First publ. in: Annals of the New York Academy of Science 986 (2003), pp. 229-231

Negative Changes of the Membrane Capacitance due to Electrogenic Na Transport by the Na,K-ATPase

V. S. SOKOLOV,[a] A. A. LENZ,[a] AND H.-J. APELL[b]

[a]*A. N. Frumkin Institute of Electrochemistry RAS, 117071, Moscow, Russia*

[b]*Department of Biology, University of Konstanz, 78457 Konstanz, Germany*

KEYWORDS: Na,K-ATPase; electrogenicity; capacitance; Na^+ movements; ion binding

Electrogenic Na^+ transport was investigated in membrane fragments containing Na,K-ATPase adsorbed to bilayer lipid membranes (BLM) triggered by fast ATP release from caged ATP.[1] The influence of voltage on the transport after ATP release was determined as small increments of capacitance and conductance by applying an alternating voltage to the membrane.[2] An electrogenic Na^+ transport through a cytoplasmic access channel of the Na,K-ATPase was detected that disappeared after enzyme phosphorylation, and thus produced a negative capacitance and conductance increments at Na^+ concentrations below 5 mM.[3] This effect was studied now in more detail by measuring the frequency dependence of the capacitance and conductance increments at different Na^+ concentrations (FIG. 1). Fitting these data by the sum of Lorentzians allowed the discrimination of the separate steps in Na^+ transport and the determination of their parameters.[4]

$$\Delta C = C_0 \frac{\omega_0^2}{\omega^2 + \omega_0^2} + C_1 \frac{\omega_1^2}{\omega^2 + \omega_1^2} - C_2 \frac{\omega_2^2}{\omega^2 + \omega_2^2} + C_{\text{lim}} \quad (1)$$

$$\Delta G = C_0 \omega_0 \frac{\omega^2}{\omega^2 + \omega_0^2} + C_1 \omega_1 \frac{\omega^2}{\omega^2 + \omega_1^2} - C_2 \omega_2 \frac{\omega^2}{\omega^2 + \omega_2^2} \quad (2)$$

At high Na^+ concentration the frequency dependence could be fitted by the sum of two Lorentzians with the amplitudes C_0 and C_1, and a constant term, C_{lim}. The Lorentzian with the lowest corner frequency ω_0 (about 30 s^{-1}) corresponds to the

Address for correspondence: Dr. V.S. Sokolov, A.N. Frumkin Institute of Electrochemistry RAS, Leninsky pr., 31, 117071, Moscow, Russia. Voice and fax: +7-095-952-5582.
sokolov@netra.elchem.ac.ru

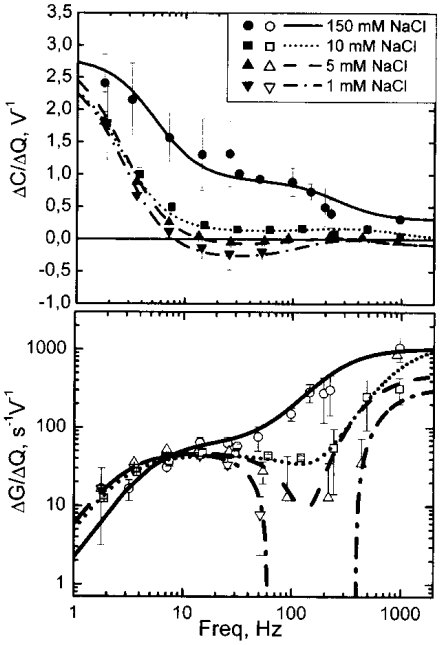

FIGURE 1. Frequency dependencies of capacitance and conductance increments, normalized to the net charge transferred through the membrane after release of ATP from caged-ATP. The solution contained various Na^+ concentrations (as indicated) and 30 mM imidazole, 10 mM $MgCl_2$, and 1 mM EDTA, pH 6.5. The lines were plotted according to the Eqs. (1) and (2) using following parameters: **150 mM NaCl**: $C_0 = 1.9\ V^{-1}$, $\omega_0 = 33\ s^{-1}$, $C_1 = 0.6\ V^{-1}$, $\omega_1 = 1600\ s^{-1}$, $C_{lim} = 0.3\ V^{-1}$; **10 mM NaCl:** $C_0 = 2.5\ V^{-1}$, $\omega_0 = 16\ s^{-1}$, $C_1 = 0.2\ V^{-1}$, $\omega_1 = 6000\ s^{-1}$ $C_2 = -0.08\ V^{-1}$, $\omega_2 = 1000\ s^{-1}$; **5 mM NaCl**: $C_0 = 2.9\ V^{-1}$, $\omega_0 = 17\ s^{-1}$, $C_1 = 0.2\ V^{-1}$, $\omega_1 = 3000\ s^{-1}$, $C_{lim} = -0.1\ V^{-1}$, $C_2 = -0.2\ V^{-1}$, $\omega_2 = 800\ s^{-1}$; **1 mM NaCl:** $C_0 = 2.9\ V^{-1}$, $\omega_0 = 17\ s^{-1}$, $C_1 = 0.2\ V^{-1}$, $\omega_1 = 3000\ s^{-1}$, $C_{lim} = -0.1\ V^{-1}$, $C_2 = -0.4\ V^{-1}$, $\omega_2 = 800\ s^{-1}$.

slowest step of Na^+ transport, the conformation transition E_1/E_2. The corner frequency coincides with the reciprocal time constant of an exponent decay of the falling phase of the ATP-induced current transient. The Lorentzian with a corner frequency ω_1 of about 2000 s^{-1} corresponds to the "intermediate" step, the release of the third Na^+ ion through an extracellular access channel. The constant term, C_{lim}, is assigned to the fast Na^+ ion release through an access channel; its rate could not be resolved so far.

The negative increments at low Na^+ may be explained by electrogenic Na^+ transport through a cytoplasmic access channel. This process is suppressed at saturating Na^+ concentrations. The negative changes depend on frequency, and this dependence can be described by an additional Lorentzian with negative amplitude, C_2. Its corner frequency, ω_2 (about 800 s^{-1}), was attributed to the rate of electrogenic Na^+ transport. The ratio of the "positive" and "negative" Lorentzian, C_0/C_2, is about 10, and the square root of this ratio, ~3, gives an estimate of the ratio of the depths of extracellular and cytoplasmic access channels. With the well-documented dielectric depth

of the extracellular access channel of 0.7–0.75, the cytoplasmic depth is then calculated about 0.25.

ACKNOWLEDGMENTS

This work was supported financially by RFBR No. 01-04-49246 and INTAS (Project 01-0224)

REFERENCES

1. BORLINGHAUS, R., H.-J. APELL & P. LÄUGER. 1987. Fast charge translocations associated with partial reactions of the Na,K-Pump: I. Current and voltage transients after photochemical release of ATP. J. Membr. Biol. **97:** 161–178.
2. SOKOLOV, V.S., K.V. PAVLOV, K.N. DZHANDZHUGAZYAN & E. BAMBERG. 1992. Capacitance and conductivity changes during Na^+,K^+-ATPase action in model membrane. Biol. Membr. **6:** 1263–1272.
3. SOKOLOV, V.S., S.M. STUKOLOV, A.S. DARMOSTUK & H.-J. APELL. 1998. Influence of sodium concentration on changes of membrane capacitance associated with the electrogenic ion transport by the Na,K-ATPase. Eur. Biophys. J. **27:** 605–617.
4. SOKOLOV, V.S., A.G. AYUAN & H.-J. APELL. 2001. Assignment of charge movements to electrogenic reaction steps of the Na,K-ATPase by analysis of salt effects on the kinetics of charge movements. Eur. Biophys. J. **30:** 515–527.