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part of their model for the action potential in the squid giant axon. More detailed recent models for channel gating are not discussed at any length. The interested reader is referred to Hille (1992), Armstrong (1981), Armstrong and Bezanilla (1973, 1974, 1977), Aldrich et al. (1983), and Finkelstein and Peskin (1984) for a selection of models of how channels can open and close in response to changes in membrane potential. An important question that we do not consider here is how channels can discriminate between different ions. Detailed discussions of this and related issues are in Hille (1992) and the references therein.

3.2   Independence, Saturation, and the Ussing Flux Ratio

One of the most fundamental questions to be answered about an ion channel is whether the passage of an ion through the channel is independent of other ions. If so, the channel is said to obey the *independence principle*.

Suppose a membrane separates two solutions containing an ion species S with external concentration c_e and internal concentration c_i. If the independence principle is satisfied, the flow of S is proportional to its local concentration, independent of the concentration on the opposite side of the membrane, and thus the flux from outside to inside, J_{in}, is

$$J_{in} = k_e c_e, \tag{3.5}$$

for some constant k_e. Similarly, the outward flux is given by

$$J_{out} = k_i c_i, \tag{3.6}$$

where in general, $k_e \neq k_i$. We let V_S denote the Nernst potential of the ion S, and let V denote the potential difference across the membrane. Now we introduce a hypothetical concentration c_e^* defined as that external concentration necessary to maintain a Nernst potential V. Thus

$$\frac{c_e}{c_i} = \exp\left(\frac{V_S F}{RT}\right), \tag{3.7}$$

and

$$\frac{c_e^*}{c_i} = \exp\left(\frac{VF}{RT}\right). \tag{3.8}$$

When the external concentration is c_e^* and the internal concentration is c_i, then the voltage is V, and there is no net flux across the membrane; i.e., the outward flux equals the inward flux, and so

$$k_e c_e^* = k_i c_i. \tag{3.9}$$

It follows that the flux ratio is given by

$$
\begin{aligned}
\frac{J_{\text{in}}}{J_{\text{out}}} &= \frac{k_e c_e}{k_i c_i} \\
&= \frac{k_e c_e}{k_e c_e^*} \\
&= \frac{c_e}{c_e^*} \\
&= \frac{\exp\left(\frac{V_S F}{RT}\right)}{\exp\left(\frac{VF}{RT}\right)} \\
&= \exp\left[\frac{(V_S - V)F}{RT}\right].
\end{aligned}
\tag{3.10}
$$

This expression for the ratio of the inward to the outward flux is usually called the *Ussing flux ratio*. It was first derived by Ussing (1949), although the derivation given here is due to Hodgkin and Huxley (1952a). Alternatively, the Ussing flux ratio can be written as

$$
\frac{J_{\text{in}}}{J_{\text{out}}} = \frac{c_e}{c_i} \exp\left(\frac{-VF}{RT}\right).
\tag{3.11}
$$

Note that when $V = 0$, the ratio of the fluxes is equal to the ratio of the concentrations, as might be expected intuitively.

As an illustration of the application of the Ussing flux ratio, suppose the Na^+ current is measured when the cell is immersed in a high Na^+ solution and then compared to the Na^+ current measured in a low Na^+ solution. The membrane potential and the internal Na^+ concentration are assumed to be the same in both cases. We let a prime denote quantities measured in the high Na^+ solution, and then

$$
\frac{I'_{\text{Na}}}{I_{\text{Na}}} = \frac{J'_{\text{out}} - J'_{\text{in}}}{J_{\text{out}} - J_{\text{in}}}.
\tag{3.12}
$$

Since the internal concentrations are the same, it follows from (3.6) that $J_{\text{out}} = J'_{\text{out}}$, and from (3.5) we find $J'_{\text{in}}/J_{\text{in}} = [Na^+]'_e/[Na^+]_e$. Substituting these into (3.12) and using the Ussing flux ratio, we find

$$
\frac{I'_{\text{Na}}}{I_{\text{Na}}} = \frac{([Na^+]'_e/[Na^+]_e)\exp[\frac{(V_{\text{Na}}-V)F}{RT}] - 1}{\exp[\frac{(V_{\text{Na}}-V)F}{RT}] - 1}.
\tag{3.13}
$$

Alternatively, this can be written as

$$
\frac{I'_{\text{Na}}}{I_{\text{Na}}} = \frac{[Na^+]_i - [Na^+]'_e \exp(\frac{-VF}{RT})}{[Na^+]_i - [Na^+]_e \exp(\frac{-VF}{RT})}.
\tag{3.14}
$$

By measuring the current ratio as a function of membrane potential, the Na^+ channel can thus conveniently be tested for independence.

Although many ion channels follow the independence principle approximately over a range of ionic concentrations, most show deviations from independence when the

ionic concentrations are sufficiently large. This has motivated the development of models that show saturation at high ionic concentrations. For example, one could assume that ion flow through the channel can be described by a barrier-type model, in which the ion jumps from one binding site to another as it moves through the channel. If there are only a limited number of binding sites available for ion passage through the channel, and each binding site can bind only one ion, then as the ionic concentration increases there are fewer binding sites available, and so the flux is not proportional to the concentration. Equivalently, one could say that each channel has a single binding site for ion transfer, but there are only a limited number of channels. However, in many of these models the Ussing flux ratio is still obeyed, even though independence is not. Hence, although any ion channel obeying the independence principle must also satisfy the Ussing flux ratio, the converse is not true. We discuss saturating models later in this chapter.

Another way in which channels show deviations from independence is in flux-coupling. If ions can interact within a channel so that, for example, a group of ions must move through the channel together, then the Ussing flux ratio is not satisfied. The most common type of model used to describe such behavior is the so-called *multi-ion model*, in which it is assumed that there are a number of binding sites within a single channel and that the channel can bind multiple ions at the same time. The consequent interactions between the ions in the channel can result in deviations from the Ussing flux ratio. A more detailed consideration of multi-ion models is given later in this chapter. However, it is instructive to consider how the Ussing flux ratio is modified by a simple multi-ion channel mechanism in which the ions progress through the channel in single file (Hodgkin and Keynes, 1955).

Suppose a membrane separates two solutions, the external one (on the right) containing an ion S at concentration c_e, and the internal one (on the left) at concentration c_i. To keep track of where each S ion has come from, all the S ions on the left are labeled A, while those on the right are labeled B. Suppose also that the membrane contains n binding sites and that S ions traverse the membrane by binding sequentially to the binding sites and moving across in single file. For simplicity we assume that there are no vacancies in the chain of binding sites. It follows that the possible configurations of the chain of binding sites are $[A_r, B_{n-r}]$, for $r = 0, \ldots, n$, where $[A_r, B_{n-r}]$ denotes the configuration such that the r leftmost sites are occupied by A ions, while the rightmost $n - r$ sites are occupied by B ions. Notice that the only configuration that can result in the transfer of an A ion to the right-hand side is $[A_n B_0]$, i.e., if the chain of binding sites is completely filled with A ions.

Now we let α denote the total rate at which S ions are transferred from left to right. Since α denotes the total rate, irrespective of labeling, it does not take into account whether an A ion or a B ion is moved out of the channel from left to right. For this reason, α is not the same as the flux of labeled ions. Similarly, let β denote the total flux of S ions, irrespective of labeling, from right to left. It follows that the rate at which $[A_r B_{n-r}]$ is converted to $[A_{r+1} B_{n-r-1}]$ is $\alpha[A_r B_{n-r}]$, and the rate of the reverse conversion is $\beta[A_{r+1} B_{n-r-1}]$. According to Hodgkin and Keynes, it is reasonable to assume that if

there is a potential difference V across the membrane, then the total flux ratio obeys the Ussing flux ratio,

$$\frac{\alpha}{\beta} = \frac{c_e}{c_i} \exp\left(\frac{-VF}{RT}\right). \qquad (3.15)$$

This assumption is justified by the fact that a flux of one ion involves the movement of a single charge through the membrane (as in the independent case treated above) and thus should have the same voltage dependence. We emphasize that α/β is not the flux ratio of labeled ions, but the total flux ratio.

To obtain the flux ratio of labeled ions, notice that the rate at which A ions are transferred to the right-hand side is $\alpha[A_n B_0]$, and the rate at which B ions are transferred to the left hand side is $\beta[A_0 B_n]$. Thus, the flux ratio of labeled ions is

$$\frac{J_{in}}{J_{out}} = \frac{\alpha}{\beta} \frac{[A_n B_0]}{[A_0 B_n]}. \qquad (3.16)$$

At steady state there can be no net change in the distribution of configurations, so that

$$\frac{[A_{r+1} B_{n-r-1}]}{[A_r B_{n-r}]} = \frac{\alpha}{\beta}. \qquad (3.17)$$

Thus,

$$\frac{J_{in}}{J_{out}} = \frac{\alpha}{\beta} \frac{[A_n B_0]}{[A_0 B_n]} = \left(\frac{\alpha}{\beta}\right)^2 \frac{[A_{n-1} B_1]}{[A_0 B_n]} = \cdots = \left(\frac{\alpha}{\beta}\right)^{n+1}, \qquad (3.18)$$

so that

$$\frac{J_{in}}{J_{out}} = \left[\frac{c_e}{c_i} \exp\left(\frac{-VF}{RT}\right)\right]^{n+1}. \qquad (3.19)$$

A similar argument, taking into account the fact that occasional vacancies in the chain arise when ions at the two ends dissociate and that these vacancies propagate through the chain, gives

$$\frac{J_{in}}{J_{out}} = \left[\frac{c_e}{c_i} \exp\left(\frac{-VF}{RT}\right)\right]^{n}. \qquad (3.20)$$

Experimental data confirm this theoretical prediction (although historically, the theory was motivated by the experimental result, as is often the case). Hodgkin and Keynes (1955) showed that flux ratios in the K^+ channel of the *Sepia* giant axon could be described by the Ussing flux ratio raised to the power 2.5. Their result, as presented in modified form by Hille (1992), is shown in Fig. 3.2. Unidirectional K^+ fluxes were measured with radioactive K^+, and the ratio of the outward to the inward flux was plotted as a function of $V - V_K$. The best-fit line on a semilogarithmic plot has a slope of 2.5, which suggests that at least 2 K^+ ions traverse the K^+ channel simultaneously.
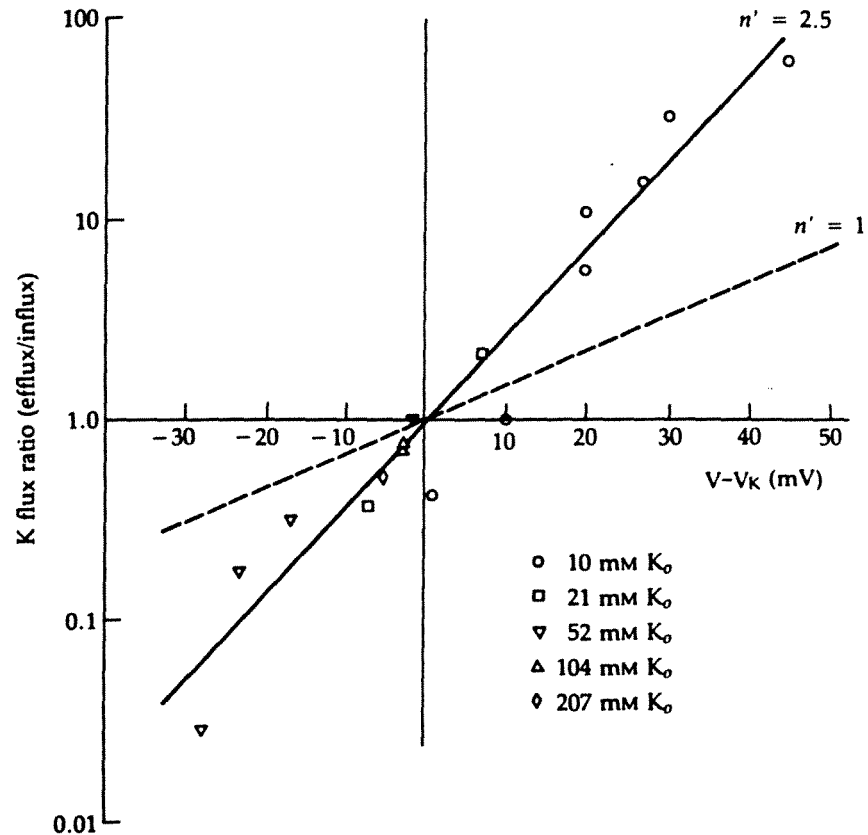
Figure 3.2 K$^+$ flux ratios as measured by Hodgkin and Keynes (1955), Fig. 7. Slightly modified into modern conventions by Hille (1992), page 375. K_o is the external K$^+$ concentration, and n' is the flux-ratio exponent, denoted by n in (3.20). (Hille, 1992, Fig. 7, p. 375.)

3.3 Electrodiffusion Models

Most early work on ion channels was based on the theory of electrodiffusion. We saw in Chapter 2 that the movement of ions in response to a concentration gradient and an electric field is described by the Nernst–Planck equation,

$$J = -D \left(\frac{dc}{dx} + \frac{zF}{RT} c \frac{d\phi}{dx} \right),$$ (3.21)

where J denotes the flux density, c is the concentration of the ion under consideration, and ϕ is the electrical potential. If we make the simplifying assumption that the field $d\phi/dx$ is constant through the membrane, then (3.21) can be solved to give the Goldman–Hodgkin–Katz current and voltage equations (2.68) and (2.71). However, in general there is no reason to believe that the potential has a constant gradient in the membrane.
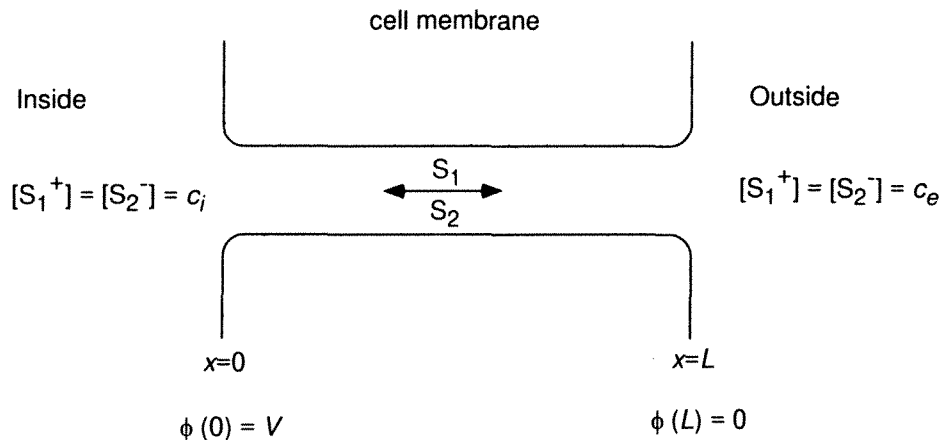
cell membrane

Inside

Outside

$[S_1{}^+] = [S_2{}^-] = c_i$

$\xleftarrow{\quad S_1 \quad}\xrightarrow{}$
S_2

$[S_1{}^+] = [S_2{}^-] = c_e$

$x=0$ $x=L$

$\phi\,(0) = V$ $\phi\,(L) = 0$

Figure 3.3 Schematic diagram of the electrodiffusion model for current through an ionic channel. Each side of the channel is electrically neutral, and both ion types can diffuse through the channel.

Ions moving through the channel affect the local electric field, and this local field in turn affects ionic fluxes. Thus, to determine the electric field and consequent ionic fluxes, one must solve a coupled problem.

3.3.1 Multi-ion Flux: The Poisson–Nernst–Planck Equations

Suppose we have two types of ions, S_1 and S_2, with concentrations c_1 and c_2, passing through an ion channel, as shown schematically in Fig. 3.3. For convenience we assume that the valence of the first ion is 1 and that of the second is -1. Then the potential in the channel $\phi(x)$ must satisfy Poisson's equation,

$$\frac{d^2\phi}{dx^2} = -\frac{q}{\epsilon}(c_1 - c_2), \tag{3.22}$$

where q is the unit electric charge and ϵ is the dielectric constant of the channel medium (usually assumed to be an aqueous solution). The flux densities J_1 and J_2 of S_1 and S_2 satisfy the Nernst–Planck equation, and at steady state dJ_1/dx and dJ_2/dx must both be zero to prevent any charge buildup within the channel. Hence, the steady-state flux through the channel is described by (3.22) coupled with

$$J_1 = -D_1\left(\frac{dc_1}{dx} + \frac{F}{RT}c_1\frac{d\phi}{dx}\right), \tag{3.23}$$

$$J_2 = -D_2\left(\frac{dc_2}{dx} - \frac{F}{RT}c_2\frac{d\phi}{dx}\right), \tag{3.24}$$

where J_1 and J_2 are constants. To complete the specification of the problem, it is necessary to specify boundary conditions for c_1, c_2, and ϕ. We assume that the channel has

length L, and that $x = 0$ denotes the left border, or inside, of the membrane. Then,

$$
\begin{aligned}
c_1(0) &= c_i, & c_1(L) &= c_e, \\
c_2(0) &= c_i, & c_2(L) &= c_e, \\
\phi(0) &= V, & \phi(L) &= 0.
\end{aligned}
\tag{3.25}
$$

Note that we have specified that the solutions on both sides of the membrane are electrically neutral. V is the potential difference across the membrane, defined, as usual, as the internal potential minus the external potential. While at first glance it might appear that there are too many boundary conditions for the differential equations, this is in fact not so, as the constants J_1 and J_2 are additional unknowns to be determined.

In general, it is not possible to obtain an exact solution to the Poisson–Nernst–Planck (PNP) equations (3.22)–(3.25). However, some simplified cases can be solved approximately. A great deal of work on the PNP equations has been done by Eisenberg and his colleagues (Chen, Barcilon, and Eisenberg, 1992; Barcilon, 1992; Barcilon, Chen, and Eisenberg, 1992; Chen and Eisenberg, 1993). Here we present simplified versions of their models, ignoring, for example, the charge induced on the channel wall by the presence of ions in the channel, and considering only the movement of two ion types, rather than three, through the channel. Similar models have also been discussed by Peskin (1991).

It is convenient first to nondimensionalize the PNP equations. We let $x^* = x/L$, $\phi^* = \phi F/RT$, $v = VF/RT$, $c_1^* = c_1/\bar{c}$, and similarly for c_2, c_i, and c_e, where $\bar{c} = c_e + c_i$. Substituting into (3.22)–(3.24) and dropping the stars, we find

$$
-\bar{J}_1 = \frac{dc_1}{dx} + c_1 \frac{d\phi}{dx},
\tag{3.26}
$$

$$
-\bar{J}_2 = \frac{dc_2}{dx} - c_2 \frac{d\phi}{dx},
\tag{3.27}
$$

$$
\frac{d^2\phi}{dx^2} = -\lambda^2(c_1 - c_2),
\tag{3.28}
$$

where $\lambda^2 = L^2 q F\bar{c}/(\epsilon RT)$, $\bar{J}_1 = J_1 L/(\bar{c}D_1)$, and similarly for $\bar{J}_2$. The boundary conditions are

$$
\begin{aligned}
c_1(0) &= c_i, & c_1(1) &= c_e, \\
c_2(0) &= c_i, & c_2(1) &= c_e, \\
\phi(0) &= v, & \phi(1) &= 0.
\end{aligned}
$$

The short-channel or low concentration limit

If the channel is short or the ionic concentrations on either side of the membrane are small, so that $\lambda \ll 1$, we can find an approximate solution to the PNP equations by setting $\lambda = 0$. This gives

$$
\frac{d^2\phi}{dx^2} = 0,
\tag{3.29}
$$

and thus

$$\frac{d\phi}{dx} = -v. \tag{3.30}$$

Hence, $\lambda \approx 0$ implies that the electric potential has a constant gradient in the membrane, which is exactly the constant field assumption that was made in the derivation of the GHK equations (Chapter 2). The equation for c_1 is then

$$\frac{dc_1}{dx} - vc_1 = -\bar{J}_1, \tag{3.31}$$

and thus

$$c_1 = \frac{\bar{J}_1}{v} + K_1 e^{vx}. \tag{3.32}$$

From the boundary conditions $c_1(0) = c_i, c_1(1) = c_e$ it follows that

$$\bar{J}_1 = v \cdot \frac{c_i - c_e e^{-v}}{1 - e^{-v}}. \tag{3.33}$$

In dimensional form, this is

$$I_1 = FJ_1 = \frac{D_1}{L}\frac{F^2}{RT} \cdot V \cdot \left(\frac{c_i - c_e \exp(\frac{-VF}{RT})}{1 - \exp(\frac{VF}{RT})}\right), \tag{3.34}$$

which is, as expected, the GHK current equation. Graphs of the concentration and voltage profiles through the membrane are shown in Fig. 3.4. It is reassuring that the widely used GHK equation for the ionic flux can be derived as a limiting case of a more general model.
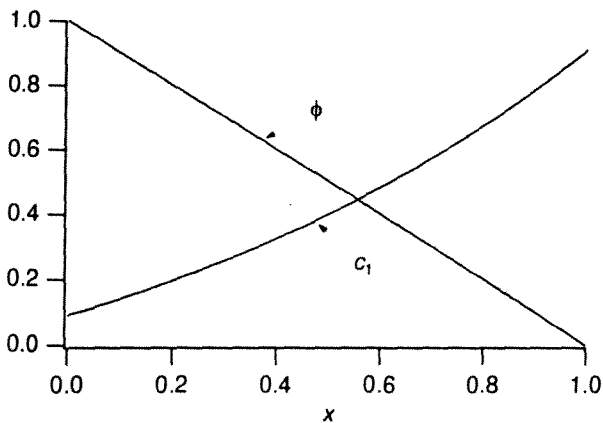

Figure 3.4 Graphs of the concentration and potential profiles for the short-channel limit of the Poisson–Nernst–Planck equations. Dimensionless parameters were set arbitrarily at $c_i = 50/550 = 0.091$, $c_e = 500/550 = 0.909$, $v = 1$. In this limit the electric field is constant through the channel (the potential has a constant slope), the concentration profile is nonlinear, and the GHK I–V curve is obtained.

The long-channel limit

Another interesting limit is obtained by letting the length of the channel go to infinity. If we let $\eta = 1/\lambda$ denote a small parameter, the model equations are

$$-\bar{J}_1 = \frac{dc_1}{dx} + c_1\frac{d\phi}{dx}, \tag{3.35}$$

$$-\bar{J}_2 = \frac{dc_2}{dx} - c_2\frac{d\phi}{dx}, \tag{3.36}$$

$$-\eta^2\frac{d^2\phi}{dx^2} = (c_1 - c_2). \tag{3.37}$$

Since there is a small parameter multiplying the highest derivative, this is a singular perturbation problem. The solution obtained by setting $\eta = 0$ does not, in general, satisfy all the boundary conditions, as the degree of the differential equation has been reduced, resulting in an overdetermined system. In the present case, however, this reduction of order is not a problem.

Setting $\eta = 0$ in (3.37) gives $c_1 = c_2$, which happens to satisfy both the left and right boundary conditions. Thus, c_1 and c_2 are identically equal throughout the channel. From (3.35) and (3.36) it follows that

$$\frac{d}{dx}(c_1 + c_2) = -\bar{J}_1 - \bar{J}_2. \tag{3.38}$$

Since both $\bar{J}_1$ and $\bar{J}_2$ are constants, it follows that dc_1/dx is a constant, and hence, from the boundary conditions,

$$c_1 = c_2 = c_i + (c_e - c_i)x. \tag{3.39}$$

We are now able to solve for ϕ. Subtracting (3.37) from (3.36) gives

$$2c_1\frac{d\phi}{dx} = 2\bar{J}, \tag{3.40}$$

where $2\bar{J} = \bar{J}_2 - \bar{J}_1$, and hence

$$\phi = \frac{\bar{J}}{c_e - c_i}\ln[c_i + (c_e - c_i)x] + K, \tag{3.41}$$

for some other constant K. Applying the boundary conditions $\phi(0) = v, \phi(1) = 0$ we determine $\bar{J}$ and K, with the result that

$$\phi = -\frac{v}{v_1}\ln\left[\frac{c_i}{c_e} + \left(1 - \frac{c_i}{c_e}\right)x\right], \tag{3.42}$$

where $v_1 = \ln(c_e/c_i)$ is the dimensionless Nernst potential of ion S_1. The flux density of one of the ions, say S_1, is obtained by substituting the expressions for c_1 and ϕ into (3.35) to get

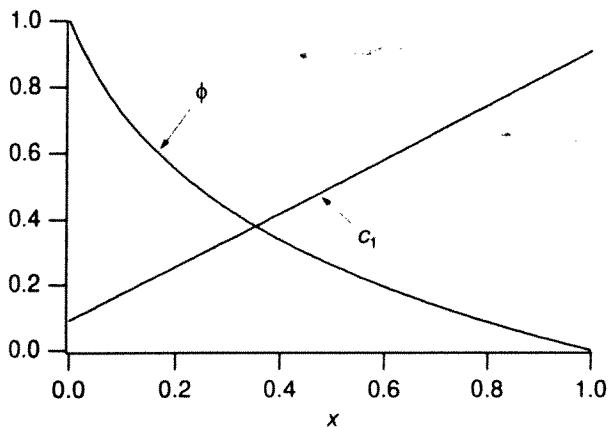$$\bar{J}_1 = \frac{c_e - c_i}{v_1}(v - v_1), \tag{3.43}$$

Figure 3.5 Graphs of the concentration and potential profiles for the long-channel limit of the Poisson–Nernst–Planck equations. Dimensionless parameters were set arbitrarily at $c_i = 50/550 = 0.091$, $c_e = 500/550 = 0.909$, $v = 1$. In this limit the concentration profile has a constant slope, the potential profile is nonlinear, and the linear I-V curve is obtained.

which is the linear I-V curve that we met previously. Graphs of the corresponding concentration and voltage profiles through the channel are shown in Fig. 3.5.

In summary, by taking two different limits of the PNP equations we obtain either the GHK I-V curve or a linear I-V curve. In the short-channel limit, ϕ has a constant gradient through the membrane, but the concentration does not. In the long-channel limit the reverse is true, with a constant gradient for the concentration through the channel, but not for the potential. It is left as an exercise to prove that although the GHK equation obeys the independence principle and the Ussing flux ratio, the linear I-V curve obeys neither. Given the above derivation of the linear curve, this is not surprising. A linear I-V curve is obtained when either the channel is very long or the ionic concentrations on either side of the channel are very high. In either case, one does not expect the movement of each ion through the channel to be independent of other ions, and so one expects the independence principle to fail. Conversely, the GHK equation is obtained in the limit of low ionic concentrations or short channels, in which case the independent movement of ions is not unexpected.

3.4 Barrier Models

The second type of model that has been widely used to describe ion channels is based on the assumption that the movement of an ion through the channel can be modeled as the jumping of an ion over a discrete number of free-energy barriers (Eyring et al., 1949; Woodbury, 1971; Läuger, 1973). It is assumed that the potential energy of an ion passing through a channel is described by a potential energy profile of the general form shown in Fig. 3.6. The peaks of the potential energy profile correspond to barriers that impede the ion flow, while the local minima correspond to binding sites within the channel.

To traverse the channel the ion must hop from one binding site to another. According to the theory of chemical reaction rates, the rate at which an ion jumps from one