Effects of cholesterol depletion on the partial reactions of the Na^+, K^+-ATPase

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Cholesterol plays a significant role in several biological processes including the proper organisation of the phospholipid bilayer in animal cells. Determining the effects of cholesterol on Na^+, K^+-ATPase (NKA) activity has been extensively studied in enzyme reconstituted in phospholipid vesicles. The use phospholipid vesicles allows for the specific control of the molar ratio of components within the bilayer. As a result of these studies, several binding sites for phospholipids have been postulated which support specific partial reactions in the NKA reaction cycle. However, determining the effect of cholesterol on the pump is not a straightforward task. The difficulty in accessing both sides of the membrane of phospholipid vesicle preparations makes measurements of certain partial reactions problematic. Apparently conflicting results have been reported, with both stimulation and inhibition of NKA activity being observed with different combinations of phospholipids and cholesterol. As such we set out to determine the effect of cholesterol depletion on the NKA in a system as close to the native phospholipid environment as possible by using purified pig kidney membrane fragments. In the series of experiments presented we depleted cholesterol from the purified pig kidney fragments with methyl-beta-cyclodextrin and measured the partial reactions of the enzyme using the stopped-flow experimental procedure with the voltage sensitive fluorescent dye RH421. Methyl-beta-cyclodextrin treated enzyme had a significantly reduced overall activity. We observed a significant reduction in the forward reaction rate of ATP phosphorylation ($E1Na^+_3$ to E2P) as well as a significant reduction in the forward reaction rate for K^+ occlusion by the phosphorylated enzyme and subsequent dephosphorylation (E2P to $E2K^+_2$). This method enables us to determine the effect of membrane cholesterol on NKA activity in the enzyme's physiologically relevant phospholipid environment.