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THE THEORY OF TRANSPORT PHENOMENA IN BIOLOGICAL MEMBRANES. II. THE ACTIVE TRANSPORT OF IONS

M. V. VOLKENSTEIN AND S. N. FISHMAN

Institute of Molecular Biology Academy of Sciences U.S.S.R., Moscow (U.S.S.R.)

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SUMMARY

A model is suggested coupling the processes of the passive and active transport of the Na^+ and K^+ in biological membranes. It is shown that the mechanism of active transport has features common to the mechanism of passive transport. However, it differs in the driving force maintaining the directed flow of ions. In the case of passive transport, the driving force is the gradient of electrochemical potential of the ions of given species; in the case of active transport it is the gradient of the potential of the complex resulting from the biochemical reaction. The specific feature of active transport is the cooperativity of enzymatic exchange reaction, determining the transmission of ions from one center to another.

INTRODUCTION

The mechanism of the passive transport of ions across cellular membranes and its connection with the resting potential was examined in the preceding paper[1]. The motion of ions in the direction of gradient of their electrochemical potential causes a gradual decrease in this potential and the establishment of an equilibrium. The support of the constant difference of electrochemical potentials requires an active transport of ions in the direction opposite to that of the gradient. It is known from the theory of irreversible processes (see *e.g.* ref. 2) that the active transport diminishes the entropy of the system and it can occur only as the result of coupling with other processes in such a way that the total change of entropy is not negative.

The scheme of the coupling of the active transport of ions with metabolic processes in a cell was described by KATCHALSKY AND SPANGLER[3] in terms of the thermodynamics of irreversible processes. The expressions for phenomenological coefficients were obtained, valid for small deflections from equilibrium. However, there remains a series of observed phenomena, connected with the active transport, which were not examined.

The experimental study of the active flux of Na^+, J, from the cell into surrounding medium has shown that this value depends on the intracellular Na^+ concentration c_{Na}' (refs. 4, 5) and on the external K^+ concentration c_K'' (refs. 5, 6). According to ROMANENKO *et al.*[4] and HARRIS[5] the curve $J(c_{Na}')$ has a sigmoid form. HOROWICZ AND GERBER[6] observed the same form of the curve $J(c_K'')$.

The aim of this work is the kinetic analysis of active transport.

Works of recent years have established the source of energy used for the support of the steady state. It is the energy of macroergic compounds, especially ATP. As has been observed by CALDWELL *et al.*[7], ATP increases the active transport if it is introduced into the cell, but an increase of ATP concentration in surrounding solution does not produce any effect. SKOU[8] obtained an ATPase from membrane. This enzyme splits ATP only in the presence of Na^+ and K^+. The action of ATPase in membrane is strongly coupled with active transport, ouabain inhibits the ATPase at the same concentration at which it stops the work of the Na^+–K^+ pump. The hydrolysis of ATP *in vitro* by the ATPase activated by Na^+ and K^+ occurs in two stages[9]. At first ADP is liberated and P_i remains bound to enzyme. This stage is activated by Na^+. The second stage needs K^+ and consists in the breakdown of P_i from enzyme. A similar asymmetry was observed in the action of the pump: at the internal surface of membrane its activity depends on the concentration of Na^+ and at the external surface on K^+ (ref. 10). The splitting of ATP results in the transition of the marked phosphorus from ATP into phosphoproteins of membrane[11,12]. SQUIRES[13] investigated the kinetics of the action of (Na^+-K^+)-activated ATPase *in vitro*. The dependence of the rate of enzymatic reaction on the concentrations of Na^+, K^+ and ATP has a pronounced S-like form. Finally there are data obtained by different methods (see *e.g.* ref. 14) showing that the splitting of one macroergic bond of ATP is followed by the removal of 2–3 Na^+ from the cell.

It is necessary that the theoretical model takes into account all these different experimental data. OPIT AND CHARNOCK[15] used these data in their molecular model of a Na^+ pump. This model describes many features of active transport. It takes into account the coupling of enzymatic reaction with ion exchange and the cooperative character of such a reaction. However, the proposed mechanism of ion transport seems to be artificial as it requires the shift of big portions of enzyme containing several active ion-exchange sites from one surface of membrane to another. In the work of STONE[16] a model of active Na^+ transport is proposed involving a carrier which can exist in several forms. The model is built for the particular case of an electrogenic pump where the splitting of one ATP molecule results in the removal of 3 Na^+ from the cell and the introduction of 2 K^+. The theory contains nineteen independent parameters and therefore cannot be verified experimentally.

A model operating with two carrier forms was investigated in the work of ROSENBERG AND WILBRANDT[17]. The kinetic study of the model in this work was, however, limited by study of the special case when the processes occurring at the surface of membrane do not determine the rate of transport but correspond to the instantaneous establishment of equilibrium. However, the experimental data suggest that the enzymatic reaction at the membrane surface determines the active flow of ions.

In our work investigation of the scheme of transport is based on the experimental data of refs. 7–14. An analysis of the kinetics of the active transport processes is proposed to explain the observed concentrational dependencies of the active flows.

Description of the model

Our basic assumption consists in the existence of ion-exchange sites in membrane which can bind and exchange Na^+ and K^+. There are two kinds of such sites. The first kind of site is connected with the phospholipid portion of membrane and with

nonspecific proteins. These centers take part in the passive transport, as was examined in the preceding paper[1]. The other sites belong to the protein–enzyme portion and determine the active transport. The motion of ions occurs according to the "relay-race mechanism", *i.e.* transmission of the ions from one immovable site to another one.

The enzyme containing such exchange sites has the ability to catalyze the splitting of some chemical substance X into products Y and Z at the internal surface of membrane. The product Z is liberated and product Y remains bound to the enzyme. The binding of Y to enzyme simultaneously helps the exchange of K^+ for Na^+ at the ion-exchange sites (ATP can play the role of X, Z corresponds to ADP or AMP, Y to P_i). The coupling of ion exchange and enzymatic process can be written in the following way

$$(Na^+)' + A'K + X \underset{k_1}{\overset{k_1'}{\rightleftarrows}} Z + A'NaY \quad (K^+)' \tag{1}$$

Index $'$ marks the internal surface of membrane and the intracellular concentrations. A signifies enzyme. At the external surface of membrane (index $''$) the exchange sites of enzyme are such that Na^+ in the complex NaY is exchanged for K^+. The complex splits into Na^+ and product Y:

$$(K^+)'' + A''NaY \underset{k_2}{\overset{k_2'}{\rightleftarrows}} Y + A''K + (Na^+)'' \tag{2}$$

The complex NaY is shifted from the place of its formation at the internal membrane surface towards the place of its breakdown at the external surface, *i.e.* along the gradient of its concentration. If the affinity of the exchange sites to ions is high and there are no free sites, then the shift of the complex NaY and its exchange for K^+ can be written in the form of an exchange reaction:

$$A'NaY + A''K \underset{k_3}{\overset{k_3'}{\rightleftarrows}} A'K + A''NaY \tag{3}$$

Therefore the generalized force driving K^+ and Na^+ is the gradient of concentration of the product Y supported by enzymatic reaction.

We further suggest that the exchange at the enzymatic sites and the enzymatic reaction are cooperative. It means that the enzymatic sites interact in such a way that an exchange of a ligand occurs practically simultaneously at several sites. Such a cooperative exchange has been observed, *e.g.* in ceolythes[18] where it is determined by the change of the lattice structure resulting from exchange of one ion for another. A description of the cooperativity requires the introduction into equations of reaction stoichiometric coefficients unequal to Eqn. 1.

The general scheme of the molecular model for the ion transport can be presented in the following way:

$$\begin{array}{ccccccc} n(Na^+)' & - & A'K_m & + & pX \underset{k_1}{\overset{k_1'}{\rightleftarrows}} A'Na_nY_p & + & m(K^+)' + pZ \\ I_{Na}\downarrow\uparrow & & k_3'\downarrow\uparrow k_3 & & k_3\downarrow\uparrow k_3' & & \uparrow\downarrow I_K \\ n(Na^+)'' & - & A''K_m & + & pY \underset{k_2}{\overset{k_2'}{\rightleftarrows}} A''Na_nY_p & + & m(K^+)'' \end{array} \tag{4}$$

I_{Na} and I_K denote the passive fluxes of corresponding ions; n, m and p are the stoichiometric coefficients; k_i, k_i', the rate constants corresponding to the arrows of reactions. The coupling of directed fluxes in this model is shown in Fig. 1.

The reactions involved in Scheme 4 can be described by the following system of kinetic equations.

$$\frac{1}{S}\frac{dc_X}{dt} = -k_1 c_{Na}'^n c_X^p a_K' - k_1' c_K'^m c_Z^p a_{Na}' \quad (5.1)$$

$$\frac{1}{S}\frac{dc_{Na}'}{dt} = -k_1 c_{Na}'^n c_X^p a_K' + k_1' c_K'^m c_Z^p a_{Na}' + I_{Na} \quad (5.2)$$

$$\frac{1}{S}\frac{dc_{Na}''}{dt} = k_2 c_K''^m a_{Na}'' - k_2' c_{Na}''^n c_Y^p a_K'' - I_{Na} \quad (5.3)$$

$$\frac{1}{S}\frac{dc_K'}{dt} = -k_1 c_{Na}'^n c_X^p a_K' - k_1' c_K'^m c_Z^p a_{Na}' + I_K \quad (5.4)$$

$$\frac{da_K'}{dt} = -k_1 c_{Na}'^n c_X^p a_K' + k_1' c_K'^m c_Z^p a_{Na}' + S k_3 a_{Na}' a_K'' + S k_3' a_K' a_{Na}'' \quad (5.5)$$

$$a_K' + a_{Na}' = T_A' \quad (5.6)$$

$$a_K'' + a_{Na}'' = T_A'' \quad (5.7)$$

$$S a_K' + S a_K'' = N' c_K' + N'' c_K'' = T_K \quad (5.8)$$

$$S a_{Na}' + S a_{Na}'' = N' c_{Na}' + N'' c_{Na}'' = T_{Na} \quad (5.9)$$

The following notations are used: c_i', c_i'': dimensionless concentrations of the ions of species i inside and outside the cell, equal to the ration of the total number of ions i to the number of solvent (water) molecules in a corresponding volume (N', N''); T_A', T_A'': the number of exchange sites of enzyme per unit internal and external surface of membrane; a_i', a_i'': the number of exchange sites of enzyme per unit membrane surface occupied by ions i; T_i: the total number of ions i in the system; S: the membrane surface; k_i', k_i'': the rate constants.

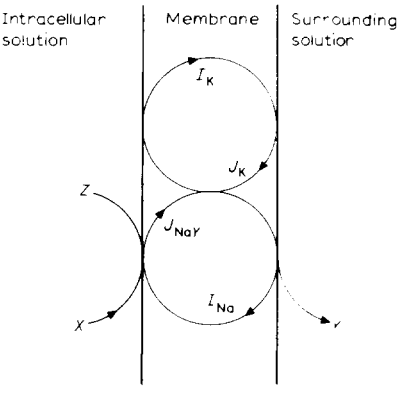

Fig. 1. The coupling of the passive and active fluxes of K^+ and Na^+ with a chemical reaction. I_K, passive flow of K^+ from the cell; I_{Na}, passive flow of Na^+ into the cell; J_K and J_{Na}, active fluxes of K^+ and Na^+; X, the substance transformed at the internal membrane surface into product Z, liberated in the internal solution; and product Y, transmitted in the form of a complex with Na^+ towards the external membrane surface.

Eqns. 5.6–5.9 describe the maintenance of the number of exchange sites at both surfaces of membrane and of the total number of ions of both species.

In steady-state conditions the right sides of Eqns. 5.2–5.5 are equal to zero, as in the state of rest only the substance X is used in the cell and all other concentrations remain constant.

This model demonstrates that there are no contradictions between the ideas of TASAKI AND SINGER[19] concerning the ion-exchange properties of membranes and the concepts of HODGKIN[20] regarding Na^+ and K^+ "channels" in membrane. The sequence of ion-exchange centers determining the transport of ions acts as the channels. This sequence can be called a kinetic channel.

The dependence of active flow on concentrations

Eqns. 5.1 and 5.2 show that the active removal of Na^+ from the cell, J, is determined by the rate of biochemical reaction, dc_X/dt. In the steady state this current is counterbalanced by the passive flow I_{Na}.

Let us consider the enzymatic reactions as irreversible. This means that in Reactions 1–3 $k_1 \gg k_1'$, $k_2 \gg k_2'$ and $k_3 \gg k_3'$. For simplicity we shall take $k_1' = k_2' = k_3' = 0$.

For the resting state we can use the following system of equations taken from Eqns. 5

$$\begin{aligned} &J = k_1 c_{Na}' {}^n c_X{}^p a_K' - k_2 a_{Na}'' c_K''^m = S k_3 a_{Na}' a_K'' ; \\ &a_{Na}' + a_K' = T_A' ; \; a_{Na}'' + a_K'' = T_A'' \end{aligned} \tag{6}$$

As was suggested in our preceding paper[1], variations of concentrations at one side of the membrane do not influence the concentrations at the other side. We shall consider the concentrations in solutions as independent. We get from Eqn. 6 the expression for the concentration c_{Na}' as a function of the flux J if c_K'' and c_X are given

$$c_{Na}' = \left[\frac{k_3 S}{k_1 c_X{}^p (k_3 S T_A' + k_2 c_K''^m)} \cdot \frac{J(J_0 - J)}{J_\infty - J} \right]^{1/n} \tag{7}$$

where

$$J_0 = k_2 T_A'' c_K''^m \tag{8}$$

and

$$J_\infty = \frac{S k_3 k_2 T_A' T_A'' c_K''^m}{S k_3 T_A' + k_2 c_K''^m} \tag{9}$$

We get from Eqn. 6 also that

$$a_{Na}' = \frac{J k_2 c_K''^m}{S k_3 (k_2 T_A'' c_K''^m - J)} \leqslant T_A' \tag{10}$$

The inequality Eqn. 10 shows that only such values of J which satisfy the condition $0 \leqslant J \leqslant J_\infty$ have physical meaning. Knowing the dependence $c_{Na}'(J)$ in the explicit form of Eqn. 7 we get the derivative

$$\frac{dc_{Na}'}{dJ} = \frac{1}{n} \left[\frac{S k_3}{k_1 c_X{}^p (S k_3 T_A' + k_2 c_K''^m)} \right]^{1/n} \frac{J_0 J_\infty - 2 J J_\infty + J^2}{J^{1-1/n} (J_\infty - J)^{1-1/n} (J_0 - J)^{1-1/n}} \tag{11}$$

The condition of extremum is:

$$J_{\text{extr.}} = J_\infty \pm \sqrt{J_\infty (J_\infty - J_0)} \tag{12}$$

Comparing Eqns. 8 and 9 we see that $J_0 > J_\infty$. The function $c_{Na}'(J)$ has no extreme value. But, as is seen from Eqn. 11, the derivative becomes infinite if $J \to 0$, $J \to J_\infty$, $J \to J_0$ if $n > 1$. It means that the curve $c_{Na}'(J)$ possesses at least one inflection point (or an odd number of them). The second derivative becomes zero at the inflection point. This condition is expressed by the equation of the fourth order

$$(n-1)(J_0 J_\infty - 2JJ_\infty - J^2)^2 = 2nJ_\infty (J_0 - J_\infty)J(J_0 - J) \tag{13}$$

As the polynomial $J_0 J_\infty - 2JJ_\infty + J^2$ has no real roots, its square is a smooth curve with one minimum at the point $J = J_\infty$. At the right side of Eqn. 13 we have the curve of the second order with the roots $J = 0$ and $J = J_0$ and with a maximum at the point $J = J_0/2$. Both curves can intersect only at two points. Therefore Eqn. 13 has no more than two real roots, and at the interval $0 < J < J_\infty$ the curve $c_{Na}'(J)$ has only one inflection point. The curve must be sigmoid, in agreement with experiment (Fig. 2a).

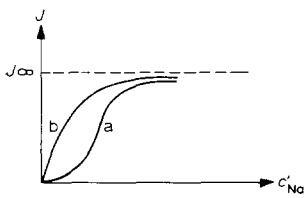

Fig. 2. Theoretical dependence $J(c_{Na}')$: a, in the case of the cooperative ion exchange; b, in the case of the non-cooperative ion exchange.

In a similar way we can calculate the function $c_K''(J)$ from Eqn. 6. The qualitative result is the same: the curve $J(c_K'')$ increases monotonously till some limit and possesses one inflection point.

It is easy to see that if $n = m = 1$, *i.e.* when there is no cooperative interaction between the active sites of enzyme, the first derivatives of the functions $J(c_{Na}')$ and $J(c_K'')$ have finite values at the beginning of coordinates and the second derivatives do not change their sign at the interval $0 > J > J_\infty$, *i.e.* the curves do not have any inflection points (see Fig. 2b).

Thus the enzymatic reaction coupled with ion exchange and resulting in the active removal of Na^+ from the cell has the same kinetic features as the usual enzymatic reactions. The non-cooperative exchange is described by the smooth curve for the dependence of rate on the ion concentrations with saturation. If the exchange is cooperative, the curve has a sigmoid form.

CONCLUSION

Scheme 4 shows the interaction of kinetic phenomena in the cell and their coupling with metabolic process. The mechanism of the active transport has much in common with the mechanism of the passive transport: the ions are bound by ion-exchange sites and shifting from one site to another cross the membrane. The

important peculiarity of active transport is the cooperativity of exchange reaction at the enzyme.

The following hypothesis can be suggested. Both the passive permeability and the spike generation can be considered as purely physical processes, which are not coupled with specific cell metabolism. Metabolic processes maintain only the necessary concentration gradients. Obviously the exchange sites which are transmitting the ions belong to the phospholipid part of membrane and to nonspecific membrane proteins. This is confirmed by the data obtained by GAMMACK[21] who has shown that the phospholipases, breaking down the lipid layers, make the axon unexcitable. This explains the recent successes in obtaining a spike at artificial phospholipid membranes[22] and even in glasses possessing ion-exchange properties. The primary specific role of the protein in biological membranes is the maintenance of the coupling of the transport and metabolic processes, *i.e.* in the creation of active transport.

Further development of the theory requires study of the changes in passive flows of ions in the presence of an external electrical field at the cell membrane and an explanation of the mechanism of the generation of the nerve impulse.

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