Rapid Ca\(^{2+}\) Extrusion Via the Na\(^{+}/Ca^{2+}\) Exchanger of the Human Platelet

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Summary. This communication reports the kinetics of the Na\(^{+}/Ca^{2+}\) exchanger and of the plasma membrane (PM) Ca\(^{2+}\) pump of the intact human platelet. The kinetic properties of these two systems were deduced by studying the rate of Ca\(^{2+}\) extrusion and its Na\(^{+}\) dependence for concentrations of cytoplasmic free Ca\(^{2+}\) (\([Ca^{2+}]_{cyt}\)) in the 1-10\(\mu\)M range. The PM Ca\(^{2+}\)-ATPase was previously characterized (Johansson, J.S. Haynes, D.H. 1988. J. Membrane Biol. 104:147-163) for \([Ca^{2+}]_{cyt}\) \(\leq 1.5\) \(\mu\)M with the fluorescent Ca\(^{2+}\) indicator quin2 (\(K_d = 115\) riM). That study determined that the PM Ca\(^{2+}\) pump in the basal state has a \(V_{max} = 0.098\) mM/min, a \(K_m = 80\) nm and a Hill coefficient = 1.7. The present study extends the measurable range of \([Ca^{2+}]_{cyt}\) with the intracellular Ca\(^{2+}\) probe, rhod2, \(K_d = 500\) nm, which has almost a fivefold lower affinity for Ca\(^{2+}\). An Appendix also describes the Mg\(^{2+}\) and pH dependence of the \(K_f\) and fluorescence characteristics of the commercially available dye, which is a mixture of two molecules. Rates of active Ca\(^{2+}\) extrusion were determined by two independent methods which gave good agreement: (i) by measuring Ca\(^{2+}\) extrusion into a Ca\(^{2+}\)-free medium (above citation) or (ii) by the newly developed "ionomycin short-circuit" method, which determines the ionomycin concentration necessary to short circuit the PM Ca\(^{2+}\) extrusion systems. Absolute rates of extrusion were determined by knowledge of how many Ca\(^{2+}\) ions are moved by ionomycin per minute. The major findings are as follows: (i) The saturable is with respect to Ca\(^{2+}\) with a \(K_m = 0.97 \pm 0.31\) \(\mu\)M and \(V_{max} = 1.0 \pm 0.6\) mM/min. (ii) At high \([Ca^{2+}]_{cyt}\), the exchanger works at a rate 10 times as large as the basal \(V_{max}\) of the PM Ca\(^{2+}\) extrusion pump. (iii) The exchanger can work in reverse after Na\(^{+}\) loading of the cytoplasm by monensin. (iv) The PM Ca\(^{2+}\) extrusion pump is activated by exposure to \([Ca^{2+}]_{cyt} \geq 1.5\) \(\mu\)M for 20-50 sec. Activation raises the pump \(V_{max}\) to 1.6 \pm 0.6 \(\mu\)M/min and the \(K_m = 0.55 \pm 0.24\) \(\mu\)M. The PM Ca\(^{2+}\) buffering capacity of the cytoplasm is 3.6 \(\mu\)M in the 0.1 to 3 \(\mu\)M range of \([Ca^{2+}]_{cyt}\). In summary, the results show that the human platelet can extrude Ca\(^{2+}\) very rapidly at high \([Ca^{2+}]_{cyt}\). Both the Na\(^{+}/Ca^{2+}\) exchanger and Ca\(^{2+}\) pump activation may prevent inappropriate platelet activation by marginal stimuli.

Key Words Na\(^{+}/Ca^{2+}\) exchanger · plasmalemmal Ca\(^{2+}\) - Mg\(^{2+}\) ATPase · platelets, human · fluorescent Ca\(^{2+}\) indicators (rhod2 & quin2) · kinetics of Ca\(^{2+}\) extrusion · calmodulin activation

Introduction

Cytoplasmic Ca\(^{2+}\) activity (\([Ca^{2+}]_{cyt}\)) plays an important regulatory role in platelet function. Figure 1 is a schematic of the mechanisms involved in main-
of Ca\(^{2+}\) ions moved into the cytoplasm; \(\Delta [\text{rhod2-Ca}]_{\text{cyt},t}\) change in concentration of total intracellular high-affinity rhod2 complexed to Ca\(^{2+}\); \(\Delta [B-\text{Ca}]_t\), change in concentration of total cytoplasmic binding sites complexed to Ca\(^{2+}\); \(\Delta [\text{quin2}]_{\text{cyt},t}\), change in concentration of total intracellular quin2 complexed to Ca\(^{2+}\); \(\Delta \alpha\), change in the degree of intracellular quin2 saturation; \(\Delta \alpha/\Delta t\), rate of change in degree of saturation of cytoplasmic high-affinity rhod2; \(\Delta \alpha/\Delta t\), rate of change in degree of saturation of cytoplasmic high-affinity rhod2; \(V_{\text{obs}}\), observed rate of Ca\(^{2+}\) removal from the rhod2-Ca complex; \(V_{\text{qu}}\), rate of Ca\(^{2+}\) removal from the high-affinity rhod2-Ca complex at [Ca\(^{2+}\)]\(_{\text{cyt}}\) = 8.3 \(\mu M\); \(\Delta \alpha/\Delta t\), rate of change in the degree of quin2 saturation; \(\alpha\), initial linear rate of ionomycin-mediated Ca\(^{2+}\) influx; EC\(_{50}\), effective concentration giving a half-maximal effect; [Na\(^{+}\)]\(_{\text{cyt}}\), cytoplasmic Na\(^{+}\) activity; CAM, calmodulin; ACN, acetonitrile; and TFA, trifluoroacetic acid.

The earliest evidence for the presence of a Na\(^{+}/\)Ca\(^{2+}\) exchanger in the human platelet was provided by Rengasamy, Soura and Feinberg (1987). These investigators showed that plasma membrane vesi-