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Incorporation of membrane potential into theoretical analysis of electrogenic ion pumps

(active transport/Na,K-ATPase/current–voltage curves)

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ABSTRACT **The transport rate of an electrogenic ion pump, and therefore also the current generated by the pump, depends on the potential difference ($\Delta\Psi$) between the two sides of the membrane. This dependence arises from at least three sources: (*i*) charges carried across the membrane by the transported ions; (*ii*) protein charges in the ion binding sites that alternate between exposure to (and therefore electrical contact with) the two sides of the membrane; (*iii*) protein charges or dipoles that move within the domain of the membrane as a result of conformational changes linked to the transport cycle. Quantitative prediction of these separate effects requires presently unavailable molecular information, so that there is great freedom in assigning voltage dependence to individual steps of a transport cycle when one attempts to make theoretical calculations of physiological behavior for an ion pump for which biochemical data (mechanism, rate constants, etc.) are already established. The need to make kinetic behavior consistent with thermodynamic laws, however, limits this freedom, and in most cases two points on a curve of rate versus $\Delta\Psi$ will be fixed points independent of how voltage dependence is assigned. Theoretical discussion of these principles is illustrated by reference to ATP-driven Na,K pumps. Physiological data for this system suggest that all three of the possible mechanisms for generating voltage dependence do in fact make significant contributions.**

We present here an elementary discussion of how the various parts of the reaction cycle of an ATP-driven ion transport system can be affected by changes in membrane potential and of the consequent effects on the voltage dependence of the steady-state rate of the transport reaction. We assume that the transport reaction has a fixed stoichiometry, so that ATP hydrolysis, ion fluxes, and the ion pump contribution to physiological current–voltage curves are all at fixed ratios to the overall cycling rate. We illustrate the principles involved by reference to ATP-driven Na,K pumps, which are assumed to be represented by the stoichiometric equation,

$$\mathrm{ATP} + 3\ \mathrm{Na^+(in)} + 2\ \mathrm{K^+(out)} \rightleftharpoons \mathrm{ADP} + \mathrm{P_i} + 3\ \mathrm{Na^+(out)} + 2\ \mathrm{K^+(in)}, \quad [1]$$

where "in" refers to the cytosol and "out" to the extracellular solution.

We define membrane potential ($\Delta\Psi$) as the measurable difference in electrostatic potential between *bulk* solutions on opposite sides of the membrane, $\Delta\Psi = \Psi(\mathrm{in}) - \Psi(\mathrm{out})$. Potentials at locations within the domain of the membrane, in the region of the membrane spanned by the transport protein, are not experimentally measurable, but need to be discussed in a theoretical analysis. We define $\Psi(x)$ as the potential at a defined location (x) and $\delta\Psi(x, y)$ as the potential difference between any two locations.

Any step in a reaction cycle in which charges are moved across the membrane involves electrical work proportional to $\Delta\Psi$, and equilibrium and rate constants for the step necessarily become $\Delta\Psi$ dependent. Transitions that involve charge movements between points x and y within the membrane involve electrical work proportional to $\delta\Psi(x, y)$. Such work terms can be intrinsically large but may depend only weakly on $\Delta\Psi$ because $\delta\Psi(x, y)$ may be largely independent of $\Delta\Psi$.

THEORETICAL RESTRICTIONS

Any step in a transport cycle may involve charge movements and any step may therefore in principle contribute to the $\Delta\Psi$ dependence of the overall rate. Theoretical analysis of $\Delta\Psi$ dependence is, however, subject to restrictions that leave fewer degrees of freedom than one might at first suppose. In particular, we can normally expect that two points on a curve of pump current versus $\Delta\Psi$ will be fixed in advance—i.e., before we make any assumptions about the contribution of individual cycle steps to the overall $\Delta\Psi$ dependence. One of the fixed points is the reversal potential (the value of $\Delta\Psi$ at which the rate is zero); the other is the pump rate when $\Delta\Psi = 0$. In addition, elementary thermodynamic laws provide a third constraint.

Equilibrium in the Overall Reaction: Reversal Potential. The overall catalyzed reaction (if it has a fixed stoichiometry) has a rigorously defined equilibrium state, which involves only the reactants and products in the bulk solutions and which must be independent of the mechanism whereby the transport protein catalyzes the reaction. The membrane potential $\Delta\Psi$ is a factor in the equation for the equilibrium state, but $\delta\Psi(x, y)$ values cannot be involved. For Na,K pumps (Eq. 1), for example (1, 2), the equilibrium state is defined by the equation

$$\frac{[\mathrm{ADP}]\,[\mathrm{P_i}]\,[\mathrm{Na^+(out)}]^3\,[\mathrm{K^+(in)}]^2}{[\mathrm{ATP}]\,[\mathrm{Na^+(in)}]^3\,[\mathrm{K^+(out)}]^2} = K_{eq}\exp\,(nF\Delta\Psi/RT), \quad [2]$$

where F is the Faraday constant, n is the net number of charges moved outward across the membrane per reaction cycle (here $n = 1$), and K_{eq} is the equilibrium constant for the reaction in the absence of a membrane potential, which in this case is equal to the equilibrium constant for ATP hydrolysis, roughly 2.4×10^5 M at pH 7 in the presence of a physiological level of Mg^{2+} (2).

One of the properties of the equilibrium point is that it defines the conditions where the direction of the pump reaction reverses itself as a variable parameter is changed (1). For example, if we consider the effect of changing $\Delta\Psi$ at constant concentrations of all reactants, we have (with $n = 1$)

$$(\Delta\Psi)_{eq} = \frac{RT}{F}\ln\frac{[\mathrm{ADP}]\,[\mathrm{P_i}]\,[\mathrm{Na^+(out)}]^3\,[\mathrm{K^+(in)}]^2}{K_{eq}\,[\mathrm{ATP}]\,[\mathrm{Na^+(in)}]^3\,[\mathrm{K^+(out)}]^2}. \quad [3]$$

The pump will operate in the forward direction (active transport) if $\Delta\Psi$ exceeds the equilibrium value and in the

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reverse direction (ATP synthesis) if $\Delta\Psi < (\Delta\Psi)_{eq}$. The important point is that this requirement is independent of the reaction mechanism, associated rate, and equilibrium constants, etc.

Cycling Rate or Current at $\Delta\Psi = 0$. The biochemical reaction cycle for electrogenic ion pumps and other active transport systems is usually already known from test tube experiments before an analysis of potential effects is carried out. All steps of the reaction cycle can normally be expected to have been identified, and equilibrium and rate constants for each step can be expected to be available. These data will normally be based on experiments done not only in the absence of a membrane potential but usually also in leaky vesicles or membrane fragments where concentrations of transported ions are the same on both sides of the membrane. Assignment of "sidedness" with respect to interaction with the transported ions is, however, always a part of the mechanistic interpretation of the biochemical data, so that the biochemical parameters can be used to calculate the expected pump turnover rate (at $\Delta\Psi = 0$) in the presence of physiological ion concentration gradients. If the density of pump molecules in the membrane is known, then the corresponding pump current per unit membrane area is obtained as well.

The biochemical parameters one is using may of course be not yet firmly established and subject to change. In that case the rate at $\Delta\Psi = 0$ would also be tentative, subject to comparison with experimental rates. In this sense physiological measurements can be an important criterion for assessing the validity of biochemical reaction mechanisms, a criterion that is often neglected.

Equilibrium Constants for Partial Reactions. The catalysis of transport is carried out by a reaction cycle such as the Post–Albers cycle for Na,K pumps shown in Fig. 1. Aspects of the mechanism such as random order of binding or release of ligands can introduce alternate pathways into parts of such a cycle, but for a reaction of fixed stoichiometry all complete productive cycles lead to the identical overall reaction (3).

Every step of a reaction cycle can in principle involve electrical work and have a $\Delta\Psi$-dependent equilibrium constant, analogous to Eq. **2** for the overall K_{eq}, but it is possible that positions within the domain of the membrane may be involved, where the dependence on $\Delta\Psi$ is secondary via $\delta\Psi(x, y)$. We thus write the equilibrium constant K_j for each cycle transition j as

$$K_j = K_j^0 \exp(\beta_j F \Delta\Psi / RT), \qquad [4]$$

where K_j^0 is the equilibrium constant in the absence of a membrane potential and β_j contains both the number of charges translocated in the step under consideration and a parameter that relates $\delta\Psi(x, y)$, if applicable, to $\Delta\Psi$. The β_j are not subject to any inherent theoretical limit. The number of charges moved across the membrane in a single step can be larger than n in Eq. **2** and can be positive (movement in → out) or negative (movement out → in). In Fig. 1, for example (for the moment excluding protein charges), 3 Na^+ ions move in a positive direction in step 1 and 2 K^+ ions move in a negative direction in step 2. The β_j also need not be integral—e.g., the $\Delta\Psi$-dependent part of $\delta\Psi(x, y)$ terms can often be expected to be only a fraction of the total potential difference across the membrane.

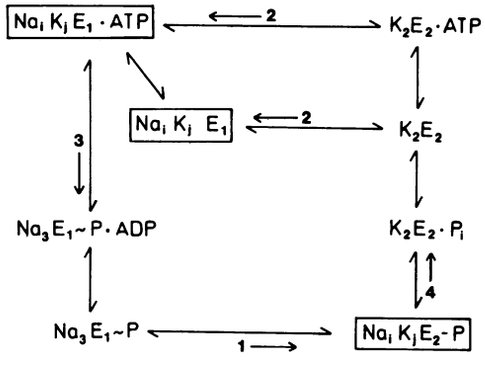

FIG. 1. The Post–Albers reaction sequence for Na,K pumps, with random order for Na^+ and K^+ binding and with allowance for low-affinity binding of ATP to the E_2 state. Steps 1–4 identify the cycle transitions most likely to be affected by membrane potential. Boxes represent competitive binding of Na^+ and K^+ in random sequence, with $0 \le i \le 3$ and $0 \le j \le 2$.

The important point here, in relation to thermodynamic constraints, is that the product of equilibrium constants around an entire cycle must be equal to the equilibrium constant for the overall reaction catalyzed by the cycle, and this condition must be satisfied at all values of the membrane potential. This means that the product of all exponential terms around a cycle must be equal to the exponential term in Eq. **2**. Regardless of the possible complexity of electrical factors affecting individual β_j values, we must have, for any complete cycle,

$$\Sigma\beta_j = n, \qquad [5]$$

with $n = 1$ for the Na,K pumps here used as illustrative example.

Where random order of addition creates variable pathways, there will then be more than one possible way of completing a "cycle." With the assumption of invariant stoichiometry, n must be the same and $\Sigma\beta_j$ must have the same value for each possible cycle. In mechanisms that allow for variable stoichiometry—e.g., as discussed in one of our previous papers (3)—different cycles may have different values of n, and a separate equation of the form of Eq. **5** would then apply to each cycle that can be completed, but the principle that the values of n are thermodynamic parameters, with fixed values independent of all other assumptions, continues to apply.

Rate Constants for Partial Reactions. The equilibrium constant for any cycle step is equal to the ratio of forward and reverse rate constants ($k_{f,j}$ and $k_{r,j}$) for that step, and the potential dependence of K_j must therefore be distributed between the two rate constants. The manner of making this distribution has been discussed in numerous places (4–6), and the normal procedure is to assume that the effect of the electric field is symmetrical, which means that the reverse reaction is slowed down (or accelerated) by the same factor by which the forward reaction is accelerated (or slowed down). This leads to

$$k_{f,j} = k_{f,j}^0 \exp(\beta_j F \Delta\Psi / 2RT) \qquad [6]$$

$$k_{r,j} = k_{r,j}^0 \exp(-\beta_j F \Delta\Psi / 2RT), \qquad [7]$$

where superscript 0 refers to the rate constant at $\Delta\Psi = 0$. These equations formally express the fact that Eq. **5** exercises as severe a constraint on rate constants as it does on equilibrium constants.

PARTICIPATION OF PROTEIN-DERIVED CHARGES

If the transport protein were an electrically inert component of the membrane, then movement of the transported ions would be the sole contributing factor for estimating effects of $\Delta\Psi$ on transport kinetics. In fact, all proteins are highly charged molecules. Transport proteins, moreover, actively

participate in the reactions they catalyze. In particular, they must alternate in the reaction cycle between a minimum of two conformational states, in which binding sites for the transported ions (expected to include protein groups with charges of opposite sign to that of the ion) are exposed to opposite sides of the membrane (7, 8). Clearly, protein charges move from Ψ(in) to Ψ(out) and back again during each transport cycle. All or part of that movement may come from rearrangement of the protein structure around the transport site rather than an actual movement of the site itself over a distance comparable to the membrane thickness (9), but this has no effect on the present considerations. The work done in moving an isolated charge across a potential difference ΔΨ does not depend on the distance traveled.

Protein charges are derived from acidic and basic groups of the protein in dynamic equilibrium with adjacent aqueous solution. This raises the possibility that protein charges that move across the membrane can also, in the form of H^+ ions, be released into or bound from the adjacent solutions. (This might occur, for example, if binding of transported ions to the protein is in part competitive with binding of H^+ ions.) As a result, the number of protein charges moving from Ψ(in) to Ψ(out) in each cycle need not be the same as the number that returns from Ψ(out) to Ψ(in). It is important to realize, however, that such inequality would mean *net* transport of H^+ ions across the membrane as part of the catalytic process, which would require the inclusion of H^+ ions in the equation for the overall reaction. If we can take it for granted that the stoichiometry of a transport reaction is an established fact and that (as in Eq. **1**) H^+ ions are not involved, then there can be no net transport of protons and no net movement of protein charges over an entire transport cycle. (It should be emphasized that pH dependence of transport kinetics cannot be used as a criterion for or against H^+ translocation. A typical transport protein molecule contains >100 acidic and basic groups, all of which change their charge state as a function of pH and thereby affect rates and equilibria of binding, conformational change, etc.)

Uncharged portions of protein molecules contain a large number of dipoles, many of which are likely to undergo changes in orientation when a conformational change occurs. Many such movements are likely to occur within the plane of the membrane, at positions of unknown electrostatic potential relative to Ψ(in) and Ψ(out), and how big a contribution they might make to the potential dependence of steps that involve conformational change is at present unknown. One can conclude, however, that interactions of these dipole movements with ΔΨ will generally lead to nonintegral values for the β_j of Eq. **4**. We can also be certain that movements of this kind must be reversed when the protein returns to its original state at the end of a cycle, but this condition is automatically satisfied by application of Eq. **5**.

APPLICATION TO Na,K PUMPS

Our version of the generally accepted Post–Albers reaction cycle is shown in Fig. 1. The cation binding sites face the inside of the cell in conformation E_1 and face the extracellular medium in conformation E_2. The cycle allows for random order in the exchange between Na^+ and K^+ ions in states E_1 and E_2–P and for the existence of a weak binding site for ATP in state E_2 (10). Individual cycle steps may be divided into four categories.

(*i*) Translocation steps. Three Na^+ ions are translocated in step 1 and two K^+ ions are moved in the opposite direction in step 2. It is clear from the preceding section that this does not mean that we should set $\beta_1 = 3$ and $\beta_2 = -2$, since protein charges of opposite sign are likely to move across the membrane together with the translocated ions. Quantitative charge compensation is, however, improbable and we can be reasonably certain that a major part of the potential dependence of the overall rate will come from these steps.

(*ii*) Steps in which ATP, ADP, or P_i is bound or dissociated. Binding is entirely from the cytoplasmic side of the membrane, and there is no theoretical reason or experimental evidence to suggest a ΔΨ dependence of the binding process. (It then follows rigorously from thermodynamic conservation laws that the ΔΨ dependence of step 2 must be the same with and without bound ATP.)

(*iii*) Phosphorylation and dephosphorylation. The formation of covalent phosphoenzyme derivatives from bound ATP or P_i in steps 3 and 4 occurs at the substrate binding site and should be inherently potential independent for the same reason that binding per se is presumed to be potential independent. It is known, however, that these interconversions affect the rate of exchange of bound Na^+ or K^+ with the adjacent aqueous media (11). This means that some kind of conformational change, presumably involving charge or dipole movements, is transmitted through the protein in steps 3 and 4 and is likely to make these steps ΔΨ dependent.

(*iv*) Steps in which Na^+ or K^+ is bound or dissociated to or from one or the other side of the membrane. Because the bound ions are to be transported, it is conceivable that binding sites may be in aqueous crevices of the protein that extend into the domain of the membrane, where the local electrostatic potential might differ from Ψ(in) or Ψ(out) in a manner dependent on ΔΨ. We consider it relatively unlikely that this possibility can significantly affect the ΔΨ dependence of overall kinetics and will ignore it in the initial illustrative calculations made in this paper (see *Discussion*).

There is as yet no consensus in the literature with respect to the equilibrium and rate constants associated with the reaction cycle of Fig. 1. A number of different parameter sets can therefore be chosen that are equally consistent with reliable biochemical experimental data, such as the dependence of the overall pump rate on Na^+, K^+, and ATP concentrations, and the apparent equilibrium between the E_1 and E_2 states determined by fluorescence changes. We have here chosen to use the following set, previously used by us to describe ATP activation in isolated cell membranes (10). Equilibrium binding constants (M^{-1}): 500 for K^+(in), 800 for K^+(out), 560 for Na^+(in), 3.15 for Na^+(out), 5×10^6 for ATP (to E_1), 1750 for ATP (to E_2), 1400 for ADP (to $Na3E_1{\sim}P$), 500 for P_i (to E_2); equilibrium constants (K_j) for numbered transitions in Fig. 1, in the direction of the arrows, $K_1 = 3$, K_2 (without bound ATP) $= 1.95 \times 10^{-3}$, K_2 (with bound ATP) $= 5.58$, $K_3 = 0.4$, $K_4 = 1$; forward rate constants for the same transitions (in sec^{-1}), $k_1 = 500$, k_2 (without bound ATP) $= 10$, k_2 (with bound ATP) $= 400$, $k_3 = 2000$, $k_4 = 1330$. Rate constants (k_{on}) for ligand binding were assumed to correspond to values for diffusion-controlled association, except that for ATP binding $k_{on} = 1 \times 10^7$ $M^{-1}{\cdot}sec^{-1}$. With these choices, the cycling rate at $\Delta\Psi = 0$ at physiological (asymmetric) ion concentrations is 5.6 sec^{-1} and becomes a fixed point in the analysis of voltage dependence. The reversal potential ($\Delta\Psi = -204$ mV) is fixed by Eq. **3** and the standard values for concentrations of transported ions, ATP, etc.

Fig. 2 shows the dependence of overall rate on ΔΨ if 100% of the ΔΨ dependence required by Eq. **5** (with $n = 1$) is assigned to a single cycle step—i.e., $\beta = 1$ for one step and $\beta = 0$ for all others. Each curve is seen to have a steep segment in the physiologically important region of $\Delta\Psi = 0$ to -100 mV.

Curve 4 in Fig. 2 is obtained with $\beta_1 = \beta_2 = 0.5$. It illustrates the fact that ΔΨ effects are not additive—i.e., one cannot predict the behavior when several steps are made ΔΨ dependent by combination of curves for a single $\beta_i = 1$. The reason for this is the complex relation between overall rate and kinetic parameters, which involves steady-state levels of cycle intermediates as well as effective rate constants.

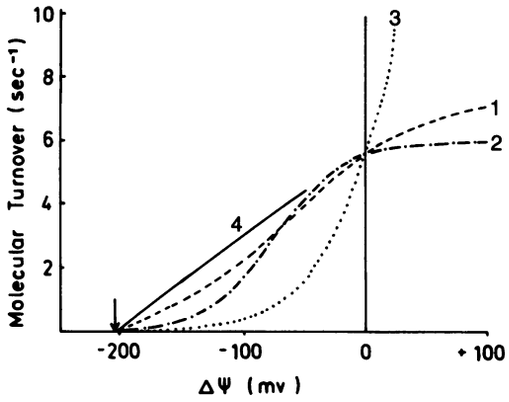

FIG. 2. Effect of putting all $\Delta\Psi$ dependence on a single step. Curve 1, $\beta_1 = 1$; curve 2, $\beta_2 = 1$; curve 3, $\beta_3 = 1$. The plot for $\beta_4 = 1$ (not shown) is very close to curve 2. Curve 4 is for $\beta_1 = \beta_2 = 0.5$, $\beta_3 = \beta_4 = 0$, and is included to show lack of additivity of curves with $\beta_i = 1$. The arrow in this and the following figures represents the fixed reversal potential, −204 mV.

Fig. 3 shows that setting $\beta_1 = +3$ and $\beta_2 = -2$—i.e., ignoring the contribution of protein charges—is not only theoretically unrealistic (see above) but also practically unacceptable. It leads to severe repression of the rate except very close to $\Delta\Psi = 0$.

Fig. 4 shows that considerable flexibility is obtained if we allow all four of the potentially $\Delta\Psi$-dependent steps to contribute. Curve 2 closely approximates the oft-cited observation that the pump current is nearly independent of $\Delta\Psi$ between 0 and −100 mV (12, 13), and it should be noted that it is obtained by "mixing in" only small contributions from the phosphorylation and dephosphorylation steps (steps 3 and 4 in Fig. 1).

Because the measured weak dependence of pump current on $\Delta\Psi$ is subject to considerable quantitative experimental error, recent accurate measurements by Gadsby (14) of the effect of extracellular K^+ at constant $\Delta\Psi$ may be an equally good test of our ability to reproduce experimental data under physiological conditions. Fig. 5 shows Gadsby's experimental results, together with a theoretical curve based on exactly the same parameters as were used for curve 2 of Fig. 4. The fit is satisfactory.

As noted earlier, the set of equilibrium and kinetic parameters on which Figs. 2–5 are based is not unique, and we have therefore repeated some of the calculations with a different parameter set. [The major difference is the use of a much higher equilibrium binding constant for K^+(out), $2 \times 10^4\ M^{-1}$, but the requirement that the theoretical parameters be able to account for biochemical data leads of course to compensating alterations in other binding constants and small changes in

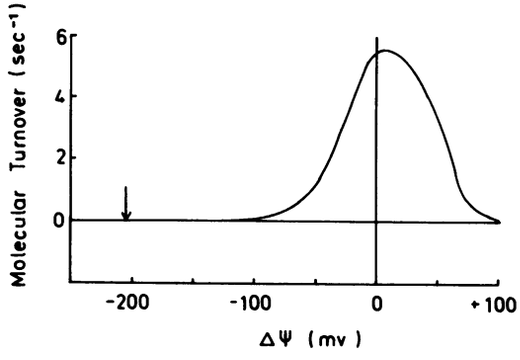

FIG. 3. Effect of assuming no protein contribution to $\Delta\Psi$ dependence: $\beta_1 = -2$; $\beta_2 = 3$; $\beta_3 = \beta_4 = 0$.

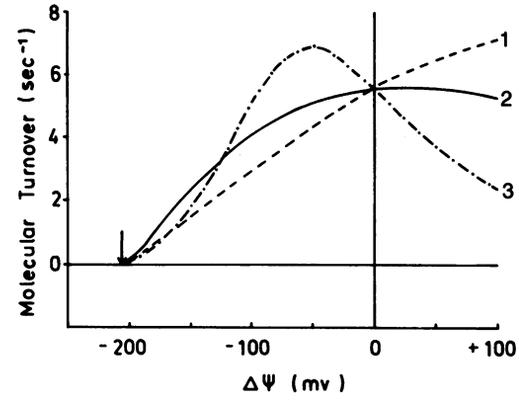

FIG. 4. Effect of allowing steps 3 and 4 to contribute to the $\Delta\Psi$ dependence. Curve 1, $\beta_1 = 0.6$, $\beta_2 = 0.4$, $\beta_3 = \beta_4 = 0$ (result close to curve 1 of Fig. 2); curve 2, $\beta_1 = 0.6$, $\beta_2 = 0.4$, $\beta_3 = -0.15$, $\beta_4 = 0.15$; curve 3, $\beta_1 = 0.5$, $\beta_2 = 0.5$, $\beta_3 = -0.5$, $\beta_4 = 0.5$.

the rate constants for steps 3 and 4.] This particular group of rate and equilibrium constants, though fitting biochemical data as well as the set of parameters listed above, shows a significantly different pump cycle rate even at $\Delta\Psi = 0$ (Fig. 6) when physiological (asymmetric) ion concentrations are used. More important, as is illustrated in Fig. 6 for $\beta_1 = \beta_2 = 0.5$, $\beta_3 = \beta_4 = 0$, the $\Delta\Psi$ dependence can be altered even when identical β values are used. However, the ability to vary four β_i parameters (as in Fig. 4), without any *a priori* theoretical reason for a particular assignment, again permits us to generate a theoretical curve that is consistent with experimental data—i.e., similar to curve 2 of Fig. 4. The β values needed in the present example are $\beta_1 = 0$, $\beta_2 = 1$, $\beta_3 = -0.1$, $\beta_4 = +0.1$.

DISCUSSION

The principal purpose of this paper has been to discuss the physicochemical principles that relate to the incorporation of membrane potential dependence into the theoretical equations (derived from test tube experiments) for ion transport by electrogenic ion pumps. Movement of protein charges across the membrane or within the membrane can be expected to make significant contributions to the $\Delta\Psi$ dependence. Using Na,K pumps as the example, we have shown that calculated data consistent with experimental results can be obtained only if we allow protein charges to contribute (*i*) by largely compensating for the charges carried by the trans-

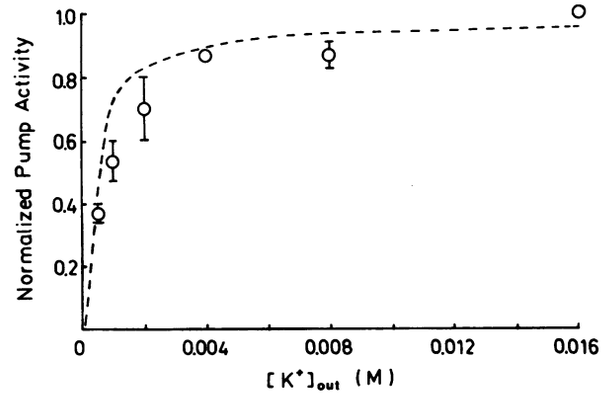

FIG. 5. Activation of pump current by external K^+ at a constant $\Delta\Psi$ of −35 mV, using the same parameters and assignment of β values as for curve 2 of Fig. 5. Points are experimental data of Gadsby (14).

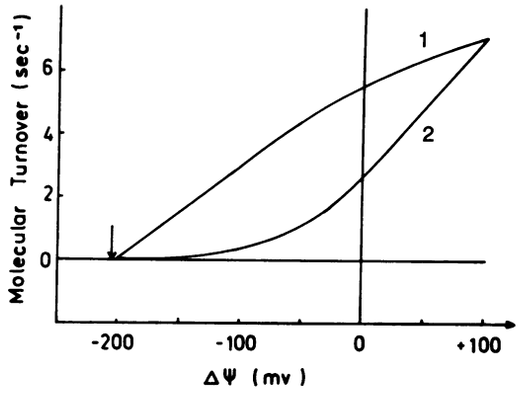

FIG. 6. Effect of different parameter sets, both of which adequately describe biochemical steady-state results for broken membranes. Both curves assume that only the ion translocation steps are $\Delta\Psi$ dependent, with $\beta_1 = \beta_2 = 0.5$ and $\beta_3 = \beta_4 = 0$. Curve 1 is obtained with the parameter set listed in the text.

ported ions in translocation steps and (*ii*) by generating a modest $\Delta\Psi$ dependence for protein conformational changes linked to the transport cycle (phosphorylation and dephosphorylation steps).

In the calculations for Na,K pumps we made the simplifying assumption that the steps in which the transported ions are initially bound to the transport protein (or ultimately dissociated from it) are not significantly $\Delta\Psi$ dependent, but we did not mean thereby to exclude the possibility of a modest contribution from this source. Even greater flexibility is of course obtained if this assumption is discarded. One could then, for example, readily obtain rate versus $\Delta\Psi$ curves that are even flatter between 0 and -100 mV than curve 2 of Fig. 4.

The calculations for the Na,K pump in this paper are based on the Post–Albers model. There appears to be no difficulty in obtaining agreement between theoretical and experimental $\Delta\Psi$ dependence of the pump rate by means of this model, but, because of inability to predict individual β_i in advance, this result is not very parameter specific. We can therefore conclude that the Post–Albers model is consistent with such physiological data as we have available, but there is no reason to believe that this conclusion is specific to that model any more than it is specific to a particular parameter set. What is needed to make physiological data more discriminating in regard to biochemical models is structural information that would permit quantitative prediction of the protein contribution to $\Delta\Psi$ dependence. Alternatively, it would be useful to have transient kinetic data for purified pump protein in reconstituted and well-sealed vesicles, by which the $\Delta\Psi$ dependence of individual cycle transitions could be determined experimentally. This would remove the present arbitrariness from the assignment of values to the β_i parameters.

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1. Chapman, J. B. (1973) *J. Gen. Physiol.* **62**, 643–646.
2. Tanford, C. (1981) *J. Gen. Physiol.* **77**, 223–229.
3. Johnson, E. A., Tanford, C. & Reynolds, J. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5352–5356.
4. Glasstone, S., Laidler, K. J. & Eyring, H. (1941) *The Theory of Rate Processes* (McGraw-Hill, New York).
5. Chapman, J. B., Johnson, E. A. & Kootsey, J. M. (1983) *J. Membr. Biol.* **74**, 139–153.
6. Läuger, P. (1984) *Biochim. Biophys. Acta* **779**, 307–341.
7. Jencks, W. P. (1980) *Adv. Enzymol.* **51**, 75–106.
8. Tanford, C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3701–3705.
9. Tanford, C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2882–2884.
10. Reynolds, J. A., Johnson, E. A. & Tanford, C. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3658–3661.
11. Beaugé, L. A. & Glynn, I. M. (1979) *Nature (London)* **280**, 510–512.
12. Marmor, M. F. (1971) *J. Physiol. (London)* **218**, 599–608.
13. Isenberg, G. & Trautwein, W. (1974) *Pflügers Arch., Eur. J. Physiol.* **350**, 41–54.
14. Gadsby, D. C. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4035–4039.