwan University School of Medicine, Suwon 440-746, Korea

[2]Department of Physiology, The University of Texas Southwestern Medical Center at Dallas, Dallas, Tx 75390, U.S.A.

Without a definitive resolution of stoichiometry of cardiac Na^+-Ca^{2+} exchange (NCX), we cannot proceed to any quantitative analysis of exchange function as well as cardiac excitation-contraction coupling. The stoichiometry of cardiac NCX, however, is presently in doubt because reversal potentials determined by various groups range between those expected for a 3-to-1 and a 4-to-1 flux coupling. For a new perspective on this problem, we have used ion-selective microelectrodes to quantify directly exchanger-mediated fluxes of Ca^{2+} and Na^+ in giant membrane patches. Ca^{2+}- and Na^+-selective microelectrodes, fabricated from quartz capillaries, are placed inside of the patch pipettes to detect extracellular ion transients associated with exchange activity. Ion changes are monitored at various distances from the membrane, and the absolute ion fluxes through NCX are determined via simulations of ion diffusion and compared with standard ion fluxes (Ca^{2+} fluxes mediated by Ca^{2+} ionophore, and Na^+ fluxes through gramicidin channels and Na^+/K^+ pumps). Both guinea pig myocytes and NCX1-expressing BHK cells were employed, and for both systems the calculated stoichiometries for inward and outward exchange currents range between 3.2- and 3.4-to-1. The coupling ratios do not change significantly when currents are varied by changing cytoplasmic [Ca^{2+}] or by adding cytoplasmic Na^+. The exchanger reversal potentials, measured in both systems under several ionic conditions, range from 3.1- to 3.3-to-1. Taken together, a clear discrepancy from a NCX stoichiometry of 3-to-1 was obtained. Further definitive experiments are required to acquire a fixed number, and the present working hypothesis is that NCX current has an extra current via 'conduction mode'.