Calcium and ATP-Dependent Potentiation of Na/Ca Exchange Function in Intact Cells: Comparison of NCX1 and NCX3 Exchangers

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Na+/Ca2+ exchangers (NCX) mediate bidirectional Ca2+ fluxes across cell membranes and contribute to Ca2+ homeostasis in many cell types. Exchangers are regulated by gating reactions that depend on Na and Ca binding to both transport and regulatory sites. A Na+i-dependent inactivation is prominent in all isoforms, whereas Ca2+i-dependent regulation varies among isoforms. Here we describe differences between NCX3 and NCX1 regulation by Ca and the long-term stimulation (potentiation) of exchange function by Ca transients in intact cells. To compare isoforms, we employed BHK cells expressing NCX1 and NCX3 constitutively. Outward currents, reflecting Ca influx, are activated by applying extracellular Ca (Cao) in the presence of Na on the cytoplasmic side. Outward NCX3 currents decay over seconds and then develop a second transient peak when cytoplasmic Ca2+ is not heavily buffered. When NCX3 current is terminated by removing Cao and then reactivated after a delay, the reactivated current is potentiated with respect to initial currents for minutes. Both the second transient peak and the stimulation of exchange function are suppressed by replacing cytoplasmic ATP with AMP-PNP or ATPγS, even though initial exchange currents remain large. In contrast, in BHK cells expressing NCX1, outward exchange current decays to a steady-state level during single Cao application, and peak currents typically decline during multiple activation episodes. However, an ATP-dependent stimulation of NCX1 current is unmasked by increasing cytoplasmic Ca buffering in BHK cells, and current stimulation is then also resolved in cardiac myocytes and iCell myocytes. Finally, we describe for the first time that inward (forward) exchange currents can inactivate in the absence of Na+i. In patches from BHK cells, inward current inactivation is pronounced for NCX3, and inactivation is alleviated by chymotrypsin treatment. In conclusion, our results suggest that NCX function is regulated more richly than appreciated heretofore, possibly including processes that are lost in excised membrane patches.