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ARTICLE

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A new interpretation of flux ratio exponents using statistical rate theory

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Abstract By viewing permeation as a three-step process (movements between the bulk intracellular medium and the channel, within the channel, and between the channel and the extracellular bulk medium), and describing each step in the process with a statistical rate theory (SRT) approach, we can envisage different permeation scenarios which consider whether movements across certain interfaces can be approximated to be at equilibrium. A new interpretation of the flux ratio exponent is presented and its value can be used to predict whether the rate-limiting region occurs within the channel or at the interface. By considering saturation profiles, estimates for SRT exchange rates can be obtained, and a comparison with the more well-known Eyring rate theory is provided.

Key words Ion channels · Nonequilibrium thermodynamics · Saturation · Unidirectional fluxes · Current-voltage curve

Introduction

A fundamental characteristic of ion channels that remains poorly understood is their ability to exhibit both a high conductance and a high degree of selectivity. Simply speaking, a high conductance implies that channels consist of large pores without restrictive regions, while a high degree of selectivity can be generated by restrictive regions in the channel pore. Any explanation of selectivity must include a description of how and why certain species of ions permeate more easily than others, and as such, an understanding of the permeation process itself is necessary.

There are two approaches that are traditionally used in the study of permeation mediated by ion channels. The first approach uses continuum theory or electrodiffusion with the Nernst-Planck equations to describe the concentration of ions within a pore, and the second approach uses barrier kinetics or Eyring rate theory (ERT) to describe the movement of ions within a pore (Dani and Levitt 1990; Hille 1992). Since it is difficult to incorporate ion-ion and ion-channel interactions within an electrodiffusion approach, several workers have chosen to use ERT to describe their biological data. However, the basic assumptions of this theory have been questioned when applied to ion channels (e.g., see Cooper et al. 1985).

In physical kinetics, a nonequilibrium thermodynamic approach, statistical rate theory (SRT), has been developed to describe molecular transport across the interface between macroscopic phases (Ward 1977, 1982; Elliott and Ward 1987a). SRT may be used to derive the expression for the rate of particle transport, where the transport process can be a chemical reaction, transport within a phase, or transport across an interphase. This approach is based on the concept of quantum mechanical transition probabilities and allows the rate to be expressed in terms of the equilibrium exchange rate of the process and the change in the electrochemical potential experienced by the particle undergoing the specific process under consideration. SRT has been examined critically in several experimental systems which include the rates of gas absorption at a liquid-gas interface (Tikuisis and Ward 1992), the rates of adsorption at a gas-solid interface (Ward and Elmoselhi 1986; Elliott and Ward 1997a, b, c), electron exchange between ionic isotopes in solution (Ward 1983; Elliott and Ward 1997a), and the rates of crystal growth or dissolution (Dejmek and Ward 1998). It is particularly interesting to note that where SRT has been successful in predicting the kinetic behaviour, absolute or conventional rate theory (on which ERT is based) has not (Ward 1983; Elliott and Ward 1997a). This is not surprising, given that the application of absolute rate theory to adsorption and desorption processes has been found to be inadequate (Clark 1970).

Using SRT, Skinner et al. (1993) considered permeation through the channel system as a three-step process: movement from the bulk medium into the channel, through

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the channel, and from the channel to the other bulk medium. The channel system consists of the adjacent bulk media in addition to the protein channel itself. Each step through the channel system was described using the SRT formulation. Skinner et al. (1993) were able to obtain nonlinear current-voltage curves in symmetric solutions, nonunity flux ratio exponents, and they underlined the importance of allowing the intracellular and extracellular partition coefficients to be different.

In this paper, we develop SRT flux equations based on a consideration of whether movements at the intracellular interface, at the extracellular interface, or within the channel can be approximated to be at equilibrium. Measurements of flux ratio exponents can then be interpreted in light of where the rate-limiting region in the channel system is located. By considering saturation profiles, an estimate of the SRT exchange rates can be obtained. Finally, we do a comparison with the traditional ERT approach to illustrate the differences between the two approaches.

Theory

Use of SRT

The details of ion channel permeation processes are not known at present. It is unknown how many binding sites are present, how long ions reside there, how much interaction between ions, ions and water, and ions and the channel wall goes on in the pore, and so on. Simply based on this, SRT or any other theory cannot be *directly* examined in ion channel permeation processes.

SRT has been critically examined in several well-defined physical systems (see review by Elliott and Ward 1997a), and it is well established. In particular, these systems include:

1. Carbon monoxide adsorbing onto nickel (Ward and Elmoselhi 1986; Elliott and Ward 1997a, b, c); in this case, only the material properties at equilibrium were used in the SRT equations to predict the experimental outcome, i.e., no fitting parameters were required.
2. Gas absorption at a liquid-gas interface (Ward et al. 1982; Tikuisis and Ward 1992).
3. Electron exchange between ionic isotopes in solution (Ward 1983; Elliott and Ward 1997c); in this case, a long-standing paradox of the need for a “zero-time exchange” was solved.
4. Crystal growth or dissolution at a solid-liquid interface (Dejmek and Ward 1998).

In each of these systems, where it was possible to test SRT *directly*, it was found to accurately *predict* the outcome of experiments. This presents a very strong case for applying SRT to molecular transport in various systems. Therefore, it is certainly reasonable to apply SRT to ion channel systems which certainly involve molecular transport and likely involve processes similar to adsorption and ion exchange mechanisms. Furthermore, SRT does not exclude diffusive motion. It is part of the transport problem, and we showed in our earlier work (Skinner et al. 1993) that the Nernst-Planck (NP) equation could be derived from SRT.

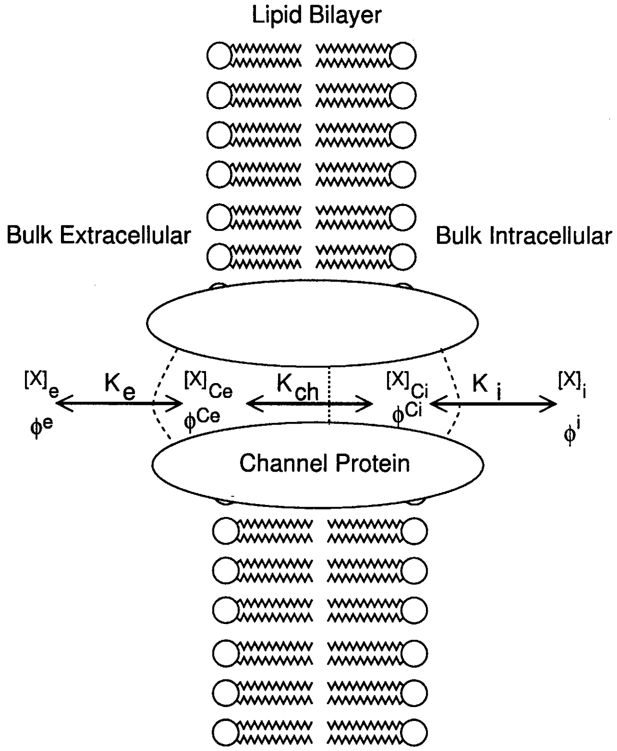

Fig. 1 Schematic of the channel system. The channel system consists of the adjacent bulk media and the pore generated by the open conformation of the ion channel protein. K_e, K_i, K_{ch} are the equilibrium exchange rates between the bulk extracellular medium and the channel on the extracellular side, between the bulk intracellular medium and the channel on the intracellular side, and within the channel, respectively. The *dashed curves* represent the interfaces between the bulk media and the channel interior, and the *dotted line* signifies an arbitrary interface between the two ends of the channel. $[X]_e$, $[X]_{Ce}$, $[X]_{Ci}$, $[X]_i$ are the concentrations of species X in the extracellular medium, in the channel on the extracellular side, in the channel on the intracellular side, and in the intracellular medium, respectively. ϕ^e, ϕ^{Ce}, ϕ^{Ci}, ϕ^i are the electrical potentials in the extracellular medium, in the channel on the extracellular side, in the channel on the intracellular side, and in the intracellular medium, respectively

Review of SRT applied to ion permeation

We briefly review our earlier work in which SRT was used to model permeation in ion channels (Skinner et al. 1993).

Among other processes, SRT may be used to derive expressions for the rate of transport of ions across an interface between fluid phases or within a phase. As illustrated in Fig. 1, we consider the channel and bulk media to be aqueous phases with different properties. There are three phases, one for the channel medium, one for the intracellular medium, and one for the extracellular medium. In

Fig. 1 the dashed curves represent the interfaces between the bulk phases and the channel phase, and the dotted line represents an arbitrary interface between the two ends of the channel.

Following the SRT procedure, one obtains as the expression for the net rate of transport of species X across the interface between phase 1 and phase 2 as (Ward 1982, 1983):

$$j_{1,2}=K_{1,2}\,(\delta_{1,2}-\delta_{1,2}^{-1}) \tag{1}$$

where $K_{1,2}$ is the equilibrium exchange rate across a unit area of the interface (i.e., mol/s per unit area) and depends on material properties of the system, and

$$\delta_{1,2}=\exp\left(\frac{\mu_X^1-\mu_X^2}{RT}\right) \tag{2}$$

where μ_X^1, μ_X^2 is the electrochemical potential of species X in phase 1 or 2 respectively, R is the gas constant, and T is the temperature. The SRT rate expression can be understood from a more physical point of view, as previously described in Skinner et al. (1993) and shown in Appendix A. To give some insight into the equilibrium exchange rate parameter, we provide a comparison with conventional or absolute rate theory in Appendix A. It is important to note that since $K_{1,2}$ is an equilibrium parameter, the nonequilibrium aspect of this SRT approach is encompassed in the $(\delta_{1,2}-\delta_{1,2}^{-1})$ part of Eq. (1).

Applying SRT and using a standard form for the electrochemical potential, we obtain three fluxes: $j_{\mathrm{i,Ci}}$, from the intracellular medium to the channel; $j_{\mathrm{Ci,Ce}}$, across the channel; and $j_{\mathrm{Ce,e}}$, from the channel to the extracellular medium:

$$j_{\mathrm{i,Ci}}=K_{\mathrm{i}}\left[-\frac{u\,(\mathrm{Ci})}{u\,(\mathrm{i})\,\beta_X^{\mathrm{i}}}+\frac{u\,(\mathrm{i})\,\beta_X^{\mathrm{i}}}{u\,(\mathrm{Ci})}\right] \tag{3}$$

$$j_{\mathrm{Ci,Ce}}=K_{\mathrm{ch}}\left[\frac{u\,(\mathrm{Ci})}{u\,(\mathrm{Ce})}-\frac{u\,(\mathrm{Ce})}{u\,(\mathrm{Ci})}\right] \tag{4}$$

$$j_{\mathrm{Ce,e}}=K_{\mathrm{e}}\left[\frac{u\,(\mathrm{Ce})}{u\,(\mathrm{e})\,\beta_X^{\mathrm{e}}}-\frac{u\,(\mathrm{e})\,\beta_X^{\mathrm{e}}}{u\,(\mathrm{Ce})}\right] \tag{5}$$

where

$$u\,(\mathrm{Ci})=[\mathrm{X}]_{\mathrm{Ci}}\exp\,(\theta_X\,\phi^{\mathrm{Ci}}) \tag{6}$$

$$u\,(\mathrm{Ce})=[\mathrm{X}]_{\mathrm{Ce}}\exp\,(\theta_X\,\phi^{\mathrm{Ce}}) \tag{7}$$

$$u\,(\mathrm{i})=[\mathrm{X}]_{\mathrm{i}}\exp\,(\theta_X\,\phi^{\mathrm{i}}) \tag{8}$$

$$u\,(\mathrm{e})=[\mathrm{X}]_{\mathrm{e}}\exp\,(\theta_X\,\phi^{\mathrm{e}}) \tag{9}$$

and the intracellular and extracellular partition coefficients, β_X^{i}, β_X^{e}, are defined as (Skinner et al. 1993):

$$\beta_X^{\mathrm{i}}=\frac{[\mathrm{X}]_{\mathrm{Ci,\,equil}}}{[\mathrm{X}]_{\mathrm{i,\,equil}}}\exp\,(\theta_X\,(\phi_{\mathrm{equil}}^{\mathrm{Ci}}-\phi_{\mathrm{equil}}^{\mathrm{i}})) \tag{10}$$

$$\beta_X^{\mathrm{e}}=\frac{[\mathrm{X}]_{\mathrm{Ce,\,equil}}}{[\mathrm{X}]_{\mathrm{e,\,equil}}}\exp\,(\theta_X\,(\phi_{\mathrm{equil}}^{\mathrm{Ce}}-\phi_{\mathrm{equil}}^{\mathrm{e}})) \tag{11}$$

[X] is the concentration of ionic species X; the superscripts and subscripts i, e, Ci, Ce refer to the intracellular side, the extracellular side, the intracellular side in the channel, and the extracellular side in the channel, respectively; the subscript equil refers to the equilibrium value of the parameter; $\theta_X=z_X F/RT$, where z_X is the valency of ionic species X, and F is Faraday's constant; ϕ is the electrical potential; K_{i}, K_{ch}, K_{e} are the equilibrium exchange rates for the three fluxes as defined above.

The definition of the partition coefficient is standard except that we have included the equilibrium surface potentials here rather than introducing separate parameters. The ratio of the partition coefficients is equivalent to the ratio of activity coefficients if the reference electrochemical potentials are the same on both sides of the membrane (see Skinner et al. 1993). Therefore, the partition coefficient, β, depends on activity coefficients, solubilities, and surface potentials. Also, since we assume that there is a single phase within the channel, the partition coefficient is unity there, and β does not appear in Eq. (4). Even though β is unity within the channel, it is still possible to have a gradient within the channel so that a non-equilibrium description is required.

If we assume steady-state conditions (which does *not* imply a constant field since this is not a continuum theory), then the fluxes given by Eqs. (3)–(5) are equal ($j_{\mathrm{i,Ci}}=j_{\mathrm{Ci,Ce}}=j_{\mathrm{Ce,e}}$), and the flux can be determined numerically (or analytically in certain situations) for given exchange rates, concentrations, voltages, and partition coefficient values (see Skinner et al. 1993).

Different permeation scenarios

When nonequilibrium exists throughout the channel system, the net flux is given by equating Eqs. (3)–(5) and the flux equations must be solved numerically (see Skinner et al. 1993). If nonequilibrium exists throughout the channel system and the exchange rates are similar, i.e., $K_{\mathrm{i}}\approx K_{\mathrm{e}}\approx K_{\mathrm{ch}}=K$, then Eqs. (3)–(5) can be simplified to give the net flux, j, as:

$$j=K\left[\left(\frac{\beta_X^{\mathrm{i}}\,[\mathrm{X}]_{\mathrm{i}}}{\beta_X^{\mathrm{e}}\,[\mathrm{X}]_{\mathrm{e}}}\exp\,(\theta_X\,V)\right)^{1/3}-\left(\frac{\beta_X^{\mathrm{e}}\,[\mathrm{X}]_{\mathrm{e}}}{\beta_X^{\mathrm{i}}\,[\mathrm{X}]_{\mathrm{i}}}\exp\,(-\,\theta_X\,V)\right)^{1/3}\right] \tag{12}$$

where $V=\phi^{\mathrm{i}}-\phi^{\mathrm{e}}$, is the voltage. However, if we consider that equilibrium exists (to a good approximation) at certain interfaces of the channel system, then Eqs. (3)–(5) can be simplified in the following manner. If equilibrium can be assumed to exist at the extracellular interface, i.e., $K_{\mathrm{e}}\gg K_{\mathrm{i}}$ and $K_{\mathrm{e}}\gg K_{\mathrm{ch}}$, then to keep Eq. (5) bounded at large values of K_{e}, Eq. (5) can be replaced by:

$$u\,(\mathrm{Ce})=\beta_X^{\mathrm{e}}\,u\,(\mathrm{e}) \tag{13}$$

Similarly, if equilibrium can be assumed to exist at the intracellular interface, i.e., $K_{\text{i}} \gg K_{\text{e}}$ and $K_{\text{i}} \gg K_{\text{ch}}$, then Eq. (3) can be replaced by:

$$u(\text{Ci}) = \beta_{\text{X}}^{\text{i}}\, u(\text{i}) \tag{14}$$

and, if equilibrium can be assumed to exist across the channel interior (see dotted line signifying interface in Fig. 1) i.e., $K_{\text{ch}} \gg K_{\text{i}}$ and $K_{\text{ch}} \gg K_{\text{e}}$, then Eq. (4) can be replaced by:

$$u(\text{Ci}) = u(\text{Ce}) \tag{15}$$

Another way of stating this is that the net flux is controlled by certain rate-limiting regions of the channel system. We consider three different scenarios below.

1. Nonequilibrium exists only within the channel interior. $K_{\text{ch}} \ll K_{\text{i}}$ and $K_{\text{ch}} \ll K_{\text{e}}$, and equating Eqs. (3)–(5) can be simplified as described above to give the net flux, j:

   $$j = K_{\text{ch}} \left[\frac{\beta_{\text{X}}^{\text{i}}\, [\text{X}]_{\text{i}}}{\beta_{\text{X}}^{\text{e}}\, [\text{X}]_{\text{e}}} \exp(\theta_{\text{X}} V) - \frac{\beta_{\text{X}}^{\text{e}}\, [\text{X}]_{\text{e}}}{\beta_{\text{X}}^{\text{i}}\, [\text{X}]_{\text{i}}} \exp(-\theta_{\text{X}} V) \right] \tag{16}$$

2. Nonequilibrium exists across two interfaces and the exchange rates for these processes are similar. There are three possibilities in this category: (i) $K_{\text{i}} \gg K_{\text{ch}}$ and $K_{\text{i}} \gg K_{\text{e}}$, and let $K_{\text{ch}} \approx K_{\text{e}} = K$; (ii) $K_{\text{e}} \gg K_{\text{ch}}$ and $K_{\text{e}} \gg K_{\text{i}}$, and let $K_{\text{ch}} \approx K_{\text{i}} = K$; or (iii) $K_{\text{ch}} \gg K_{\text{i}}$ and $K_{\text{ch}} \gg K_{\text{e}}$, and let $K_{\text{i}} \approx K_{\text{e}} = K$. (Note that since the channel is considered to be a single phase, if $K_{\text{ch}} \gg K_{\text{i}}$ and $K_{\text{ch}} \gg K_{\text{e}}$, then it must be that $K_{\text{i}} = K_{\text{e}}$ since they represent the same process.) Using the described simplifications, the net flux, j, for the three possibilities in this category is:

   $$j = K \left[\left(\frac{\beta_{\text{X}}^{\text{i}}\, [\text{X}]_{\text{i}}}{\beta_{\text{X}}^{\text{e}}\, [\text{X}]_{\text{e}}} \exp(\theta_{\text{X}} V) \right)^{1/2} - \left(\frac{\beta_{\text{X}}^{\text{e}}\, [\text{X}]_{\text{e}}}{\beta_{\text{X}}^{\text{i}}\, [\text{X}]_{\text{i}}} \exp(-\theta_{\text{X}} V) \right)^{1/2} \right] \tag{17}$$

   where K refers to the appropriate exchange rate for the particular case.

3. Nonequilibrium exists across two interfaces and the exchange rates for these processes are different. There are only two possibilities here since a single phase in the channel implies that $K_{\text{i}} = K_{\text{e}}$: (i) $K_{\text{i}} \gg K_{\text{ch}}$ and $K_{\text{i}} \gg K_{\text{e}}$; or (ii) $K_{\text{e}} \gg K_{\text{ch}}$ and $K_{\text{e}} \gg K_{\text{i}}$. The net flux, j, for these two possibilities is:

   $$j = K_{\text{ch}} \left[\left(\frac{\beta_{\text{X}}^{\text{i}}\, [\text{X}]_{\text{i}}}{\beta_{\text{X}}^{\text{e}}\, [\text{X}]_{\text{e}}} \exp(\theta_{\text{X}} V) \right)^{1/2} (\mathit{factor})^{1/2} - \left(\frac{\beta_{\text{X}}^{\text{e}}\, [\text{X}]_{\text{e}}}{\beta_{\text{X}}^{\text{i}}\, [\text{X}]_{\text{i}}} \exp(-\theta_{\text{X}} V) \right)^{1/2} (1/\mathit{factor})^{1/2} \right] \tag{18}$$

   where

   $$\mathit{factor} = \frac{(K_{\text{int f}}/K_{\text{ch}})(\beta_{\text{X}}^{\text{i}}\, [X]_{\text{i}}/\beta_{\text{X}}^{\text{e}}\, [X]_{\text{e}}) \exp(\theta_{\text{X}} V) + 1}{(\beta_{\text{X}}^{\text{i}}\, [X]_{\text{i}}/\beta_{\text{X}}^{\text{e}}\, [X]_{\text{e}}) \exp(\theta_{\text{X}} V) + (K_{\text{int f}}/K_{\text{ch}})} \tag{19}$$

and $K_{\text{int f}} = K_{\text{e}}$ for possibility (i) or $K_{\text{int f}} = K_{\text{i}}$ for possibility (ii) above.

The conductance, g, is defined by $g = \mathrm{d}I/\mathrm{d}V$, where I is the current and $I = z_{\text{X}}\, F\, \text{j}$. For the special case of symmetric solutions (i.e., $[\text{X}]_{\text{i}} = [\text{X}]_{\text{e}}$), the conductance at 0 mV is:

$$g = \frac{z_{\text{X}}\, F\, \theta_{\text{X}}\, K_{\text{ch}}}{2} \cdot \left[(\beta_{\text{X}}^{\text{i}}/\beta_{\text{X}}^{\text{e}})^{1/2} (\mathit{factor})^{-1/2} \left(1 + \frac{(\beta_{\text{X}}^{\text{i}}/\beta_{\text{X}}^{\text{e}})[(K_{\text{int f}}/K_{\text{ch}})^2 - 1]}{[(\beta_{\text{X}}^{\text{i}}/\beta_{\text{X}}^{\text{e}}) + (K_{\text{int f}}/K_{\text{ch}})]^2} \right) + (\beta_{\text{X}}^{\text{i}}/\beta_{\text{X}}^{\text{e}})^{-1/2} (\mathit{factor})^{1/2} \left(1 + \frac{(\beta_{\text{X}}^{\text{i}}/\beta_{\text{X}}^{\text{e}})[(K_{\text{int f}}/K_{\text{ch}})^2 - 1]}{[(\beta_{\text{X}}^{\text{i}}/\beta_{\text{X}}^{\text{e}})(K_{\text{int f}}/K_{\text{ch}}) + 1]^2} \right) \right] \tag{20}$$

and

$$\mathit{factor} = \frac{(K_{\text{int f}}/K_{\text{ch}})(\beta_{\text{X}}^{\text{i}}/\beta_{\text{X}}^{\text{e}}) + 1}{(\beta_{\text{X}}^{\text{i}}/\beta_{\text{X}}^{\text{e}}) + (K_{\text{int f}}/K_{\text{ch}})} \tag{21}$$

If in addition, the partition coefficient ratio, $(\beta_{\text{X}}^{\text{i}}/\beta_{\text{X}}^{\text{e}})$, is unity the conductance is further simplified to:

$$g = z_{\text{X}}\, F\, \theta_{\text{X}}\, K_{\text{ch}} \left[1 + \frac{(K_{\text{int f}}/K_{\text{ch}})^2 - 1}{[1 + (K_{\text{int f}}/K_{\text{ch}})]^2} \right] \tag{22}$$

$$g = \frac{2\, z_{\text{X}}^2\, F^2}{RT} \frac{K_{\text{ch}}\, K_{\text{int f}}}{K_{\text{ch}} + K_{\text{int f}}} \tag{23}$$

Flux ratios

Classical

With passive diffusion through the channel and the absence of any flux coupling, i.e., independent ion movements, the ratio of the unidirectional fluxes (flux ratio) is equal to the ratio of the electrochemical activities of the ion in the bulk intracellular and extracellular solutions, i.e.,

$$\frac{\text{Efflux}}{\text{Influx}} = \left(\frac{[\text{X}]_{\text{i}}}{[\text{X}]_{\text{e}}} \exp(\theta_{\text{X}} V) \right)^{n'} \tag{24}$$

where the flux ratio exponent, n', equals one and the partition coefficients are assumed to be the same (see Aidley and Stanfield 1996, p. 131; or Hille 1992, p. 302). For example, using the Goldman-Hodgkin-Katz (GHK) current equation derived from an electrodiffusion approach (i.e., Nernst-Planck equations), and where the partition coefficient is considered to be the same on both sides of the membrane and no ion-ion or ion-channel interactions are included, a flux ratio exponent of unity is obtained. However, it has been known for several decades that the flux ratio is better described with an exponent different from one (Hodgkin and Keynes 1955; and see Hille 1992, p. 302), and models based on a barrier kinetic approach have been developed in which the flux ratio exponent can be roughly equated with the maximum number of ions allowed in the channel (Hille and Schwartz 1978; Kohler and Heckmann 1979; Levitt 1984).

SRT approach

From the basic SRT flux equation, Eq. (1), the efflux is given by $K_{1,2}\ \delta_{1,2}$ and the influx is given by $K_{1,2}\ \delta_{1,2}^{-1}$. Therefore, the flux ratio is $\delta_{1,2}^{2}$. Now consider the various permeation scenarios. Note that when the exchange rates are similar, the flux ratio does not depend on the exchange rates. For example, in scenario 2, where the influx and efflux are:

$$\mathrm{Efflux} = K\left(\frac{\beta_X^i\,[X]_i}{\beta_X^e\,[X]_e}\exp\left(\theta_X V\right)\right)^{1/2} \tag{25}$$

$$\mathrm{Influx} = K\left(\frac{\beta_X^e\,[X]_e}{\beta_X^i\,[X]_i}\exp\left(-\theta_X V\right)\right)^{1/2} \tag{26}$$

the flux ratio is then given by:

$$\frac{\mathrm{Efflux}}{\mathrm{Influx}} = \frac{\beta_X^i\,[X]_i}{\beta_X^e\,[X]_e}\exp\left(\theta_X V\right) \tag{27}$$

However, if the exchange rates are different, as in scenario 3, then the flux ratio *does* depend on the exchange rates. In this case, the efflux, influx, and flux ratio are given by:

$$\mathrm{Efflux} = K_{\mathrm{ch}}\left(\frac{\beta_X^i\,[X]_i}{\beta_X^e\,[X]_e}\exp\left(\theta_X V\right)\right)^{1/2}(\mathit{factor})^{1/2} \tag{28}$$

$$\mathrm{Influx} = K_{\mathrm{ch}}\left(\frac{\beta_X^e\,[X]_e}{\beta_X^i\,[X]_i}\exp\left(-\theta_X V\right)\right)^{1/2}(\mathit{factor})^{1/2} \tag{29}$$

$$\frac{\mathrm{Efflux}}{\mathrm{Influx}} = \frac{\beta_X^i\,[X]_i}{\beta_X^e\,[X]_e}\exp\left(\theta_X V\right)(\mathit{factor}) \tag{30}$$

where (*factor*) is given by Eq. (19) and depends on the particular exchange rates.

Results

Flux ratio exponents

To compare with the classical Using flux-ratio criterion, we calculate the flux ratio exponent based on the following form:

$$\frac{\mathrm{Efflux}}{\mathrm{Influx}} = \left(\frac{\beta_X^i\,[X]_i}{\beta_X^e\,[X]_e}\exp\left(\theta_X V\right)\right)^{n''} \tag{31}$$

where (compared to the classical approach) the partition coefficients are not assumed to be the same on the both sides of the membrane. We symbolize the flux ratio exponent in this more general formulation as n'' (to distinguish it from n' used in the classical approach; see previous section).

Using the SRT flux equations for the different scenarios (see above), the flux ratio exponent, n'' is calculated (analytically in some circumstances, otherwise numerically, see previous section). The results are shown in Table 1. Based on the different scenarios considered, n'' can be related to whether equilibrium can be assumed to exist in different regions of the channel system and the relative sizes of the exchange rates. In other words, it gives a measure of the degree of disequilibrium that exists during channel permeation. For example, a value of two implies that nonequilibrium exists only within the channel interior, whereas a value greater than one (but less than two) implies that either nonequilibrium exists within the channel and at the extracellular interface or that nonequilibrium exists within the channel and at the intracellular interface, but in either case the channel exchange rate is *less* than the interfacial exchange rate.

Table 1 Flux ratio exponents and their interpretation

Flux ratio exponent, n''	Physical meaning/interpretation
2	Nonequilibrium only within channel ($K_{\mathrm{ch}} \ll K_i$ and $K_{\mathrm{ch}} \ll K_e$)
1	Nonequilibrium within channel and at extracellular interface, with $K_{\mathrm{ch}} \approx K_e$ ($K_i \gg K_{\mathrm{ch}}$ and $K_i \gg K_e$)
1	Nonequilibrium within channel and at intracellular interface, with $K_{\mathrm{ch}} \approx K_i$ ($K_e \gg K_{\mathrm{ch}}$ and $K_e \gg K_i$)
1	Nonequilibrium at intracellular and extracellular interfaces, with $K_e \approx K_i$ ($K_{\mathrm{ch}} \gg K_i$ and $K_{\mathrm{ch}} \gg K_e$)
2/3	Nonequilibrium at all interfaces, with $K_{\mathrm{ch}} \approx K_i \approx K_e$
< 2/3	Nonequilibrium at all interfaces, with K's all different
< 1	Nonequilibrium within channel and at extracellular interface, with $K_{\mathrm{ch}} > K_e$ ($K_i \gg K_{\mathrm{ch}}$ and $K_i \gg K_e$) or Nonequilibrium within channel and at intracellular interface, with $K_{\mathrm{ch}} > K_i$ ($K_e \gg K_{\mathrm{ch}}$ and $K_e \gg K_i$)
> 1	Nonequilibrium within channel and at extracellular interface, with $K_{\mathrm{ch}} < K_e$ ($K_i \gg K_{\mathrm{ch}}$ and $K_i \gg K_e$) or Nonequilibrium within channel and at intracellular interface, with $K_{\mathrm{ch}} < K_i$ ($K_e \gg K_{\mathrm{ch}}$ and $K_e \gg K_i$)

In general, if we only consider situations where nonequilibrium exists within the channel interior and across one of the interfaces, then we can say that as the interfacial exchange rate decreases relative to the channel exchange rate, there is a decrease in n''. This is illustrated in Table 2: if the interfacial exchange rate is greater than the channel exchange rate, then $n'' > 1$; if the channel exchange rate is greater than the interfacial exchange rate, then $n'' < 1$; and $n'' = 1$ if the rates are the same. The smaller exchange rate would constitute the rate-limiting region of the system.

Table 1 shows that the flux ratio exponent can range from zero to two, and in Appendix B we prove that $0 \le n'' \le 2$. Flux ratio exponent values in excess of two have been obtained for potassium channels (Begenisich and DeWeer 1980; Vestergaard-Bogind et al. 1985; Stampe and Begenisich 1996). However, in those cases, the flux ratio exponent was calculated using the classical approach [Eq. (24)]. With our formulation, we know that n'' cannot exceed two, and the only difference between the classical flux ratio exponent and n'' is that the partition coefficients are allowed

Table 2 Flux ratio exponent values for different channel and interfacial exchange rates

K_{ch}	$K_{\mathrm{int\,f}}$	$K_{\mathrm{ch}}/K_{\mathrm{int\,f}}$	Flux ratio exponent, n''
1.0×10^{-5}	∞ or $\gg K_{\mathrm{ch}}$	0	2
0.001×10^{-6}	1.0×10^{-6}	0.001	1.997
0.005×10^{-6}	1.0×10^{-6}	0.005	1.983
0.01×10^{-6}	1.0×10^{-6}	0.01	1.967
0.02×10^{-6}	1.0×10^{-6}	0.02	1.936
0.05×10^{-6}	1.0×10^{-6}	0.05	1.854
0.1×10^{-6}	1.0×10^{-6}	0.1	1.741
0.2×10^{-6}	1.0×10^{-6}	0.2	1.571
0.5×10^{-6}	1.0×10^{-6}	0.5	1.267
1.0×10^{-6}	1.0×10^{-6}	1.0	1
2.0×10^{-6}	1.0×10^{-6}	2.0	0.733
3.0×10^{-6}	1.0×10^{-6}	3.0	0.589
5.0×10^{-6}	1.0×10^{-6}	5.0	0.429
8.0×10^{-6}	1.0×10^{-6}	8.0	0.307
9.0×10^{-6}	1.0×10^{-6}	9.0	0.281
10.0×10^{-6}	1.0×10^{-6}	10.0	0.26
20.0×10^{-6}	1.0×10^{-6}	20.0	0.146
50.0×10^{-6}	1.0×10^{-6}	50.0	0.064
70.0×10^{-6}	1.0×10^{-6}	70.0	0.046
100.0×10^{-6}	1.0×10^{-6}	100.0	0.0033

Table 3 Classical flux ratio exponents at different partition coefficient ratios and concentrations[a]

$[\mathrm{K}]_e$	$[\mathrm{K}]_i$	$\beta^i_{\mathrm{K}^+}/\beta^e_{\mathrm{K}^+}$	n''	n'
20	350	1.2	2.0	2.3
20	350	1.5	2.0	2.6
40	350	1.2	2.0	2.5
40	350	1.5	2.0	3.1
20	200	1.2	2.0	2.4
20	200	1.5	2.0	2.9

[a] $V=-38$ mV; $K_i/K_{\mathrm{ch}}=1500$. Concentration and voltage value chosen to be similar to those in Begenisich and DeWeer (1980)

to be different. Therefore, if flux ratio exponents greater than two are measured, then the partition coefficient ratio *must* be different from one. In other words, the partition coefficients cannot be considered to be the same on both sides of the membrane.

To illustrate how much of an error could be obtained if a non-unity partition coefficient ratio is ignored, we calculate some examples of n'' and n'. These values are shown in Table 3 for the situation where one approximates equilibrium conditions at the extracellular interface, but not within the channel or at the intracellular interface, and $K_{\mathrm{ch}} \ll K_i$. In general, the error depends on the voltage, the ratio of the intracellular and extracellular concentrations, and the ratio of the partition coefficients. For example, a partition coefficient ratio of 1.2 yields an n' value of 2.4 at −38 mV when the extracellular and intracellular potassium concentrations are 20 mM and 200 mM, respectively. With a partition coefficient ratio of 1.5, n' is 2.6 at −38 mV with extracellular and intracellular concentrations of 20 mM and 350 mM, respectively. This can be compared with Begenisich and DeWeer's (1980) data in which they obtained flux ratio exponents that ranged from 1.8 to 3.65 (depending on the particular method) at −38 mV and extracellular and intracellular concentrations of 20 mM and 350 mM, respectively.

We indicated in our previous work (Skinner et al. 1993) that unequal partition coefficients could lead to a current reversal at a voltage that is different from the Nernst potential, where the Nernst potential is classically defined as:

$$V_{\mathrm{X,\,Nernst}} = \frac{1}{\theta_{\mathrm{X}}} \ln\left(\frac{[\mathrm{X}]_e}{[\mathrm{X}]_i}\right) \tag{32}$$

As is well known, this classical description does not allow for the existence of different activity or partition coefficients on either side of the membrane. To put the above calculations into perspective: a partition coefficient ratio value, β^i/β^e, of 1.2 can produce a measured reversal potential that is 5 mV less than the calculated Nernst potential [Eq. (32)]. The above calculations simply serve to demonstrate the possibility that unequal partition coefficients can be responsible for flux ratio exponents being greater than two.

Implications for a GHK approach

In deriving the GHK current equation from the Nernst-Planck equation, equilibrium is assumed to exist at the channel interfaces with equal intracellular and extracellular partition coefficients (e.g., see Hille 1992, p. 345). This allows the permeability, P, to be defined as $P=D\beta/l$, where D is the diffusion coefficient, l is the membrane thickness, and β is the extracellular or intracellular partition coefficient. Thus, from the work described above, if the measured flux ratio exponent exceeds two, then the partition coefficients on the intracellular and extracellular sides *must* be different and, strictly speaking, using permeabilities and permeability ratios to refer to the data would be incorrect.

Saturation

The classical theory of membrane permeation was based on independent ion movements and the most commonly used formalism is the GHK current equation. However, a common characteristic of ion fluxes through channels which is not exhibited by the GHK equation is saturation, in which the conductance reaches a maximal value and stays there as the ion concentrations are increased. This discrepancy caused workers to turn to alternate descriptions, most commonly an ERT formalism (Eyring et al. 1949; Woodbury 1971). However, it now seems likely and possible that the assumptions underlying such models are invalid (e.g., see Eisenberg 1996; Nonner and Eisenberg 1998).

Let us consider the channel conductance using the SRT-based equations and the different permeation scenarios described above. We restrict the investigation to situations where nonequilibrium does not exist throughout the channel system (i.e., $n'' > 2/3$), which means that equilibrium can be assumed to exist across one of the interfaces. This restriction seems reasonable since, based on the experimental literature, flux ratio exponent values much less than one are not commonly reported (but see Schagina et al. 1983). Therefore, unless measured reversal potentials and Nernst potentials differ by more than 10–15 mV, nonequilibrium throughout the channel system (i.e., $n'' \leq 2/3$) is unlikely. Thus, we have scenarios 1–3 (see above) where scenario 3 with nonequilibrium existing within the channel and one of the interfaces, and the exchange rates being different. Roughly speaking, scenarios 1 and 2 can be thought of as special cases of scenario 3. In scenario 2, the exchange rates are not very different, and in scenario 1 the exchange rates are very different with the interfacial exchange rate being significantly larger than the channel exchange rate so that equilibrium can be assumed to exist at the interface, i.e., $K_{\mathrm{int\,f}} \gg K_{\mathrm{ch}}$.

Saturation is described such that, for a given voltage, the conductance first increases and then saturates as the ionic concentrations are symmetrically raised. If we assume that current (or conductance) changes that occur with changes in concentration are not mostly due to changes in the partition coefficient ratio, then we know that when the current (or conductance) changes with *symmetric* variations in concentration, it is due to changes in $K_{\mathrm{int\,f}}$ and K_{ch}. Therefore, as the concentrations are increased, the current and conductance increases could be due to changes in K_{ch}, $K_{\mathrm{int\,f}}$, or both. Therefore, for a given voltage (say, 0 mV), the conductance for the three different scenarios are given by:

- Scenario 1:

$$g = 2\,\frac{z_{\mathrm{X}}^2\,F^2}{RT}\,K_{\mathrm{ch}} \tag{33}$$

- Scenario 2:

$$g = \frac{z_{\mathrm{X}}^2\,F^2}{RT}\,K \tag{34}$$

- Scenario 3:

$$g = 2\,\frac{z_{\mathrm{X}}^2\,F^2}{RT}\,\frac{K_{\mathrm{ch}}\,K_{\mathrm{int\,f}}}{K_{\mathrm{ch}} + K_{\mathrm{int\,f}}} \tag{35}$$

Figure 2 shows that increases in either K_{ch} or $K_{\mathrm{int\,f}}$ lead to increases in the conductance. For now, if we do not consider the possibility that both exchange rates saturate (or do not change with concentration), then we can say that as the concentration increases, either (1) only one of the exchange rates increases significantly so that the other exchange rate can be considered to be unchanging, or (2) both exchange rates change with increasing concentration.

If case (1) is true, then it is impossible to get saturation with scenarios 1 or 2 (scenario 2 is not valid since the rates should be the same, and scenario 1 only has one exchange rate affecting conductance). The saturating conductance is given by [lim $(K_{\mathrm{int\,f}} \to \infty)$, Eq. (35)]:

$$g = 2\,\frac{z_{\mathrm{X}}^2\,F^2}{RT}\,K_{\mathrm{ch}} \tag{36}$$

if $K_{\mathrm{int\,f}}$ is the significantly changing exchange rate with increasing concentration, or by [lim $(K_{\mathrm{ch}} \to \infty)$, Eq. (35)]:

$$g = 2\,\frac{z_{\mathrm{X}}^2\,F^2}{RT}\,K_{\mathrm{int\,f}} \tag{37}$$

if K_{ch} is the significantly changing exchange rate with increasing concentration. In this case, one can use the saturating conductance value to estimate one of the exchange rates at the given concentration. The other exchange rate can then be calculated from the full conductance equation [Eq. (35)] above.

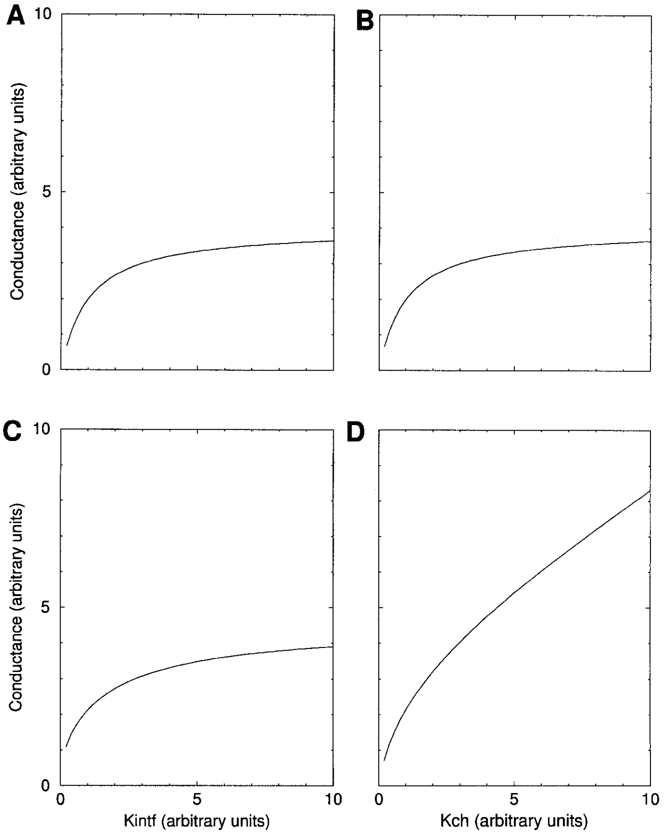

Fig. 2A–D Conductance changes as exchange rates are varied. **A, B** A unity partition coefficient ratio is used in Eq. (20) (symmetric solutions and 0 mV). Nonequilibrium exists within the channel and at one of the interfaces. In **A**, $K_{\mathrm{int\,f}}$ is varied and K_{ch} is fixed at 1.0. In **B**, K_{ch} is varied and $K_{\mathrm{int\,f}}$ is fixed at 1.0. The particular units are not critical (arbitrary units) but rather the increasing trend of the conductance as the exchange rates are increased. **C, D** Respectively the same as **A, B** except now the ratio of the intracellular to the extracellular partition coefficient ratio is not unity but is 2

However, if case (2) is true, then one could consider that one of the exchange rates increases and the other decreases with increasing concentration, leading to a net conductance increase and eventual saturation. This would only apply to scenario 3 since scenario 1 already assumes that $K_{\mathrm{int\,f}} \gg K_{\mathrm{ch}}$, and in scenario 2 the two exchange rates are similar (and they would not be if one were increasing and the other decreasing). For saturation there would have to be a precise balance between the increasing and decreasing exchange rates.

The only other way for saturation to occur is if one or both of the exchange rates themselves saturates. In this view, any of the three permeation scenarios would be possible. Since we have restricted this study to not having nonequilibrium throughout the channel system, other possibilities such as K_{ch} not changing but both interfacial exchange rate changing equally with concentration changes are not considered here. (Recall that changes in the partition coefficient ratios are not considered to contribute significantly to current or conductance changes when the concentration is changed.)

Example calculations

Based on the above, it seems reasonable that the saturating conductance is given by:

$$g = 2\,\frac{z_X^2\,F^2}{RT}\,K \qquad (38)$$

where K is either K_{ch} or $K_{int\,f}$ as described above. If we know what the flux ratio exponent is at the given concentration, then one could deduce which exchange rate should be used in the saturating conductance expression. Specifically, if the flux ratio exponent is larger than one, then the calculations should yield exchange rates such that the channel exchange rate is less than the interfacial exchange rate.

Example 1: Shaker K^+ channel. In Fig. 1 of Heginbotham and MacKinnon (1993), the saturating conductance (at 1.15 m K^+) is approximately 47 pS. Although unknown at this concentration and voltage, it is reasonable to assume that the flux ratio exponent would be greater than one (see Stampe and Begenisich 1996). Thus, $K_{ch} < K_{int\,f}$. From Eq. (38) above, we can calculate one of the exchange rates as (using $T = 22\,°C$): $K = 6.2 \times 10^{-6}$ pmol/s. This would be the exchange rate that is not changing much as the concentration increases. Consider that the conductance at a different concentration, say 43 mM, is approximately 11 pS. The full conductance equation [Eq. (35)] can then be used to calculate the other exchange rate, which we obtain as: $K = 1.9 \times 10^{-6}$ pmol/s. Since $K_{ch} < K_{int\,f}$, then $K_{int\,f} = 6.2 \times 10^{-6}$ pmol/s and $K_{ch} = 1.9 \times 10^{-6}$ pmol/s.

Arguing that voltage changes should not affect the exchange rates as much as concentration changes [since “full sized electrical signals can be generated with relatively few pores per unit area and only minute ionic fluxes” (Hille 1992, p. 20), we can then use Eq. (18) to generate the current-voltage (*I–V*) curve at 43 mM concentration. The curve is shown in Fig. 3A, and there is reasonable agreement with the *I–V* data (Heginbotham and MacKinnon 1993), suggesting that the various assumptions made along the way are valid.

Example 2: Cl^- in CFTR channels. In Fig. 2 of Tabcharani et al. (1997), the saturating conductance is about 10 pS. Similar to the previous example, one of the exchange rates (the nonchanging one) can be calculated to be [using $T = 22\,°C$ and Eq. (38)]: $K = 1.3 \times 10^{-6}$ pmol/s. Given that the conductance at 150 mM is 7 pS, the other exchange rate can be calculated from Eq. (35) as: $K = 3.1 \times 10^{-6}$ pmol/s. Since we do not have a value for the flux ratio exponent, we cannot determine which exchange rate is the channel or interfacial one. However, we can plot the *I–V* curve from Eq. (18), as shown in Fig. 3B. The agreement is not as good this time, suggesting either that the modulation of the exchange rates with voltage cannot be ignored, so that at the larger voltage values, different exchange rates should be used, and/or we cannot assume that only one of the exchange rates changes significantly with increasing concentration.

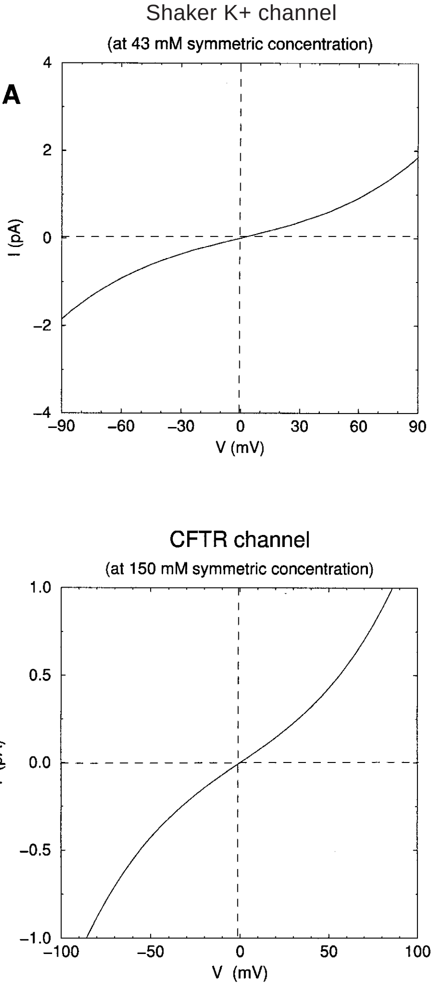

Fig. 3A, B Current-voltage (*I–V*) curves for two examples. **A** The *I–V* relationship calculated from Eq. (18) using the estimated exchanges rates (see text) for the *Shaker* K^+ channel at a symmetric concentration of 43 mM. **B** The *I–V* relationship calculated from Eq. (18) using the estimated exchange rates (see text) for the CFTR channel at a symmetric concentration of 150 mM

Comparison with ERT

ERT and SRT are based on fundamentally different theories, and so, strictly speaking, they cannot be directly com-

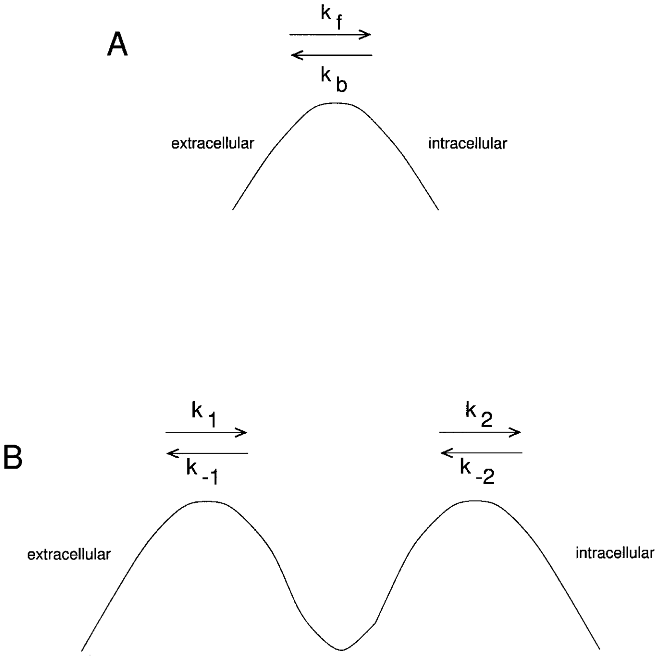

Fig. 4A, B Schematic of barrier models. **A** A one-barrier model with forward and backward rates as indicated. **B** A two-barrier/one-binding-site model with forward and backward rates over each barrier as indicated

pared. SRT is a nonequilibrium thermodynamic approach (Ward 1982) whereas ERT is based on absolute rate theory in which nonequilibrium situations are not considered (Glasstone et al. 1941; Eyring et al. 1949). However, the use of ERT is so commonly used in the ion channel permeation literature that it is useful to make some sort of comparison. One might roughly consider the three SRT equilibrium exchange rates (which are not necessarily the same) as corresponding to three "barriers" of different heights. However, we would like to stress that the meaning of the rates are different. In particular, the SRT rates *do* depend on concentrations. This difference is illustrated in Appendix A for the specific example of a chemical reaction.

Comparison 1: one barrier. Let us first consider the simplest possible case of one barrier symmetrically located in the channel (Fig. 4a). For the ERT approach, the current is given by:

$$I = z_X F \lambda \, [k_b \, [X]_i - k_f \, [X]_e] \tag{39}$$

where λ is the membrane thickness, [X] is the concentration on the extracellular (e) or intracellular (i) sides, and other parameters have their usual meanings (as defined earlier). k_f, k_b are the forward and backward rates, respectively, and are given by:

$$k_f, k_b = \kappa \frac{kT}{h} \exp(-\Delta G / kT) \exp(-\delta \, z_X \, eV / kT) \tag{40}$$

where κ is a "transmission coefficient" which corresponds to the frequency of attempted jumps, k is Boltzmann's constant, h is Planck's constant, ΔG is the height of the energy barrier, δ is the fractional distance across the channel where the energy barrier is located, e is the elementary charge, and the other parameters have been defined before. κ is typically taken to be unity and if the barrier is symmetrically located in the channel such that $\delta = 0.5$, then the current is given by:

$$I = z_X F \lambda \, [k'_b \, [X]_i \exp(z_X FV/2RT) - [k'_f \, [X]_e \exp(-z_X FV/2RT)] \tag{41}$$

where k'_f, $k'_b = \kappa (kT/h) \exp(-\Delta G/kT)$. Differentiating this equation to obtain the conductance, we find that at 0 mV and symmetric concentrations, the conductance is given by:

$$g = \lambda \frac{z_X^2 F^2}{2RT} [X] (k'_f + k'_b) \tag{42}$$

For the SRT approach, we could think about the above situation as the situation when equilibrium can be assumed to exist at the channel interfaces but not within the channel, so that we are only dealing with one exchange rate, K_{ch}. In this case, the current [from Eq. (16)] is given by (assuming that the partition coefficient ratio is unity):

$$j = K_{ch} \left[\frac{[X]_i}{[X]_e} \exp(\theta_X V) - \frac{[X]_e}{[X]_i} \exp(\theta_X V) \right] \tag{43}$$

and the conductance at 0 mV and symmetric concentrations is:

$$g = 2 \frac{z_X^2 F^2}{RT} K_{ch} \tag{44}$$

Now, similar to the chemical reaction example in Appendix A, but now for a single species ([X]), the near-equilibrium approximation of SRT can be done to obtain a relationship between the SRT exchange rates and the absolute rate theory rates. They are:

$$k'_f = \frac{2 K_{SRT}}{[X]_{e,\,equil}} \tag{45}$$

$$k'_b = \frac{2 K_{SRT}}{[X]_{i,\,equil}} \tag{46}$$

or, equivalently, and since K_{SRT} refers to K_{ch} in this case:

$$K_{ch} = \frac{k'_f \, [X]_{e,\,equil}}{2} \tag{47}$$

$$K_{ch} = \frac{k'_b \, [X]_{i,\,equil}}{2} \tag{48}$$

The ERT- and SRT-based conductance equations for this situation [Eqs. (42) and (44)] can now be seen to be equivalent by taking into account the units for the various rates and concentrations and making the substitution based on the above relationships:

$$K_{ch} = \frac{k'_f \, [X]_{e,\,equil}}{4} + \frac{k'_b \, [X]_{i,\,equil}}{4} \tag{49}$$

Therefore, in the near-equilibrium limit for a one-barrier approximation and one dominant rate-limiting region in the channel, the SRT and ERT approaches give similar conductance (or $I-V$ slope) values.

Comparison 2: two barriers/one binding site. Now consider a one-binding-site ERT model, which conceptually would consist of two barriers with the binding site in the middle. From the analysis of Kohler and Heckmann (1979), the flux ratio exponent could range from 0 to 1.

For the comparative SRT situation here, we would think about nonequilibrium across two interfaces. If the exchange rates are the same, the flux ratio exponent would be one. However, the flux ratio exponent can range from zero to two (Tables 1, 2), and its value allows one to determine whether the channel or interfacial exchange rate is smaller and so constitute the rate-limiting region.

For a two-barrier (one-binding-site) channel model (Fig. 4B), the ERT-based expression for the flux, J, is (e.g., see Aidley and Stanfield 1996, p. 136):

$$J = \frac{[\mathrm{X}](k_{-1}k_{-2} - k_1 k_2)}{k_{-1} + k_2 + [\mathrm{X}](k_1 + k_{-2})} \tag{50}$$

where the intracellular and extracellular concentrations ([X]) are assumed to be symmetric. The saturating conductance (or current) expression from a Michaelis-Menten relation has the given form:

$$g = \frac{g_{\max}}{1 + K_{\mathrm{m}}/[\mathrm{X}]} \tag{51}$$

where $g_{\max}$ is the maximal or saturating single channel conductance, K_{m} is the Michaelis constant (the concentration at which the conductance is half its maximum value), and [X] is the concentration of the ion (symmetric concentrations). With some rearrangement and absorbing various constant coefficients in Eqs. (50) and (51), $g_{\max}$ and K_{m} can be identified with the following expressions:

$$g_{\max} \propto \frac{k_{-1}k_{-2} - k_1 k_2}{k_1 + k_{-2}} \tag{52}$$

$$K_{\mathrm{m}} = \frac{k_{-1} + k_2}{k_1 + k_{-2}} \tag{53}$$

For the SRT-based equations for the case of nonequilibrium across two interfaces [scenario 3, Eq. (35)], we have:

$$g = g_{\mathrm{sat}} \frac{K_{\mathrm{ch}}}{K_{\mathrm{ch}} + K_{\mathrm{int\,f}}} \tag{54}$$

or

$$g = g_{\mathrm{sat}} \frac{K_{\mathrm{int\,f}}}{K_{\mathrm{ch}} + K_{\mathrm{int\,f}}} \tag{55}$$

where g_{sat}, the saturating conductance, is given either by Eq. (37) or Eq. (36), respectively. Comparing Eqs. (51) and (54), one can obtain approximations for the SRT exchange rates

$$K_{\mathrm{ch}} \approx (k_1 + k_{-2})\,[\mathrm{X}] \tag{56}$$

$$K_{\mathrm{int\,f}} \approx (k_{-1} + k_2) \tag{57}$$

or K_{ch} and $K_{\mathrm{int\,f}}$ would be reversed if Eqs. (51) and (55) were compared. Again, knowing the flux ratio exponent allows one to determine which case applies.

Discussion

This work has shown that measurements of unidirectional fluxes to determine flux ratio exponents can be used to establish where the rate-limiting region during the permeation process is located. It is important to note that this is a dynamic determination which may not be in accordance with a given static channel structure. Owing to its relatively simple (but general) description, this approach does not provide any detailed structural/conformational information. Instead, one determines whether movements at the interface or within the channel constitutes the rate-limiting region. The reasons why particular regions are considered to be rate-limiting are many (e.g., dehydration/rehydration, surface charges, surface receptor/channel binding sites, repulsive effects, etc.), and although this approach does not specify the reasons, identifying the rate-limiting region itself is potentially useful in focusing studies on specific parts of the channel system and/or sequence in the case of mutational studies. In summary, this work provides a useful starting point for molecular work and for more detailed theoretical work.

Why use SRT?

The SRT approach is straightforward and inclusive. In modelling permeation, electrodiffusion approaches quickly yield unwieldly expressions when Poisson's equation is included to determine the electric field (e.g., Chen et al. 1992). ERT approaches, although conceptually quite useful, do not provide unique solutions for a given set of data. More importantly, they invoke fundamental assumptions about equilibrium within each “well” or “binding site” which may not be valid, potentially giving misleading and false physical pictures of the channel profile (Cooper et al. 1985; Levitt 1986; Dani and Levitt 1990). Specifically, using the Poisson-Nernst-Planck (PNP) model by Eisenberg and colleagues, it has been demonstrated that the ERT rate constants (free energy barriers) cannot be independent of concentration (Eisenberg 1996).

Other ways of modelling permeation include Brownian dynamics (Bek and Jakobsson 1994) and hybrid electrodiffusion/reaction rate theory approaches. In one hybrid case, discrete rate constants are used but the expressions for the rate constants are described using diffusion theory (Cooper et al. 1988). In another hybrid case, a continuum description is employed but with the rate of entry and exit from the channel being described using reaction rate theory (Levitt 1982).

Along the lines of describing biological data conveniently, Dani and Levitt (1990) suggest using “a formalism simple enough to write down and implement on a laboratory computer" and whose "underlying principles should make physical sense". We feel that SRT and its use to describe permeation meets these requirements. SRT provides manageable expressions for the flux and when applied to permeation the equations include various complexities (surface charges, solubilities, etc.), albeit in a lumped fashion. The formulation of the model is straigthforward: the channel system consists of three different fluid phases (extracellular, channel, and intracellular), and similar to other workers (e.g., see Levitt 1986), three steps in the permeation process are considered. The approach is generally applicable making no a priori assumptions about narrow channels or single filing. With the SRT approach, permeation can be viewed as a "probability distribution" of ions flowing through the channel where this distribution depends on the properties of the channel system which encompasses the protein channel and the surrounding bulk media. Within this interpretative framework, interactions would necessarily occur.

It is important to point out that one should not directly compare these SRT-based permeation equations with, for example, the GHK current equation, where several assumptions are made in the reduction of the NP equation to the GHK equation (e.g., see Hille 1992, p. 345). However, since the SRT-based equations can be reduced to the NP equation with appropriate assumptions (Skinner et al. 1993), some comparisons may be possible. Although ERT and SRT are fundamentally different theories, some comparisons are possible and have been demonstrated in the results.

Similar to ERT, the use of a SRT-based approach will not be proved or disproved based on fitting the data at this stage of the model development. In the case of ERT, one can always add more barriers, etc., to obtain a better fit, but on the theoretical level, ERT seems clearly deficient. With SRT, it is unknown how the exchange rates change with voltage and concentration. However, the present formulation is rather simple and general, determines the rate-limiting region based on flux ratio exponent values, and saturating conductance values can be used to estimate values for the exchange rates. We suggest using this SRT-based approach to describe ion permeation as a starting alternative before jumping to more complex theories and situations.

SRT exchange rates may be more useful than permeabilities and permeability ratios

As demonstrated here, using permeabilities could be wrong and this is especially true for potassium channels where flux ratio exponent values exceed two. Permeability ratios may not indicate the selectivity of the channel since the underlying permeation process (i.e., where the rate-limiting region is located) may be different for the different permeating ions. Therefore, it is not surprising that a different selectivity sequence is obtained when conductance ratios are compared (Eisenman et al. 1986; Christophersen 1991; Heginbotham and MacKinnon 1993; Löffler and Hunter 1997), or that different permeability ratios are obtained when an inverse bi-ionic situation is considered (Heginbotham and MacKinnon 1993; Pérez-Cornejo and Begenisich 1994). Instead, the SRT exchange rates determined at different concentrations and voltages could be taken as a measure of the selectivity of the channel for a particular permeant species. Chen et al. (1997) also recognize the inappropriateness of using permeability ratios to define selectivity and they go on to suggest the requirement of a nonequilibrium theory of permeation. SRT is a nonequilibrium thermodynamic theory.

The new interpretation of the flux ratio exponent can circumvent conflicting experimental data

Our new interpretation of the flux ratio exponent as an indication of the degree of disequilibrium in the channel system during permeation and the relative sizes of the exchange rates may contribute to the "challenge of explaining the long pore single-filing behaviors shown by K^+ channels" (Miller 1996). Miller (1996) has not fully embraced the idea of a classical long pore for potassium channels because of molecular work suggesting a much shorter pore, and recently this has been convincingly demonstrated by Doyle et al. (1998). However, the long pore phenomenon for potassium channels is emphasized by recent measurements of high flux ratio exponents (Stampe and Begenisich 1996). Using the SRT flux equations, we have proven that if exponent values exceed two, it must be because the partition coefficients are different at the intracellular and extracellular interfaces. Thus, unlike ERT-based theories, high flux ratio exponents do not necessarily imply that there is a long pore.

It is important to note that within our SRT-based interpretation of flux ratio exponents, an exponent of one implies that two of the exchange rates are approximately the same (see Table 1), which is more information than obtained from the traditional approach in which a flux ratio exponent of one is taken to mean that there is passive diffusion with no flux coupling. Furthermore, we show (here and in Skinner et al. 1993) that an exponent of two predicts that in considering the permeation process, one can assume that equilibrium exists at the interfaces so that the rate-limiting region is within the channel and not on the intracellular or extracellular side.

Single-ion/multi-ion criteria may not be useful

It may be useful to alter the commonly used conceptual framework regarding permeation from one where ions jump over "barriers" and bind to specific sites in "wells" to one where the net current flow is seen to be controlled by certain regions in the channel system. In this way, criteria based on ERT models would be avoided. Criteria for whether a channel is multi-ion or single-ion in nature include determining whether the measured flux ratio exponent is greater than or equal to one, and whether an anomalous mole-fraction effect is present or not.

Based on a well/barrier/multiple occupancy model, Eisenman et al. (1986) suggest that anomalous mole-fraction effects could be due to different binding strengths by the different ions. Considering the various permeation scenarios presented here, it may also be that the rate-limiting region is different for the different ions. Wu (1992) has shown that flux ratio exponents greater than one as well as anomalous mole-fraction effects can occur in a single-site channel model (when ions are allowed to enter the channel transiently and repel the bound ion). We have shown that the size of the flux ratio exponent allows one to determine whether the rate-limiting region occurs within the channel or at the interface during permeation.

It may be that all channels behave as multi-ion channels under certain conditions. Instead of using criteria (based on ERT) to determine whether a channel is single-ion or multi-ion in nature, it may be more fruitful to use the method proposed in this paper to overcome some of the complexities encountered when trying to evaluate both experimental data and their theoretical interpretation (e.g., see Finkelstein and Andersen 1981).

More details?

In applying SRT to ion permeation, we assume that the ions are in solution in the different phases of the system (Skinner et al. 1993). Clearly, if the channel is not a waterfilled pore so that permeation does not occur in a fluid phase, this model would not apply. In the present model formulation, it may be considered that binding sites are present in the rate-limiting regions of the channel system. However, this is not specifically modelled. It is possible to include these details in the system via a specific representation for the electrochemical potential and equilibrium exchange rates. This has been done in certain well-defined physical kinetic situations (e.g., see Elliott and Ward 1997b, c). We would like to have expressions for the SRT exchange rates in terms of the channel properties, but given the complexity and diversity of channels, this is a significant task.

Concluding remarks

The new method presented in this paper offers us a way to obtain some understanding into the effects of different drugs and neuromodulators. The permeation processes of different substances in different channels can be compared to help evaluate their actions. Much work has concentrated on how channel behaviour is modulated, with most of the emphasis on channel gating (e.g. see Kaczmarek and Levitan 1989). It is not usual to consider permeation itself as being modulated, but several examples of conductance modification are available (Kuo and Bean 1993; Eghbali et al. 1997; Benke et al. 1998). Furthermore, if the permeation process varies for different ionic species, then modulation effects could be critically dependent on the particular process in operation or whether the channel interior or interface constitutes the rate-limiting region.

Appendix A

Physical description of the SRT expression

This is also described in Skinner et al. (1993). Consider a chemical reaction in which

$$\mathrm{A}+\mathrm{B} \rightleftharpoons \mathrm{C}+\mathrm{D} \tag{58}$$

The chemical potential is given by:

$$\mu_\gamma = \mu_\gamma^0 + RT\ln[\gamma] \tag{59}$$

where γ is species A, B, C, or D, μ^0 is a reference chemical potential, and activities and concentrations have been assumed to be the same. The equilibrium constant for the reaction is:

$$K_{\mathrm{equil}} = \frac{[\mathrm{C}]_{\mathrm{equil}}\,[\mathrm{D}]_{\mathrm{equil}}}{[\mathrm{A}]_{\mathrm{equil}}\,[\mathrm{B}]_{\mathrm{equil}}} \tag{60}$$

where $[\mathrm{X}]_{\mathrm{equil}}$ is the equilibrium concentration of X, defined by:

$$K_{\mathrm{equil}} = \exp\left(\frac{\mu_{\mathrm{A}}^0 + \mu_{\mathrm{B}}^0 - \mu_{\mathrm{C}}^0 - \mu_{\mathrm{D}}^0}{RT}\right) \tag{61}$$

Using the nonequilibrium SRT approach, the net rate of the reaction is:

$$j = K_{\mathrm{SRT}}\left(\frac{K_{\mathrm{equil}}\,[\mathrm{A}][\mathrm{B}]}{[\mathrm{C}][\mathrm{D}]} - \frac{[\mathrm{C}][\mathrm{D}]}{K_{\mathrm{equil}}\,[\mathrm{A}][\mathrm{B}]}\right) \tag{62}$$

where K_{SRT} is the equilibrium exchange rate.

The expression for the equilibrium constant [Eq. (60)] may be interpreted as saying that when the ratio of product to reactant concentration reaches the value K_{equil}, the reaction proceeds in the forward and reverse directions at the same rate. Under equilibrium conditions then, the ratio $[\mathrm{C}]_{\mathrm{equil}}\,[\mathrm{D}]_{\mathrm{equil}}/K_{\mathrm{equil}}\,[\mathrm{A}]_{\mathrm{equil}}\,[\mathrm{B}]_{\mathrm{equil}}$ would be unity and if K_{SRT} is the equilibrium exchange rate, then

$$K_{\mathrm{SRT}}\,\frac{[\mathrm{C}]_{\mathrm{equil}}\,[\mathrm{D}]_{\mathrm{equil}}}{K_{\mathrm{equil}}\,[\mathrm{A}]_{\mathrm{equil}}\,[\mathrm{B}]_{\mathrm{equil}}} \tag{63}$$

would also be the equilibrium exchange rate. The equilibrium exchange rate is a constant, and its value can be predicted in terms of the properties that are constrained to be constant. If at any instant the value of the ratio $[\mathrm{C}][\mathrm{D}]/K_{\mathrm{equil}}\,[\mathrm{A}][\mathrm{B}]$ is greater than unity, then there is an excess of products compared to reactants, and the net reaction proceeds in the reverse direction. The larger this ratio is compared to unity, the further the system is displaced from equilibrium in the direction away from the reactants and, therefore, the larger should be the magnitude of the "force" driving the system toward equilibrium, i.e., toward the reactants. Thus one might reasonably take the reverse rate of the reaction to be given by:

$$j_{\mathrm{rev}} = K_{\mathrm{SRT}}\,\frac{[\mathrm{C}][\mathrm{D}]}{K_{\mathrm{equil}}\,[\mathrm{A}][\mathrm{B}]} \tag{64}$$

If one now considers the circumstance in which there is an overabundance of reactants compared to products, the ratio $K_{\text{equil}}\,[\text{A}]\,[\text{B}]/[\text{C}]\,[\text{D}]$ will be greater than unity, the system will be displaced from equilibrium in the direction of the reactants, and the force restoring the system to equilibrium would be the latter ratio. Thus the rate at which the system proceeds in the forward direction could be reasonably assumed to be:

$$j_{\text{for}} = K_{\text{SRT}}\,\frac{K_{\text{equil}}\,[\text{A}][\text{B}]}{[\text{C}][\text{D}]} \tag{65}$$

and the net rate of the reaction is:

$$j = K_{\text{SRT}}\left(\frac{K_{\text{equil}}\,[\text{A}][\text{B}]}{[\text{C}][\text{D}]} - \frac{[\text{C}][\text{D}]}{K_{\text{equil}}\,[\text{A}][\text{B}]}\right) \tag{66}$$

This is the same expression as obtained above [Eq. (62)] using the SRT approach (Ward 1983).

Comparison of SRT and absolute rate theory

To understand how the equilibrium exchange rate in SRT theory is related to the more conventional absolute rate theory, let us perform some approximations using the chemical reaction in Eq. (58) above. This is also described in Ward (1983). Since absolute rate theory is valid in the near-equilibrium condition, define ε as a parameter that is small compared to unity given by:

$$2\,\varepsilon = \left(\frac{[\text{A}][\text{B}]}{[\text{A}]_{\text{equil}}\,[\text{B}]_{\text{equil}}} - \frac{[\text{C}][\text{D}]}{[\text{C}]_{\text{equil}}\,[\text{D}]_{\text{equil}}}\right) \tag{67}$$

Assume that the displacement from equilibrium by both reactants and products is symmetrical so that:

$$\frac{1}{2}\left(\frac{[\text{A}][\text{B}]}{[\text{A}]_{\text{equil}}\,[\text{B}]_{\text{equil}}} + \frac{[\text{C}][\text{D}]}{[\text{C}]_{\text{equil}}\,[\text{D}]_{\text{equil}}}\right) = 1 \tag{68}$$

for both nonequilibrium and equilibrium states. Eqs. (67) and (68) can be solved simultaneously to give:

$$\left(\frac{[\text{A}][\text{B}]}{[\text{A}]_{\text{equil}}\,[\text{B}]_{\text{equil}}}\;\frac{[\text{C}][\text{D}]}{[\text{C}]_{\text{equil}}\,[\text{D}]_{\text{equil}}}\right) = \frac{1+\varepsilon}{1-\varepsilon} \tag{69}$$

which when plugged into Eq. (62) becomes:

$$j = \frac{4\,\varepsilon\,K_{\text{SRT}}}{1-\varepsilon^2} \tag{70}$$

To second order in ε, this equation is approximately

$$j_{\text{approx}} = 4\,\varepsilon\,K_{\text{SRT}} \tag{71}$$

$$= 2\,K_{\text{SRT}}\left(\frac{[\text{A}][\text{B}]}{[\text{A}]_{\text{equil}}\,[\text{B}]_{\text{equil}}} - \frac{[\text{C}][\text{D}]}{[\text{C}]_{\text{equil}}\,[\text{D}]_{\text{equil}}}\right) \tag{72}$$

Absolute rate theory gives a net rate of reaction of:

$$j_{\text{abs}} = k_{\text{f}}\,[\text{A}]\,[\text{B}] - k_{\text{r}}\,[\text{C}]\,[\text{D}] \tag{73}$$

Comparing j_{approx} and j_{abs} gives:

$$k_{\text{f}} = \frac{2\,K_{\text{SRT}}}{[\text{A}]_{\text{equil}}\,[\text{B}]_{\text{equil}}} \tag{74}$$

$$k_{\text{r}} = \frac{2\,K_{\text{SRT}}}{[\text{C}]_{\text{equil}}\,[\text{D}]_{\text{equil}}} \tag{75}$$

Appendix B

Proof that n'' lies between 0 and 2

For a given ionic species, X, n'' is defined as:

$$\frac{\text{Efflux}}{\text{Influx}} = \left(\frac{\beta_{\text{X}}^{\text{i}}\,[\text{X}]_{\text{i}}}{\beta_{\text{X}}^{\text{e}}\,[\text{X}]_{\text{e}}}\exp\left(\theta_{\text{X}}\,V\right)\right)^{n''} \tag{76}$$

Values for n'' can be exactly determined from the SRT flux equations for certain cases: If nonequilibrium exists only within the channel interior, then $n''=2$; if nonequilibrium exists across two interfaces and the two exchange rates are similar, then $n''=1$; if nonequilibrium exists throughout the channel system and all of the exchange rates are similar, then $n''=2/3$. If nonequilibrium exists throughout the channel system and the exchange rates are different, then we numerically find that $n''<2/3$.

Consider the situation where nonequilibrium exists across two interfaces and the two exchange rates are different. The net flux, j, is:

$$j = \text{Efflux} - \text{Influx} \tag{77}$$

where [from Eq. (18)] we have:

$$\text{Efflux} = K_{\text{ch}}\left(\frac{\beta_{\text{X}}^{\text{i}}\,[\text{X}]_{\text{i}}}{\beta_{\text{X}}^{\text{e}}\,[\text{X}]_{\text{e}}}\exp\left(\theta_{\text{X}}\,V\right)\right)^{1/2}(\mathit{factor})^{1/2} \tag{78}$$

$$\text{Influx} = K_{\text{ch}}\left(\frac{\beta_{\text{X}}^{\text{e}}\,[\text{X}]_{\text{e}}}{\beta_{\text{X}}^{\text{i}}\,[\text{X}]_{\text{i}}}\exp\left(\theta_{\text{X}}\,V\right)\right)^{1/2}(1/\mathit{factor})^{1/2} \tag{79}$$

and

$$\mathit{factor} = \frac{(K_{\text{int f}}/K_{\text{ch}})\,(\beta_{\text{X}}^{\text{i}}\,[\text{X}]_{\text{i}}/\beta_{\text{X}}^{\text{e}}\,[\text{X}]_{\text{e}})\exp\left(\theta_{\text{X}}\,V\right)+1}{(\beta_{\text{X}}^{\text{i}}\,[\text{X}]_{\text{i}}/\beta_{\text{X}}^{\text{e}}\,[\text{X}]_{\text{e}})\exp\left(\theta_{\text{X}}\,V\right)+(K_{\text{int f}}/K_{\text{ch}})}$$

Then, from Eq. (76) above [or Eq. (31), n'' can be rewritten as:

$$n'' = \frac{\ln\left(\text{Efflux/Influx}\right)}{\ln\left(\dfrac{\beta_{\text{X}}^{\text{i}}\,[\text{X}]_{\text{i}}}{\beta_{\text{X}}^{\text{e}}\,[\text{X}]_{\text{e}}}\exp\left(\theta_{\text{X}}\,V\right)\right)}$$

$$= 1 + \frac{\ln\left(\mathit{factor}\right)}{\ln\left(\dfrac{\beta_{\text{X}}^{\text{i}}\,[\text{X}]_{\text{i}}}{\beta_{\text{X}}^{\text{e}}\,[\text{X}]_{\text{e}}}\exp\left(\theta_{\text{X}}\,V\right)\right)}$$

Let $term = (\beta_X^i\,[X]_i / \beta_X^e\,[X]_e)\,\exp\,(\theta_X V)$, and let $m = \ln\,(factor) / \ln\,(term)$. Then

$n'' = 1 + m$

and

$0 \le n'' \le 2 \Leftrightarrow -1 \le m \le 1$

Before proving that $-1 \le m \le 1$, we prove two Lemmas.

Lemma 1: $factor > term \Rightarrow term < 1$

Proof: By definition

$$factor = \frac{(K_{\mathrm{int\,f}}/K_{\mathrm{ch}})\,(term) + 1}{term + (K_{\mathrm{int\,f}}/K_{\mathrm{ch}})}$$

$$factor > term \Rightarrow \frac{(K_{\mathrm{int\,f}}/K_{\mathrm{ch}}) + 1\,(1/term)}{term + (K_{\mathrm{int\,f}}/K_{\mathrm{ch}})} > 1$$

$$\Rightarrow 1 > term^2 \Rightarrow 1 > term \Rightarrow term < 1$$

QED.

Lemma 2: $factor < (1/term) \Rightarrow term < 1$

Proof:

$$factor < (1/term) \Rightarrow \frac{(K_{\mathrm{int\,f}}/K_{\mathrm{ch}})\,(term) + 1}{1 + (K_{\mathrm{int\,f}}/K_{\mathrm{ch}})\,(1/term)} < 1$$

$$\Rightarrow term^2 < 1 \Rightarrow term < 1$$

QED.

Proposition 1: $m < -1$ (or equivalently, $n'' < 0$)

$$m < -1 \Rightarrow \frac{\ln\,(factor)}{\ln\,(term)} < -1$$

Since the natural logarithm can be negative, two cases must be considered.

Case (i): $\ln\,(factor) < 0$ and $\ln\,(term) > 0$.

$$\ln\,(factor) < 0 \Rightarrow factor < 1 \tag{80}$$

and

$$\ln\,(term) > 0 \Rightarrow term > 1 \tag{81}$$

Therefore

$m < -1 \Rightarrow |\ln\,(factor)| > \ln\,(term)$

$|\ln\,(factor)| > \ln\,(term)$ iff $\ln\,(factor) > \ln\,(term)$ or $\ln\,(factor) < -\ln\,(term)$

$\ln\,(factor) > \ln\,(term) \Rightarrow factor > term \Rightarrow term < 1$ (from Lemma 1)

Contradiction with Eq. (81).

$\ln\,(factor) < -\ln\,(term) \Rightarrow factor < (1/term) \Rightarrow term < 1$ (from Lemma 2)

Contradiction with Eq. (81).

Case (ii): $\ln\,(factor) > 0$ and $\ln\,(term) < 0$.

$$\ln\,(factor) > 0 \Rightarrow factor > 1 \tag{82}$$

and

$$\ln\,(term) < 0 \Rightarrow term < 1 \tag{83}$$

Therefore

$m < -1 \Rightarrow \ln\,(factor) > |\ln\,(term)|$

$\ln\,(factor) > |\ln\,(term)|$
iff $-\ln\,(factor) < \ln\,(term) < \ln\,(factor)$

$\ln\,(term) < \ln\,(factor) \Rightarrow term < factor \Rightarrow term < 1$
(from Lemma 1)

OK, no contradiction.

$\ln\,(term) > -\ln\,(factor) \Rightarrow term > (1/factor)$
$\Rightarrow factor > (1/\mathrm{term}) \Rightarrow term > 1$
(follows from Lemma 2)

Contradiction with Eq. (83).
Therefore, the original **Proposition 1 is false** and $\boldsymbol{m \ge 1}$.

Proposition 2: $m > 1$ (or equivalently, $n'' > 2$)

$$m > 1 \Rightarrow \frac{\ln\,(factor)}{\ln\,(term)} > 1$$

Again, since the natural logarithm can be negative, two cases must be considered.

Case (i): $\ln\,(factor) < 0$ and $\ln\,(term) < 0$.

$$\ln\,(factor) < 0 \Rightarrow factor < 1 \tag{84}$$

and

$$\ln\,(term) < 0 \Rightarrow term < 1 \tag{85}$$

Therefore

$m > 1 \Rightarrow -\ln\,(factor) > -\ln\,(term) \Rightarrow (1/factor) > (1/term)$
$\Rightarrow term > factor \Rightarrow term > 1$
(follows from Lemma 1)

Contradiction with Eq. (85).

Case (ii): $\ln\,(factor) > 0$ and $\ln\,(term) > 0$.

$$\ln\,(factor) > 0 \Rightarrow factor > 1 \tag{86}$$

and

$$\ln\,(term) > 0 \Rightarrow term > 1 \tag{87}$$

Therefore

$m > 1 \Rightarrow \ln\,(factor) > \ln\,(term) \Rightarrow factor > term \Rightarrow term < 1$
(from Lemma 1)

Contradiction with Eq. (87).
Therefore, the original **Proposition 2 is false** and $\boldsymbol{m \le 1}$.

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References

Aidley DJ, Stanfield PR (1996) Ion channels: molecules in action. Cambridge University Press, Cambridge

Begenisich T, DeWeer P (1980) Potassium flux ratio in voltage-clamped squid giant axons. J Gen Physiol 76: 83–98

Bek S, Jakobsson E (1994) Brownian dynamics study of a multiply-occupied cation channel: application to understanding permeation in potassium channels. Biophys J 66: 1028–1038

Benke TA, Lüthi A, Isaac JTR, Collingridge GL (1998) Modulation of AMPA receptor unitary conductance by synaptic activity. Nature 393: 793–797

Chen DP, Barcilon V, Eisenberg RS (1992) Constant fields and constant gradients in open ionic channels. Biophys J 61: 1372–1393

Chen D, Lear J, Eisenberg B (1997) Permeation through an open channel: Poisson-Nernst-Planck theory of a synthetic ionic channel. Biophys J 72: 97–116

Christophersen P (1991) Ca^{2+}-activated K^{+} channel from human erythrocyte membranes: single channel rectification and selectivity. J Membr Biol 119: 75–83

Clark A (1970) The theory of adsorption and catalysis. Academic Press, New York

Cooper KE, Jakobsson E, Wolynes P (1985) The theory of ion transport through membrane channels. Prog Biophys Mol Biol 46: 51–96

Cooper KE, Gates PY, Eisenberg RS (1988) Diffusion theory and discrete rate constants in ion permeation. J Membr Biol 106: 95–105

Dani JA, Levitt DG (1990) Diffusion and kinetic approaches to describe permeation in ionic channels. J Theor Biol 146: 289–301

Dejmek M, Ward CA (1998) A statistical rate theory study of interface concentration during crystal growth or dissolution. J Chem Phys 108: 8698–8704

Doyle DA, Cabral JM, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998) The structure of the potassium channel: molecular basis of K^{+} conduction and selectivity. Science 280: 69–77

Eghbali M, Curmi JP, Birnir B, Gage PW (1997) Hippocampal GABA$_{A}$ channel conductance increased by diazepam. Nature 388: 71–75

Eisenberg RS (1996) Computing the field in proteins and channels. J Membr Biol 150: 1–25

Eisenman G, Latorre R, Miller C (1986) Multi-ion conduction and selectivity in the high-conductance Ca^{++}-activated K^{+} channel from skeletal muscle. Biophys J 50: 1025–1034

Elliott JAW, Ward CA (1997a) Statistical rate theory and the material properties controlling adsorption kinetics on well defined surfaces. In: Rudziński W, Steele WA, Zgrablich G (eds) Equilibria and dynamics of gas adsorption on heterogeneous solid surfaces. (Studies in surface science and catalysis, vol 104) Elsevier, Amsterdam, pp 285–333

Elliott JAW, Ward CA (1997b) Statistical rate theory description of beam-dosing adsorption kinetics. J Chem Phys 106: 5667–5676

Elliott JAW, Ward CA (1997c) Temperature programmed desorption: a statistical rate theory approach. J Chem Phys 106: 5677–5684

Eyring H, Lumry R, Woodbury JW (1949) Some applications of modern rate theory to physiological systems. Rec Chem Prog 10: 100–114

Finkelstein A, Andersen OS (1981) The gramicidin A channel: a review of its permeability characteristics with special reference to the single-file aspect of transport. J Membr Biol 59: 155–171

Glasstone S, Laidler KJ, Eyring H (1941) The theory of rate processes. McGraw-Hill, New York

Heginbotham L, MacKinnon R (1993) Conduction properties of the cloned *Shaker* K^{+} channel. Biophys J 65: 2089–2096

Hille B (1992) Ionic channels of excitable membranes. Sinauer, Sunderland, Mass

Hille B, Schwartz W (1978) Potassium channels as multi-ion single-file pores. J Gen Physiol 72: 409–442

Hodgkin AL, Keynes RD (1955) The potassium permeability of a giant nerve fibre. J Physiol (Lond) 128: 61–88

Kaczmarek LK, Levitan IB (1987) Neuromodulation: the biochemical control of neuronal excitability. Oxford University Press, New York

Kohler H, Heckmann K (1979) Unidirectional fluxes in saturated single-file pores of biological and artificial membranes. I. Pores containing no more than one vacancy. J Theor Biol 79: 381–401

Kuo C-C, Bean BP (1993) G-protein modulation of ion permeation through N-type calcium channels. Nature 365: 258–262

Levitt DG (1982) Comparison of Nernst-Planck and reaction-rate models for multiply occupied channels. Biophys J 37:575–587

Levitt DG (1984) Kinetics of movement in narrow channels. Curr Top Membr Transp 21:181–197

Levitt DG (1986) Interpretation of biological ion channel flux data – reaction-rate versus continuum theory. Annu Rev Biophys Biophys Chem 15:29–57

Löffler K, Hunter M (1997) Cation permeation and blockade of ROMK1, a cloned renal potassium channel. Pflugers Arch 434: 151–158

Miller C (1996) Commentary: the long pore gets molecular. J Gen Physiol 107:445–447

Nonner W, Eisenberg B (1998) Ion permeation and glutamate residues linked by Poisson-Nernst-Planck theory in L-type calcium channels. Biophys J 75:1287–1305

Pérez-Cornejo P, Begenisich T (1994) The multi-ion nature of the pore in *Shaker* K^{+} channels. Biophys J 66: 1929–1938

Schagina LV, Grinfeldt AE, Lev AA (1983) Concentration dependence of bidirectional flux ratio as a characteristic of transmembrane ion transporting mechanism. J Membr Biol 73: 203–216

Skinner FK, Ward CA, Bardakjian BL (1993) Permeation in ionic channels: a statistical rate theory approach. Biophys J 65: 618–629

Stampe P, Begenisich T (1996) Unidirectional K^{+} fluxes through recombinant *Shaker* potassium channels expressed in single *Xenopus* oocytes. J Gen Physiol 107: 449–457

Tabcharani JA, Linsdell P, Hanrahan JW (1997) Halide permeation in wild-type and mutant cystic fibrosis transmembrane conductance regulator chloride channels. J Gen Physiol 110: 341–354

Tikuisis P, Ward CA (1992) Rate of gas absorption by liquids and “surface resistance.” In: Chhabra R, DeKee D (eds) Transport processes in bubbles, drops and particles. Hemisphere, New York, pp 114–132

Vestergaard-Bogind B, Stampe P, Christophersen P (1985) Single-file diffusion through the Ca^{+}-activated K^{+} channel of human red blood cells. J Membr Biol 88: 67–75

Ward CA (1977) The rate of gas absorption at a liquid interface. J Chem Phys 67: 229–235

Ward CA (1982) Statistical rate theory of interfacial transport. I. Theoretical development. J Chem Phys 76: 5599–5605

Ward CA (1983) Effect of concentration on the rate of chemical reactions. J Chem Phys 79: 5605–5615

Ward CA, Elmoselhi M (1986) Molecular adsorption at a well defined gas-solid interphase: statistical rate theory approach. Surf Sci 176: 457–475

Ward CA, Rizk M, Tucker AS (1982) Statistical rate theory of interfacial transport. II. Rate of isothermal bubble evolution in a liquid-gas solution. J Chem Phys 76: 5606–5614

Woodbury JW (1971) Eyring rate theory model of the current-voltage relationships of ion channels in excitable membranes. Adv Chem Phys 21: 601–617

Wu JV (1992) Dynamic ion-ion and water-ion interactions in ion channels. Biophys J 61: 1316–1331