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(U) Effect of Electric Fields on Membrane Bound (Na,K)-ATPase

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

~~(Na,K)~~-ATPase of human erythrocytes has been activated by an oscillating electric field of 20 V/cm. The optimum frequency for activation of the Rb^+-pump was 1.0 kHz and for activation of the Na^+-pump was 1.0 MHz. At 4°C and under the optimum conditions, the net voltage induced, ouabain sensitive Rb^+ uptake was 10 - 20 atto-mole per red cell per hour, and Na^+ efflux was 20 - 30 atto-mole per red cell per hour, depending on erythrocyte samples from different individuals. No electric fi stimulated consumption of ATP was detected. Apparently, the enzyme can absorb free energy from the applied electric field for pumping Rb^+ and Na^+ against their respectiv concentration gradient. We have proposed a mechanism "Resonance Electroconformational Coupling" to interpret these results. Some feature of these results can also be reproduced by the surface compartmental model of Blank.

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Date: 14 June 1988

PROGRESS REPORT ON CONTRACT N00014-87-K-0038

PRINCIPAL INVESTIGATOR: Tian Y. Tsong

CONTRACTOR: Johns Hopkins University

CONTRACT TITLE: Effect of Electric Fields on Membrane Bound (Na,K)-ATPase

START DATE: 1 December 1986

RESEARCH OBJECTIVE: To investigate effects of electric fields on membrane proteins; to understand mechanisms of interactions.

PROGRESS (Year II): In this second year of the project we continued to investigate frequency dependence of the voltage stimulated (Na,K)-ATPase activity. We tested for the frequency range 1 to 20 MHz. When using the optimum strength of a.c. field (20 V/cm for both Rb^+ and Na^+ pumps), the optimum frequency for activating the Na^+-pump was found to be 1.0 MHz (Fig. 1). At 4°C and under the optimum conditions, the Rb^+-pump activity ranges from 10 to 20 ions per enzyme per sec and the Na^+-pump activity ranges from 20 to 30 ions per enzyme per sec. The stoichiometry for Rb^+/Na^+ is approximately 2/3, consistent with ATP dependent activity. In our experiment, the temperature was kept at 3°C and the application of the ac field did not appreciably change the sample temperature (smaller than 0.5°C change). A control sample was always kept at 3.5°C. In the red cells, the cytoplasmic concentration of Na^+ was 6 mM and Rb^+ was 27 mM (pre-loaded), and the external medium contained 140 mM Na^+ and 10 mM Rb^+. Yet the ac stimulated only the Na^+ efflux and the Rb^+ uptake, both are transport against the concentration gradients. We have analyze ATP concentration of the red cells by using luciferin/luciferase phosphorescence assay and found that the electric field induced activity did not depend appreciably on the ATP level in the red cells. Neither was there ac stimulated hydrolysis of ATP. We concluded that (Na,K)-ATPase absorbed free energy from the oscillating field for doing chemical work.

WORK PLAN (Year 3): In the third year we will study the kinetics of the electric field activated Rb^+ and Na^+ pumping activity. K_m and V_m will be determined for the internal Na^+ and the external K^+ and compare these values with the ATP dependent activity. Other transport system will be examined to see whether any of them is also influenced by the electric field. We will also compare the efficiency of the square waveform and the sinusoidal waveform. The resonance electroconformational coupling (RECC) model we proposed earlier predicts that square wave electric field is more efficient than the sinusoidal wave. Other waveforms will also be done to test the prediction of the RECC model.

INVENTION: None.

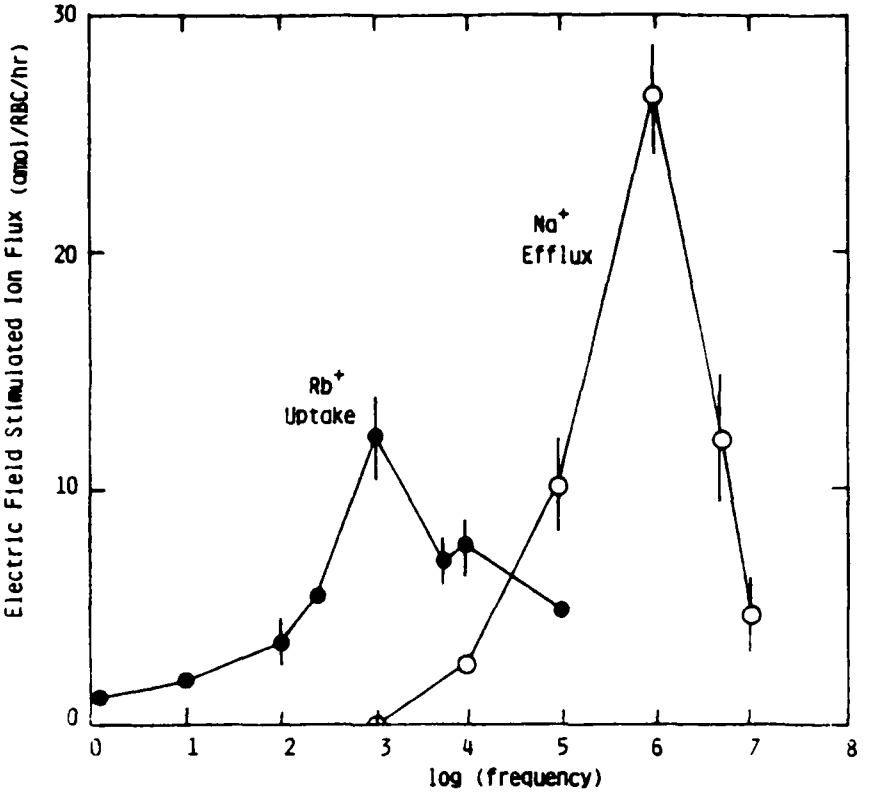

Fig. 1 Activation of the N^+- and the Rb^+ (K^+)-pumps of [Na,K]-ATPase by an oscillating electric field of 20 V/cm, at 4°C.

Human erythrocytes in an isotonic suspension were exposed to the oscillating electric field of different frequencies. This electric field can generate a maximum $\Delta\psi$ of 12 mV across the membrane, or an effective transmembrane electric field of 20 kV/cm. The ouabain sensitive Na^+ efflux (O) and Rb^+ uptake (●) are plotted against the frequency of the applied field. In the same experiment no ouabain sensitive Rb^+ efflux nor ouabain sensitive Na^+ uptake was stimulated by the electric field. The cytoplasmic concentration of Na^+ was 6 mM and Rb^+ was 27 mM, and the external concentration of Na^+ was 140 mM and Rb^+ was 10 mM. Thus, both fluxes are active transports against the respective concentration gradients. No consumption of ATP was detected. It is concluded that the enzyme absorbed free energy from the applied electric field and converted it to the chemical potential energy of the two ions. These results have been successfully explained by RECC model, although the surface compartmental model of Blank also can also reproduce some feature of the data.

PUBLICATIONS:

1. Tsong, T.Y. & Astumian, R.D. (1987). Prog. Biophys. Mole. Biol., 50, 1-45.

2. Tsong, T.Y. & Astumian, R.D. (1988). Ann. Rev. Physiol., 50, 273-290.

3. Tsong, T.Y. (1988). Methods in Enzymol., 157, 240-251.

4. Tsong, T.Y., Tomita, M. & Lo, M.M.S. (1988). In "Molecular Mechanisms of Membrane Fusion", Oki, S. Ed., Plenum, N.Y., Pp. 223-236.

5. Tsong, T.Y., Chauvin, F. & Astumian, R.D. (1987). In "Mechanistic Approaches to Interaction of Electromagnetic Fields with Living System", Blank, M. & Findl, E., Eds. Plenum, N.Y., Pp. 187-202.

And several abstracts for scientific meetings.

TRAINING ACTIVITIES: One technician and one trainee worked on the project. The trainee from China is making a good progress. The technician is working fine.

Non-citizen - 1 (China).