PLASMA MEMBRANE CA^{2+}-ATPASE SULFHYDRYL MODIFICATIONS: IMPLICATION FOR OXIDIZED RED CELL

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Abstract. A common perturbation found in cells under oxidative stress is alteration in cellular Ca^{2+} homeostasis. In order to understand the effects of such oxidative damage, human red cell plasma membrane Ca^{2+}-ATPase (PMCA) was studied by measuring PMCA activity, both in the presence and absence of calmodulin (CaM), following treatment with sulfhydryl agents, N-ethylmaleimide, iodoacetate and diamide. PMCA activity of washed red cell membrane was measured by coupling with pyruvate kinase, using phosphoenolpyruvate as substrate, and lactate dehydrogenase to convert pyruvate to lactate employing β-NADH as co-factor. All treatments inhibited basal and CaM-stimulated activity in a dose-dependent manner (0.01-1 mM), but at low concentrations, basal Ca^{2+}-ATPase activity was inhibited whereas CaM-stimulated activity was unaffected. Inhibition by diamide, a disulfide-forming agent, was reversed with dithiotreitol (DTT). Treatment with calpain, a calcium-dependent protease, elevated basal PMCA activity to CaM-stimulated level, but abolished response to CaM. Further treatment with diamide inhibited PMCA activity, which could be restored by DTT, but only to basal and not CaM-stimulated level. These studies indicated that it is necessary to protect against both sulfhydryl and proteolytic damages to red cell PMCA if perturbation to Ca^{2+} homeostasis is to be minimized. This has implications for membranes under oxidative stress, such as in the hereditary anemia, thalassemia, where membrane-bound unmatched hemoglobin chains cause oxidative damage to red blood cells.

Keywords: Ca^{2+}-ATPase, calmodulin, PMCA, sulfhydryl agent, thalassemia

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