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Characterization of the cardiac Na^+/K^+ pump by development of a comprehensive and mechanistic model

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ABSTRACT

A large amount of experimental data on the characteristics of the cardiac Na^+/K^+ pump have been accumulated, but it remains difficult to predict the quantitative contribution of the pump in an intact cell because most measurements have been made under non-physiological conditions. To extrapolate the experimental findings to intact cells, we have developed a comprehensive Na^+/K^+ pump model based on the thermodynamic framework (Smith and Crampin, 2004) of the Post–Albers reaction cycle combined with access channel mechanisms. The new model explains a variety of experimental results for the Na^+/K^+ pump current (I_{NaK}), including the dependency on the concentrations of Na^+ and K^+, the membrane potential and the free energy of ATP hydrolysis. The model demonstrates that both the apparent affinity and the slope of the substrate–I_{NaK} relationship measured experimentally are affected by the composition of ions in the extra- and intracellular solutions, indirectly through alteration in the probability distribution of individual enzyme intermediates. By considering the voltage dependence in the Na^+- and K^+-binding steps, the experimental voltage–I_{NaK} relationship could be reconstructed with application of experimental ionic compositions in the model, and the view of voltage-dependent K^+ binding was supported. Re-evaluation of charge movements accompanying Na^+ and K^+ translocations gave a reasonable number for the site density of the Na^+/K^+ pump on the membrane. The new model is relevant for simulation of cellular functions under various interventions, such as depression of energy metabolism.

1. Introduction

The Na^+/K^+ pump maintains the Na^+ gradient across the cell membrane by actively transporting 3 Na^+ ions out of the cell in exchange for 2 K^+ ions into the cell by utilizing the energy derived from ATP hydrolysis (ΔG_{ATP}). This Na^+ gradient is used to remove Ca^{2+} or H^+ from the cytosol via Na^+/Ca^{2+} exchange or Na^+/H^+ exchange, respectively. Thereby, the Na^+/K^+ pump has a central role in maintaining cellular Ca^{2+} and pH homeostasis, and excitation and contraction of cardiac muscle. Experimental characterization of the Na^+/K^+ pump current (I_{NaK}) has been conducted in cardiac myocytes using the whole-cell patch-clamp technique, and a large body of evidence on the dependence of I_{NaK} on intra- and extracellular Na^+ and K^+, ΔG_{ATP}, and membrane potential (V_m) has been accumulated. However, most of these data cannot be directly applied to physiological conditions because the experimental ionic and energetic conditions differ from those in intact cells. Therefore, development of a mathematical model of the cardiac Na^+/K^+ pump that can reconstruct major experimental findings is important for extrapolation of these findings to the pump function in intact cells. Such a model can facilitate development of working hypotheses for further experimental studies on the Na^+/K^+ pump.

Several types of Na^+/K^+ pump models have been published as one of the components in cardiac cell models, but most consist of a collection of descriptive statements. For example, in the Luo–Rudy model (Luo and Rudy, 1994), I_{NaK} was described as an empirical equation of intracellular Na^+, extracellular K^+ and V_m, which were mostly based on the work of Nakao and Gadsby (1989). According to the Post–Albers cycle reaction, which is used as one of the standard schemes for the Na^+/K^+ pump, it is clear that the activity of I_{NaK} is much more complicated. Even in models based on the Post–Albers cycle reaction there are still critical limitations; for example, our simple 6-state Na^+/K^+ pump model (Matsuoka et al., 2003) requires supplementation with the ΔG_{ATP} dependency of I_{NaK}. Recently, Smith and Crampin (2004) provided a theory for modeling active transport taking thermodynamic principles into account. However, their Na^+/K^+ pump model (Smith and Crampin, 2004; Terkildsen et al., 2007) was not optimized to reproduce the electrogenicity of the K^+/K^+ exchange

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deduced from the direct recording of charge movements (Peluffo and Berlin, 1997) and the pump activity at varying $[Na^+]$ or $[K^+]$ in the presence of Cs^+. Therefore, we attempted to improve the mathematical model of the Na^+/K^+ pump to account for the large variety of electrophysiological characteristics of the pump activity, and to produce a model that is applicable to cardiac cell models under normal conditions.

In the present study, the model parameters of the reduced Post–Albers reaction cycle were determined based on a wide range of experimental findings using the theoretical framework of Smith and Crampin (2004). By reconstructing the major experimental findings, we examined the mechanisms underlying the variation in the substrate–I_{NaK} relationship between experimental studies using different extra- and intracellular ion compositions. The variable pattern of V_m-dependency of I_{NaK} was also analyzed in relation to different experimental conditions. We also re-evaluated the charge movements accompanying Na^+ and K^+ translocation. A preliminary report of this work was presented at the 51st Annual Meeting of the Biophysical Society (Oka et al., 2007b) and at the 84th Japanese Physiological Society Annual Meeting (Oka et al., 2007a).

2. Methods

2.1. Development of the Na^+/K^+ pump model

The Na^+/K^+ pump exchanges 3 intracellular Na^+ ions for 2 extracellular K^+ ions using the free energy from hydrolysis of 1 ATP molecule. This stoichiometry is thought to be maintained by the formation of "occluded" states after ion binding. Based on this, we assumed that the activity of the Na^+/K^+ pump could be expressed with an unbranched Post–Albers reaction cycle (Fig. 1A), as suggested by Lauger and Apell (1986). For simplification, we assumed that the binding and release of Na^+, K^+ and MgATP are so fast that they could be approximated as equilibrium reactions defined by dissociation constants, K_d. Then, the scheme in Fig. 1A was simplified using a lumped 4-state model (Fig. 1B), which is the same approach as that of Smith and Crampin (2004). P_{1-6} is a sum of states whose binding sites are open to the intracellular side, and the lumped P_{8-13} represents states facing the extracellular side. P_7 and P_{14-15} are occluded states for Na^+ and K^+ ions, respectively. For the 4-state model, apparent rate constants, α, can be defined. For the forward reaction steps, α are given by

$$\begin{aligned}
\alpha_1^+ &= \frac{k_1^+ \overline{Na_i}^3}{(1+\overline{Na_i})^3+(1+\overline{K_i})^2-1},\\
\alpha_2^+ &= k_2^+,\\
\alpha_3^+ &= \frac{k_3^+ \overline{K_o}^2}{(1+\overline{Na_o})^3+(1+\overline{K_o})^2-1},\\
\alpha_4^+ &= \frac{k_4^+ \overline{MgATP}}{1+\overline{MgATP}}
\end{aligned} \tag{1}$$

and for the backward steps,

$$\begin{aligned}
\alpha_1^- &= k_1^-[MgADP],\\
\alpha_2^- &= \frac{k_2^- \overline{Na_o}^3}{(1+\overline{Na_o})^3+(1+\overline{K_o})^2-1},\\
\alpha_3^- &= \frac{k_3^-[Pi][H^+]}{1+\overline{MgATP}},\\
\alpha_4^- &= \frac{k_4^- \overline{K_i}^2}{(1+\overline{Na_i})^3+(1+\overline{K_i})^2-1},
\end{aligned} \tag{2}$$

where

$$\begin{aligned}
\overline{Na_i} &= \frac{[Na^+]_i}{K_{d,Na_i}}, \quad \overline{Na_o} = \frac{[Na^+]_o}{K_{d,Na_o}},\\
\overline{K_i} &= \frac{[K^+]_i}{K_{d,K_i}}, \quad \overline{K_o} = \frac{[K^+]_o}{K_{d,K_o}},\\
\overline{MgATP} &= \frac{[MgATP]}{K_{d,MgATP}},
\end{aligned} \tag{3}$$

where $k^{\pm}$ are the rate constants and $K_{d,X}$ are the dissociation constants for substrate X. The steady-state cycle rate of the Na^+/K^+ pump, v_{cyc}, is then given by the King–Altman method (Segel, 1993):

$$\begin{aligned}
v_{cyc} &= \frac{\alpha_1^+\alpha_2^+\alpha_3^+\alpha_4^+ - \alpha_1^-\alpha_2^-\alpha_3^-\alpha_4^-}{\Sigma},\\
\Sigma &= \alpha_1^-\alpha_2^-\alpha_3^- + \alpha_1^+\alpha_2^-\alpha_3^- + \alpha_1^+\alpha_2^+\alpha_3^- + \alpha_1^+\alpha_2^+\alpha_3^+\\
&\quad + \alpha_2^-\alpha_3^-\alpha_4^- + \alpha_2^+\alpha_3^-\alpha_4^- + \alpha_2^+\alpha_3^+\alpha_4^- + \alpha_2^+\alpha_3^+\alpha_4^+\\
&\quad + \alpha_3^-\alpha_4^-\alpha_1^- + \alpha_3^+\alpha_4^-\alpha_1^- + \alpha_3^+\alpha_4^+\alpha_1^- + \alpha_3^+\alpha_4^+\alpha_1^+\\
&\quad + \alpha_4^-\alpha_1^-\alpha_2^- + \alpha_4^+\alpha_1^-\alpha_2^- + \alpha_4^+\alpha_1^+\alpha_2^- + \alpha_4^+\alpha_1^+\alpha_2^+.
\end{aligned} \tag{4}$$

The Na^+/K^+ pump is an electrogenic transporter and is affected by V_m. In this study, we assumed that all the processes for ion binding and release in Fig. 1A are voltage-dependent, based on the

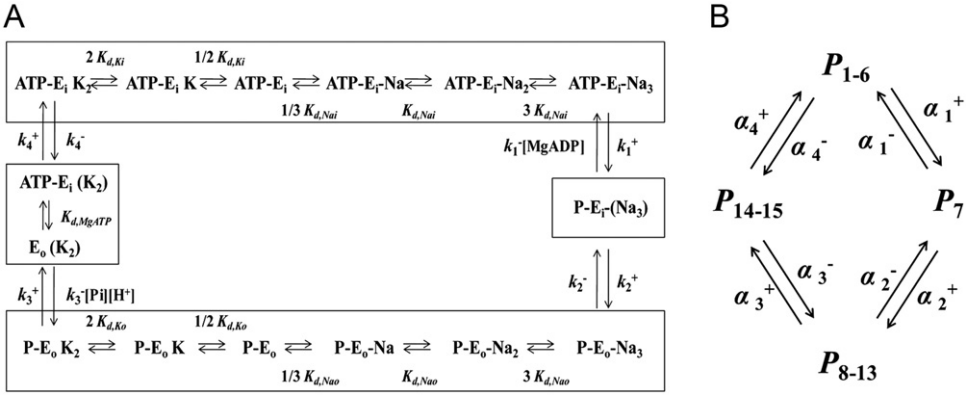

Fig. 1. Schematic representation of the Na^+/K^+ pump model. (A) The Post–Albers reaction cycle of 15 states based on Smith and Crampin (2004). E_o and E_i represent an Na^+,K^+ ATPase in which the binding site opens to the extra- and intracellular side, respectively. (B) Lumped four-state model of (A). Each state P is a sum of states which are enclosed by a corresponding black box in (A). A definition of the parameters is given in the Methods.