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# The asymmetric, rectifier-like $I–V$ curve of the Na/K pump transient currents in frog skeletal muscle fibers

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**Abstract**

The Na/K pump transient currents in skeletal muscle fiber were identified using an improved double Vaseline gap voltage clamp technique. The asymmetric characteristics of the pump current–voltage relationship were studied. The definition of the Na/K pump currents was the ouabain-sensitive currents, where ouabain is a specific Na/K ATPase inhibitor. Membrane potential was held at −90 mV, the membrane resting potential. A series of stimulation pulse-pairs symmetric to the membrane resting potential were applied to the cell membrane. The summation of the currents responding to the two pulses in each pair indicates the asymmetry of the pump currents with respect to the membrane resting potential. The voltage dependence of the Na/K pump transient currents from skeletal muscle is similar to the steady-state $I–V$ curve from either skeletal muscle fibers or cardiac muscles. It is a sigmoidal-shaped, asymmetric curve with respect to the membrane resting potential. This asymmetric, rectifier-like voltage dependence indicates that a symmetric oscillating membrane potential may generate a net, outward pump current. In other words, the Na/K pump molecules may be activated by an oscillating membrane potential.


*Keywords:* Na/K ATPase; Skeletal muscle; Voltage dependence; Oscillating electric field

## 1. Introduction

Electrogenic pump molecules in cell membrane are essential for active transport systems capable of moving ions across cell membrane against electrochemical gradients. By expending energy carried by ATP molecules, ions can be moved across the cell membrane from a low concentration compartment to a high concentration compartment.

Na/K ATPases are popular active transporters in many kinds of cell membranes. By extruding three $Na^+$ ions and pumping in two $K^+$ ions in one cycle at an expenditure of one ATP molecule, the Na/K ATPase generates a net outward transmembrane current. These molecular pumps play a significant role in maintaining the membrane resting potential, providing energy for secondary membrane transport systems and regulating the cell volume. Gadsby et al. [1] were the first to demonstrate the voltage dependence of the Na/K pump current in isolated heart cells.

The voltage dependence of the Na/K pump currents in skeletal muscle fibers has not been reported, even though the population of the pump molecules is large in skeletal muscles, especially in red muscle fibers. One of the possible reasons is the difficulty of obtaining accurate measurement of the transmembrane currents in skeletal muscle fibers using the traditional micropipette voltage clamp technique.

We have studied the voltage dependence of the Na/K ATPase currents by directly measuring the pump currents using our improved double Vaseline gap voltage clamp technique. Several methods have been used to quantitatively identify the amount of the pump current in a wide range of membrane potential. The results showed a sigmoidal-shaped, asymmetric characteristic curve of the pumps' current–voltage relationship with respect to the membrane resting potential. The current–voltage dependence of the skeletal muscle fibers is similar to the pumps' $I–V$ curve of cardiac myocytes obtained by others [2]. The pump current in skeletal muscle fiber is larger than those in cardiac muscle fiber because the pump molecular density in red skeletal muscle fiber is higher than that of cardiac muscle fibers.

However, all of these results obtained either form ventricle myocyte or skeletal muscle fibers, the currents were

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sampled 200 ms after the change of membrane potential. Consequently, the resultant voltage dependence only reflects the steady-state pump currents as a function of the membrane potential. In this paper, we now report our results in studying the transient changes of the pump currents as a function of the membrane potential. We would like to confirm whether the Na/K ATPase's current–voltage relationship retains an asymmetric, rectifier-like shape when the membrane potential quickly changes.

The pioneer work has been done by Tsissie and Tsong [3], who found that an oscillating electric field can increase the intracellular $K^+$ ions by measuring changes in radio active dye intensity. Further theories have been developed about electrocoupling of membrane proteins [4,5]. The results presented in this paper are among a series of studies in further investigation of how an oscillation electric field affects the electrogenic pump molecules by directly measuring the pump currents upon exposure to an electric field.

## 2. Materials and methods

### 2.1. Skeletal muscle fiber preparation

The protocol of single fiber preparation is followed from the others [6–9] and used in our laboratory with some improvements. Briefly, skeletal twitch muscles, *semitendinosus* and *illus*, were separated from American frogs, *Rana pipen.* A single fiber was hand-dissected and mounted in a custom-made chamber. The fiber was held by two clips at two ends. The fiber was electrically isolated into three pools by two Vaseline-seal partitions. The width of the two partitions and the width of the central pool are 100 and 300 μm, respectively. The fiber was stretched up to a sarcomere length of 3 μm. The purpose of the stretching is to avoid fiber contraction during electrical stimulations.

The sections of the fiber in two end pools were treated by a solution with 0.2% saponin for 2 min and then washed out, where the fiber segments were permeabilized electrically and ionically. The three pools were then connected to a voltage clamp by six agar bridges and three Ag/AgCl pallets. Resistance of these agar bridges and pallets was less than 1 K ohm. Stimulation pulses were delivered through a voltage clamp (Axon 2000 Whole Clamp) controlled by an IBM-compatible computer. Data were sampled by an Axon data acquisition board (AC 1200) and stored in a hard disk for further analysis.

### 2.2. Solutions

In order to compare the Na/K pump currents measured from skeletal muscle fibers and those measured from cardiac myocytes [1], the solution preparation was similar as that used in cardiac study. Even though the solution ingredients and concentration may differ because of a difference in the animals used (frog versus guinea pig), the principle of solution design is the same. The membrane conductance was purposely reduced by block $Na^+$, $K^+$ and $Ca^{2+}$ channels. The low membrane conductance can reduce the residual transmembrane current and increase the signal/noise ratio in the measurement of the Na/K pump currents. To reduce the outward $K^+$ current across the cell membrane, $K^+$ concentration in the internal solution is reduced to 10 mM by substituting with $Cs^+$ ions. In addition, 1 mM 3-4 Diaminopyridine (DAP) and 20 mM tetraethylammonium (TEA) were added in the external solution to block the $K^+$ channels. In the external solution, the nominal value of $Ca^{2+}$ is zero. To further block the residual $Ca^{2+}$ channel currents, 1 mM of $Ba^{2+}$, 0.2 mM $Cd^{2+}$ and 1 mM of $Cs^+$ were used in the external solution. The $Na^+$ channels were blocked by adding 1 μM Tetradotoxin (TTX) in the external solution.

To maximize the Na/K pump currents, about 40 mM $Na^+$ and regular $K^+$ concentration were used in the internal solution and external solution, respectively. To identify the Na/K pump current, 0.1 mM ouabain, the specific inhibitor of the Na/K pump molecule, was used in the experiments.

The ingredients and their concentration in the experimental solutions are listed as follows (in mM):

Relaxing solution: 120, K–glutamate; 1, $MgSO_4$; 0.1, EGTA; 5, PIPES.
Normal Ringer solution: 120, NaCl; 2.5, KCl; 2.15, $Na_2HPO_4$; 0.85, $NaH_2PO_4$; 1.8, $CaCl_2$.
Internal solution: 40, Na–glutamate; 22.5, Cs–glutamate; 5, $Cs_2$–PIPES; 20, $Cs_2$–EGTA; 6.8, $MgSO_4$; 5, glucose; 5.5, $K_2$–ATP; 10, Tris–Creatine Phosphate.
External solution: 120, NaCl; 5.4, KCl; 4, MOPS; 1.8, $MgCl_2$; 1, $BaCl_2$; 0.2, $CdCl_2$; 1, CsCl.

### 2.3. Electrophysiology measurements of the Na/K pump currents

The transmembrane currents were measured responding to the same stimulation pulses before and after changes of the external solution with 0.1 mM ouabain. The Na/K ATPase currents were defined as the ouabain-sensitive currents by subtracting the transmembrane currents with ouabain from those currents without ouabain. The membrane potential is held at $-90$ mV, which is the regular resting potential for skeletal muscle fibers. A sequence of stimulation pulse-pairs holding the membrane potential from $-170$ to $-10$ mV were applied to cell membrane. In each stimulation pulse-pair, two pulses were symmetric with respect to the membrane resting potential having the same magnitude but opposite polarity. The purpose of choosing these pulse-pairs is to study the asymmetry of the pump currents with respect to the membrane resting potential. If the pump's $I$–$V$ curve is symmetric with respect to $-90$ mV, the pump currents responding to the two pulses in each pair should have the same magnitude.

## 3. Results

With the Na/K pump strongly activated by a high intracellular $Na^+$ concentration (40 mM) and normal external $Na^+$ and $K^+$ concentrations (120 mM $Na^+$ and 5.4 mM $K^+$ in external solution), a sequence of 700-μs pulse-pairs symmetric to the membrane holding potential at −90 mV was applied to the cell membrane, as shown at the top of the upper panel of Fig. 1. The transmembrane currents responding to these pulses were recorded and superimposed, as plotted in the upper panel of Fig. 1. After adding 0.5 mM ouabain, a cardiotonic steroid that specifically inhibits the Na/K pump molecules, to the external solution, the same stimulation sequence was re-applied to the cell membrane. The Na/K pump currents can be defined by subtracting the transmembrane current in the presence of ouabain from those in the absence of ouabain. To study the asymmetry of the transient pump currents with respect to the membrane resting potential, we added the pump currents corresponding to two pulses in each pair. The results are shown in the lower panel of Fig. 2. These nonzero results illustrate the asymmetry of the transient pump current with respect to the membrane potential of −90 mV. The increase in pump currents elicited by the positive pulse is larger than the decrease in pump currents elicited by the negative pulse in the same pair. Fig. 2 provides strong evidence that the transient pump currents measured less than 700 μs after the changes in membrane potential retain a nonlinear voltage dependence. More specifically, the Na/K pump $I–V$ curve is asymmetric with respect to the membrane resting potential.

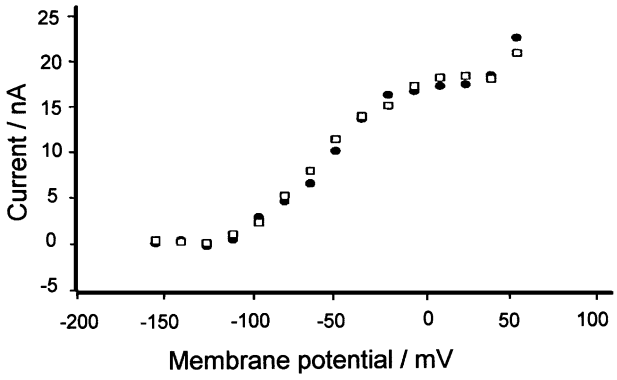


Fig. 2. The Na/K pump currents plotted as a function of membrane potential. The currents represented by open squares are defined by ouabain-sensitive currents, while the currents represented by solid circles are the pump currents obtained by eliminating the external $K^+$ ions.

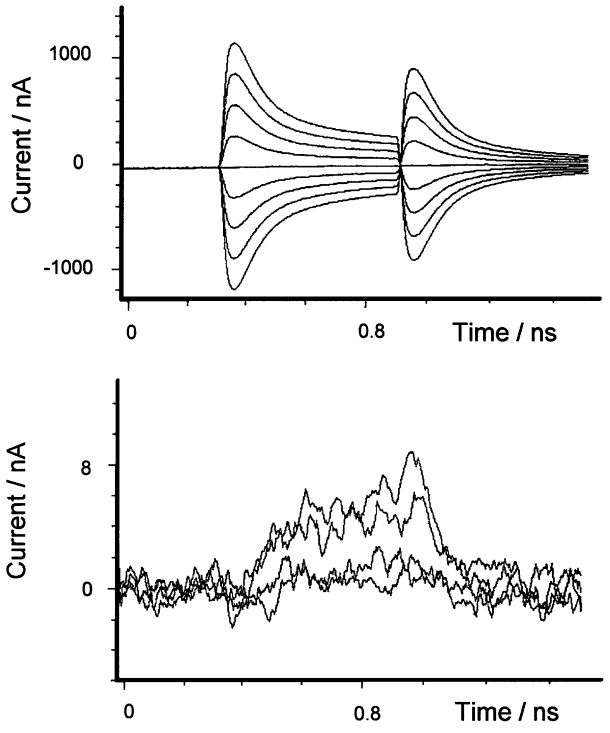


Fig. 1. The upper panel shows the transmembrane currents responding to four stimulation pulse-pairs, holding the membrane potential from −160 to −10 mV. The currents were recorded with no ouabain in the solution. Summations of the currents, $(I_i+I_{-i})_{\text{control}}$, responding to the pulses in each pair indicate the asymmetry of the current–voltage relationship for control. After 0.1 mM ouabain was applied to the external solution, the same stimulation pulse-pairs were delivered to the cell membrane and the responding currents were measured (not shown). The asymmetric features of the current–voltage relationship can be obtianed by $(I_i+I_{-i})_{\text{ouabain}}$. Finally, those summations for the currents in the presence of ouabain were subtracted from those in the absence of ouabain, shown in the lower panel of the figure. These results indicate that the Na/K pump currents responding to a positive pulse are larger than the response to a negative pulse when the membrane potential was held at −90 mV. In other words, the pumps' $I–V$ curve is asymmetric to the membrane resting potential.

## 4. Discussion

When comparing with the steady-state current–voltage relationship, we found a similar voltage dependence. Both $I–V$ curves show a sigmoidal shape and asymmetric voltage dependence with respect to the membrane resting potential.

In this paper, we mainly present our study results of the pumps' transient current–voltage relationship and do not show our results of an oscillating electric field-induced effects on the pump functions. However, the rectifier-like shape of the Na/K pumps' $I–V$ curve conveys an important point that when the membrane potential is set (biased) at the membrane resting potential, a symmetric oscillating membrane potential may generate a net, outward transmembrane current because of the asymmetric features of the $I–V$ curve. The pumps' nonlinear voltage–current relationship is similar to the $I–V$ curve of a semiconductor rectifier. When the

cell membrane is held (biased) at its resting potential of −90 mV, depolarizing cell membrane can significantly increase the pumping rate to generate a large outward current, while hyperpolarizing the membrane potential leads only to a slight decrease in pump current. By integrating the whole cycle, the net transmembrane current should be a nonzero, outward current.

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