

THEORETICAL STUDY OF THE RELAXATION CURRENTS IN VOLTAGE-CLAMP CONDITIONS AS DEDUCED FROM TWO MODELS FOR 'INWARD' RECTIFICATION: THE 'BLOCKING PARTICLE' AND THE 'ELECTROCHEMICAL GATING' HYPOTHESES

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Abstract

Two models for 'inward' rectification: the 'Blocking Particle (B.P.)' and the 'Electrochemical-Gating (E.G.)' hypotheses, previously developed to account for the steady-state conductance, are extended here to deduce the expected relaxation currents in voltage-clamp conditions. Although restrictive assumptions are considered for mathematical simplicity (e.g. a one-site pore is assumed in the (B.P.) model and a one-gating site in the other), it is nevertheless remarkable that the two viewpoints predict very different behaviors for the time constants of relaxation, suggesting thus experiments that may be of value for discriminating between alternative mechanisms: more precisely, in the (B.P.) model the time constant of relaxation increases with increasing the external potassium, while the opposite is expected in the (E.G.) model. Under appropriate conditions, and when the internal potassium concentration is constant, both models yield equations for the time constants that can be expressed as function of $\Delta V = V - V_R$, V being the actual membrane voltage and V_R the resting potential. However, also in this case, the directions of the dependence are different, the expectations of the (E.G.) hypothesis being in surprisingly good agreement with the existing data. In the Discussion section a slightly more complex version of the (E.G.) model is considered, whereby the voltage dependence of the instantaneous conductance, found experimentally, is rationalized without postulating rectification of the single pore.

1. Introduction

This paper deals with the time-dependent behavior of the membrane current deduced from two different models for 'anomalous rectification'. 'Anomalous', or 'inward rectification' is a property of certain potassium-permeable membranes that was discovered in muscle by Katz (1949), and was later analyzed in the same tissue by Hodgkin and Horowicz (1959), Adrian and Freygang (1962a,b),

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Nakajima *et al.* (1962), Adrian (1964, 1969), Horowicz *et al.* (1968), Adrian *et al.* (1970), Almers (1971, 1972), Gay and Stanfield (1977), Standen and Stanfield (1978), Hestrin (1981).

A similar rectification was found and studied also in other preparations, such as the egg membrane of a tunicate, by Takahashi *et al.* (1971) and Miyazaki *et al.* (1974), as well as in the egg membrane of certain starfish, by Hagiwara and Takahashi (1974), Miyazaki *et al.* (1975), Hagiwara *et al.* (1976) and Hagiwara and Yoshii (1979).

The typical features of membranes with inward rectification can be summarized as follows:

- (1) The ionic pathway is impermeable to anions, and the selectivity sequence for the monovalent cations is similar to that of the K^+ -channel of nerve.
- (2) When K^+ is the only permeant cation present, the membrane rectifies, showing much larger conductance for inward than outward currents.
- (3) When the internal K^+ is kept constant, the steady-state conductance, G , can be viewed as a function $\Delta V = V - V_R$, V_R being the resting potential and V the actual membrane potential. In this respect, the behavior is different from that of the delayed rectifier of nerve or muscle, where the conductance depends solely on the voltage, V .
- (4) Both in starfish egg cells (see Hagiwara *et al.*, 1976) and in muscle (see Hestrin, 1981), the transients of current elicited by steps of hyperpolarizing potentials can be fitted by a single exponential with a time constant that decreases when either hyperpolarization, or the external potassium concentration, or both, are increased. Moreover, the data on muscle indicate that this time constant is insensitive to changes of internal potassium.

Recently, two classes of physical models have been proposed to account for the steady-state properties of inward rectification. We shall refer to them as the 'Blocking Particle' and the 'Electrochemical-Gating' hypothesis, respectively. The 'Blocking Particle' hypothesis was formulated originally by Armstrong (1975), and was subsequently elaborated by Hille and Schwartz (1978), as well as by Standen and Stanfield (1978a,b). The main idea is that rectification is due to the presence, inside the cell, of large cations that can move part way into the pores, but are unable to cross them completely. Thus, when a pore is obstructed by a blocking cation, conduction in that pore will cease. It should be intuitive that lowering the membrane potential and increasing external potassium will favor removal of the blocking cation from the channels, causing the conductance to increase, while the opposite effect will result from raising the potential and decreasing the external potassium.

The alternative class of models, proposed by Ciani *et al.* (1978) and referred to as 'Electrochemical-Gating', is based on the idea that the membrane potential and the external potassium ions are both implicated in the processes of opening and closing the channels, with the important assumption, however, that the pathway

through which potassium operates in its gating role is physically separate from that of permeation. An example of this class of models, somehow similar to the one proposed by Bauman and Mueller (1974) for the gating of alamethicin, is that in which the formation of conductive pores requires a voltage-dependent orientation of membrane-bound dipoles followed by complexation with external potassium ions. This mechanism has been analysed in detail in a previous paper to account for the steady-state conductance (see Ciani *et al.*, 1978). Another example of 'Electrochemical-Gating', shown in Figs 2 and 4, and considered in this paper, is that in which one gating site, located near the inner surface of the membrane, but accessible only from the outside via a pathway different from that of permeation, must bind an external cation before the channel can open. Since the site is located in a voltage sensitive region, it is intuitive that also in this picture gating depends on both voltage and external potassium.

In order to derive testable results that may be helpful for distinguishing between alternative mechanisms, we shall now consider one example of each class of models, and shall analyze theoretically the expected time course of the current elicited by the application of voltage steps. Since the study of the time-dependent properties is mathematically more complicated than that of the steady-state ones, and given that, at this stage of still preliminary understanding of the system, the qualitative behavior of the results may be more meaningful than the quantitative details, we shall not hesitate to use simplifying assumptions that, although restrictive, will nevertheless lead us to unambiguous predictions. Thus, for example, in the 'Blocking Particle' model we shall assume that the pore has only one internal site that can be occupied either by the permeant or the blocking cation. Consequently, multi-occupancy of the pore by potassium ions (a feature that is essential to explaining both the anomalous behavior of the flux ratio and the steepness of the voltage dependence of the conductance), will not be allowed for. The justification for using this assumption all the same is, however, that the quantity of main interest in this paper, the time constant of relaxation for the onset of rectification, is probably unrelated to the mechanism of potassium permeation; in which case it seems plausible that simplifying assumptions concerning the details of such mechanism may not invalidate the results about the time constant.

Simplifying assumptions will also be used in the treatment of 'Electrochemical-Gating'. Most importantly, we shall restrict to the case of only one gating site per channel, even though the steepness of the dependence of the steady-state conductance on ΔV , as measured by Hagiwara and Takahashi (1974) in starfish egg cells, was shown by Ciani *et al.* (1978) to require that there be at least three. Although the discrepancy between the data and the results of this simplified model cannot be overlooked in the case of the starfish, it is nevertheless worth recalling that a less steep dependence of the conductance on voltage was observed in skeletal muscle by Adrian and Freygang (1962*a,b*), and even a less steep one in cardiac Purkinje fibers by McAllister *et al.* (1975), the last case being actually compatible with the assumption of one 'gating' site only.

2. Results

2.1 Relaxation currents in the 'Blocking Particle' hypothesis

As we mentioned previously, the model of Hille and Schwartz (1978) will be simplified, assuming that the pores have only one internal site that can be occupied either by potassium or the blocking cation. The latter ion is assumed to be present intracellularly and, while capable of entering the channel, is however, unable to cross it completely. As illustrated in Fig. 1, not only the height of the energy

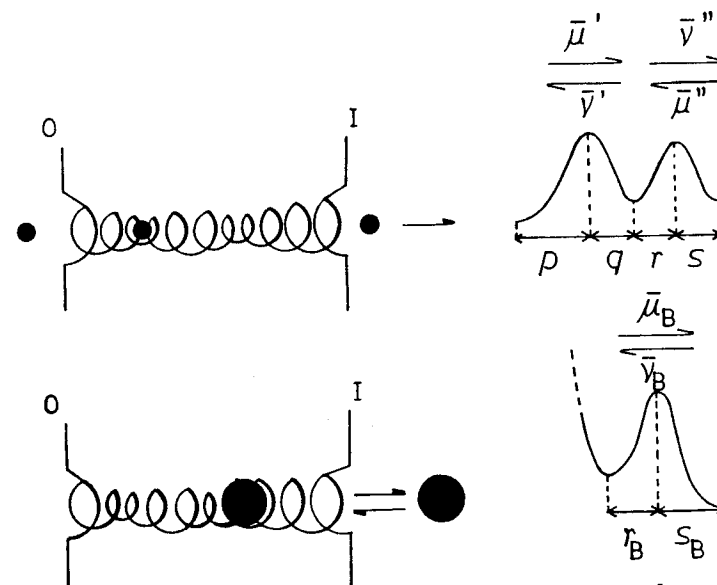


Fig. 1. Diagram for the one-site pore used in the treatment of the 'Blocking Particle' model. The blocking cation is present in the internal compartment and can move only part way across the channel.

barriers, but also the location of the site may, in principle, be different for the two ions. Consistently with this physical picture, the pore can exist in three states: empty, occupied by the permeant ion (e.g. potassium), and blocked, namely occupied by the blocking cation. The corresponding densities of pores in each state will be denoted by N_e , N , and N_B , respectively. Using the same symbols as those of Fig. 1 for the rate constants, the kinetics of transition between the three states will be described by the following equations:

$$(1) \quad \frac{dN}{dt} = \bar{\mu}' C^e N_e - \bar{v}' N + \bar{\mu}'' C^i N_e - \bar{v}'' N$$

$$(2) \quad \frac{dN_B}{dt} = \bar{\mu}_B C_B N_e - \bar{v}_B N_B$$

$$(3) \quad N^T = N_e + N + N_B,$$

where C_B is the internal concentration of the blocking cations, and C^e and C^i are the external and the internal concentrations of potassium, respectively. Using the formalism of Eyring's rate theory to express the dependence of the rate constants on potential, we have

$$(4) \quad \begin{aligned} \bar{\mu}' &= \mu' e^{-p\phi}; & \bar{v}' &= v' e^{q\phi}; & \bar{\mu}'' &= \mu'' e^{s\phi}; & \bar{v}'' &= v'' e^{-r\phi}; \\ \bar{\mu}_B &= \mu_B e^{s_B\phi}; & \bar{v}_B &= v_B e^{-r_B\phi} \end{aligned}$$

where ϕ is the total membrane potential, while the coefficients of ϕ in the exponents indicate fractions of such potential, as illustrated in Fig. 1. From microscopic reversibility it also follows that

$$(5) \quad \mu' v'' = \mu'' v'.$$

If considered positive when directed inward, the fluxes of the permeant ion across the outer and the inner energy barriers are given by

$$(6) \quad J' = \bar{\mu}' C^e N_e - \bar{v}' N$$

and

$$(7) \quad J'' = \bar{v}'' N - \bar{\mu}'' C^i N_e$$

respectively, while the flux of the blocking cations across the only barrier permeable to them (namely the barrier between the site and the intracellular space) is

$$(8) \quad J_B = \bar{v}_B N_B - \bar{\mu}_B C_B N_e.$$

Clearly, at steady state J_B vanishes and $J' = J''$. However, during relaxation, after a pulse is applied, neither does J_B vanish, nor are J' and J'' necessarily equal to each other. The problem therefore arises of finding an expression for the total density of current during the transient state. It is shown in the Appendix that such current density can be expressed, in terms of the fluxes, by

$$(9) \quad \frac{I}{F} = (p + q)J' + (r + s)J'' + (r_B + s_B)J_B,$$

where p, q, r, s, r_B and s_B are the fractional distances introduced in eq. (4), whose physical meaning should be apparent from inspection of Fig. 1. Realizing from the same figure that

$$(10) \quad p + q + r + s = 1,$$

it is clear that, at steady state, eq. (9) reduces to J' (or J''). If the rate constants are independent of the ion concentrations inside the membrane, eqs (6) to (9) show that the current density is a linear function of the three variables, N_e, N and N_B ,

so that an explicit expression for I can be obtained after integration of eqs (1) and (2). Observing, with the aid of eq. (3), that only two of the three variables, N_e, N, N_B , are independent, we can anticipate that the general expression for I will contain the sum of two exponential functions of time, characterized by two time constants, τ_1 and τ_2 . Although the mathematical derivation of the equation for I is straightforward, the exact results (in particular those for the relaxation amplitudes), are too lengthy to be useful. Fortunately, simple considerations, given below, suggest that the only case of interest for a meaningful comparison of the model with the data is that in which the kinetics of the blocking particle is much slower than that of the permeant ion (in which case the expressions deduced from the exact integration of eqs (1) and (2) can be simplified considerably). In fact, the exact integration of eqs (1) and (2) shows that, if the rate constants of the two ions are comparable, the time constants, τ_1 and τ_2 , though numerically different, will both have the order of magnitude of the reciprocal of those constants. Now, since the available data about the single channel conductance indicate that the rate constants of the permeant ions are much too large to yield experimentally resolvable relaxations, it is clear that neither time constant of the model could possibly be identified with the measured one if the kinetics of the blocking particle were as fast as that of the permeant ions. Consequently, we are forced to conclude that a necessary prerequisite for relating one of the two time constants of the model (namely, the slowest), to that seen experimentally is the condition that the rate constants for the blocking particle be much smaller than those of the permeant ions. Thus, making this assumption, and following the procedure outlined in the Appendix for the derivation of the results, the current density after the application of a step of voltage (from ϕ_o to ϕ) is given by

$$(11) \quad I(t, \phi_o, \phi) = I(\infty, \phi) \{1 + \alpha(\phi_o, \phi) e^{-t/\tau(\phi)}\}.$$

The explicit expressions for $I(\infty, \phi)$, $\tau(\phi)$ and $\alpha(\phi_o, \phi)$ can be written in a simple form using the following definitions:

$$(12) \quad \bar{K}_B(\phi) = K_B e^{(r_B + s_B)\phi}; \quad K_B = \mu_B / v_B$$

and

$$(13) \quad M(\phi) = \bar{\mu}' C^e + \bar{\mu}'' C^i; \quad P(\phi) = \bar{v}' + \bar{v}''.$$

With the aid of eqs (12) and (13) we find

$$(14) \quad I(\infty, \phi) = \frac{N^T \mu' v'' e^{(q+s)\phi}}{M(\phi) + P(\phi)[1 + \bar{K}_B(\phi)C_B]} \{C^e e^{-\phi} - C^i\}$$

$$(15) \quad 1/\tau(\phi) = \bar{v}_B(\phi) + \frac{\bar{\mu}_B(\phi)C_B}{1 + M(\phi)/P(\phi)}$$

$$(16) \quad \alpha(\phi_o, \phi) = \frac{N_B(\infty, \phi) - N_B(\infty, \phi_o)}{N^T \bar{v}_B(\phi)\tau(\phi)}$$

where

$$(17) \quad N_{\text{B}}(\infty, \phi) = \frac{N^T \bar{K}_{\text{B}}(\phi) C_{\text{B}}}{1 + \frac{M(\phi)}{P(\phi)} + \bar{K}_{\text{B}}(\phi) C_{\text{B}}}$$

and $N_{\text{B}}(\infty, \phi_0)$ is given by the same expression as eq. (17), in which ϕ is replaced by ϕ_0 , the initial value of the potential prior to the pulse. Even without discussing in detail the behavior of the above expressions, certain interesting conclusions that are directly pertinent to the objectives of this paper can be deduced from inspection of eqs (15) and (13). For example, it is apparent from such equations that $\tau(\phi)$ increases with increasing both the external and the internal potassium concentrations. This theoretical prediction is in disagreement with the existing data. Both the experiments of Hagiwara *et al.* (1976) on starfish egg cells and those of Hestrin (1981) on muscle fibers show that, for given membrane potentials, the time constant decreases with increasing external potassium. Furthermore, the experiments of Hestrin indicate that the same time constant is virtually independent of internal potassium, while eqs (15) and (13) predict that it should depend on it.

Dependence of τ and $I(\infty, \phi)$ on $\Delta\phi$ in the 'Blocking Particle' hypothesis.—There seems to be only one case in which the time constant and the current can be expressed as functions of $\Delta\phi = \phi - \phi_{\text{R}}$, ϕ being the membrane potential and ϕ_{R} the resting membrane potential, in units of RT/F . This case corresponds to the situation in which the internal concentration C^{i} is constant, and the barrier peaks shown in Fig. 1 are all so close to the internal surface that

$$(18) \quad p \simeq 1; \quad q \simeq r \simeq s \simeq r_{\text{B}} \simeq s_{\text{B}} \simeq 0.$$

In this case, $\mu' = \mu'e^{-\phi}$, while all the other constants become voltage independent: ($\bar{\mu}'' = \mu''$; $\bar{v}' = v'$; $\bar{\mu}_{\text{B}} = \mu_{\text{B}}$; $\bar{v}_{\text{B}} = v_{\text{B}}$). Recalling that the resting potential and the concentrations of the sole permeant ion must be related by

$$(19) \quad C^{\text{e}} = C^{\text{i}} e^{\phi_{\text{R}}},$$

the quantity, $M(\phi)$, defined in eq. (13) can, with the aid of eqs (18) and (19), be rewritten in the form

$$(20) \quad M(\phi) = C^{\text{i}} \{ \mu' e^{-\Delta\phi} + \mu'' \}.$$

Substituting in eq. (15), and recalling that, on account of eq. (18), the quantities, v_{B} , μ_{B} and $P(\phi)$, are all constant, we find

$$(21) \quad \frac{1}{\tau(\Delta\phi)} = v_{\text{B}} + \frac{\mu_{\text{B}} C_{\text{B}}}{1 + C^{\text{i}} \frac{\mu' e^{-\Delta\phi} + \mu''}{v' + v''}}.$$

Eq. (21) gives the explicit functional relation between τ and $\Delta\phi$. Since ϕ denotes the potential inside minus the potential outside, a simple inspection of eq. (21)

shows that τ increases with increasing hyperpolarization. In particular, for very low and very high membrane potentials, one finds

$$(22) \quad \lim_{\Delta\phi \rightarrow -\infty} \tau = \frac{1}{v_{\text{B}}}; \quad \lim_{\Delta\phi \rightarrow +\infty} \tau = \frac{1}{v_{\text{B}}} \left\{ \frac{1}{1 + \frac{K_{\text{B}} C_{\text{B}} (v' + v'')}{\mu'' C^{\text{i}} + v' + v''}} \right\}.$$

Since the quantity within parentheses is less than unity, τ is obviously smaller when the membrane is highly depolarized than when it is highly hyperpolarized. This result is in disagreement with the existing data, and particularly so with those of Hagiwara *et al.* (1976), which show most clearly that the experimental τ decreases with decreasing $\Delta\phi$. For the same locations of the barrier peaks as those defined in eq. (18) and used to deduce eqs (20) to (22), the steady-state current density, eq. (14), becomes

$$(23) \quad I(\infty, \phi) = \frac{N^T \mu' v'' C^{\text{i}} (e^{-\Delta\phi} - 1)}{C^{\text{i}} (\mu' e^{-\Delta\phi} + \mu'') + (v' + v'') (1 + K_{\text{B}} C_{\text{B}})}.$$

Eq. (23) shows that, when the internal concentration, C^{i} , and $\Delta\phi$ are fixed, the current density is completely determined, implying that its dependence on the external concentration, C^{e} , and on the potential, ϕ , can be condensed in a dependence on the sole quantity, $\Delta\phi$. Also this prediction of the model is not verified experimentally, since, when $\Delta\phi$ and C^{i} are fixed, the conductance is generally still a function of C^{e} . For example, in the case of starfish egg cells, it increases with the square root of C^{e} (see Hagiwara and Takahashi, 1974).

2.2 Relaxation currents according to the 'Electrochemical-Gating' hypothesis

It was shown in a previous paper by Ciani *et al.* (1978) that several physical mechanisms satisfy the main requirements of 'Electrochemical-Gating', giving similar results for the steady-state conductance. One of the simplest ones, though different from that considered in the paper referred to above, is illustrated in Fig. 2. This scheme implies that 'gating' is associated with a single kinetic step, whereby an external potassium ion, moving along a pathway independent of that of permeation, binds to a site located in the proximity of the internal surface. According to this model, the pore can have two states: 'closed' and 'open', that will be denoted by A_0 and A_1^* , respectively. (Consistently with the notation in a previous paper by Ciani *et al.* (1978), the subscripts indicate the number of ions bound to the gating site, and the asterisk is used to distinguish the open state, A_1^* , from an intermediate state, A_1 , that will be introduced in a slightly more elaborate version of the model to be dealt with in the discussion.) If i is the gating cation, the kinetics of opening and closing can be represented schematically by



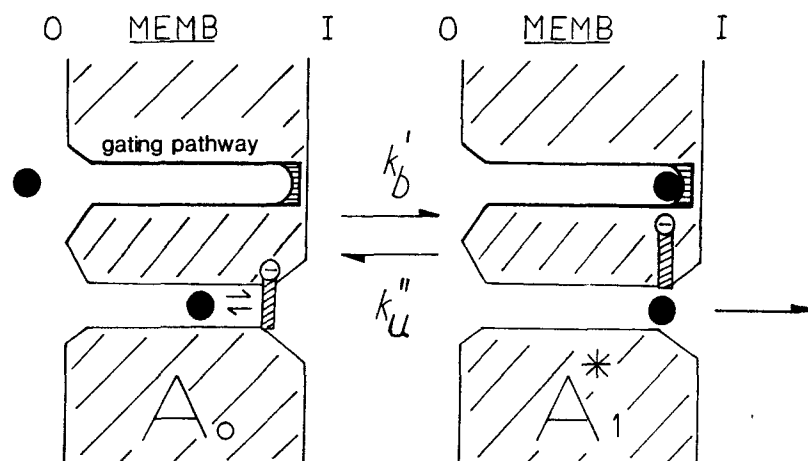


Fig. 2. Diagram for the simple version of the 'Electrochemical-Gating' model treated in the Results section. Each pore has only one gating site, and the transition between closed and open states, which requires the movement of an ion across the gating pathway as well as the opening of the gate, is assumed to be describable by a single kinetic step.

Since the ion i must move along the gating pathway across the membrane, the rate constants for binding and unbinding, k_b' and k_u'' , will generally depend on voltage. Using the formalism of Eyring's rate theory to express such dependence, we can write

$$(25) \quad k_b' = k_b e^{-\alpha\phi}; \quad k_u'' = k_u e^{(1-\alpha)\phi},$$

where ϕ is the potential difference between the location of the binding site and the external surface of the membrane, and α is the fractional 'electric distance' between the peak of the activation energy barrier in the gating pathway and the same external surface. Since the position of the gating site is assumed to be next to the internal surface (see Fig. 2), ϕ coincides practically with the membrane potential. Applying the equations of chemical kinetics to reaction (24), the rate of variation of A_1^* will be described by

$$(26) \quad \frac{d[A_1^*]}{dt} = k_b' C^e [A_o] - k_u'' [A_1^*],$$

where the brackets denote number of channels per unit area, and C^e indicates, as in the previous model, the external potassium concentration. Recalling that in this simple scheme the sum of the densities of closed and open pores must be constant, so that

$$(27) \quad [A_o] + [A_1^*] = [A^T],$$

substituting eq. (27) into eq. (26) and integrating yields

$$(28) \quad [A_1^*]_t = [A_1^*]_\infty \{1 + \beta e^{-t/\tau}\},$$

where

$$(29) \quad \tau = 1/(k_b' C^e + k_u'')$$

and

$$(30) \quad \beta = \frac{[A_1^*]_o - [A_1^*]_\infty}{[A_1^*]_\infty}.$$

On the other hand, $[A_1^*]_\infty$ (as well as $[A_1^*]_o$) can be obtained directly from eqs (26) and (27) setting the derivative on the left hand side of eq. (26) equal to zero. Thus, if ϕ is the potential of the pulse step, one finds, at steady state

$$(31) \quad [A_1^*]_\infty = \frac{k_b}{k_u} \cdot \frac{C^e e^{-\phi}}{1 + \frac{k_b}{k_u} C^e e^{-\phi}}.$$

The only difference between the explicit formulas for $[A_1^*]_\infty$ and $[A_1^*]_o$ is that, in the formula for the latter, ϕ must be replaced by the potential value prior to the pulse. If ϕ_R is the zero-current potential, determined by the gradient of the ionic species, i (which, of course, is also the gating ion), we can still use eq. (19) to relate the external and the internal potassium concentrations. With the aid of that equation, eq. (31) can be written in the more concise form

$$(32) \quad [A_1^*]_\infty = \frac{[A^T]}{1 + \exp[\Delta\phi - \Delta\phi_h]}$$

where

$$(33) \quad \exp[\Delta\phi] = e^\phi C^i / C^e; \quad \exp[-\Delta\phi_h] = k_u / k_b C^i.$$

From eqs (32) and (33) it is apparent that, as long as the internal concentration, C^i , is constant, the steady-state density of open channels can be expressed as a function of $\Delta\phi$. If $g^*(C^e, C^i, \phi)$ denotes the conductance of the individual channel, the steady-state membrane conductance per unit area will be given by the product of $g^*(C^e, C^i, \phi)$ and eq. (32), namely

$$(34) \quad G_\infty(\phi) = g^*(C^e, C^i, \phi) \cdot \frac{[A^T]}{1 + \exp[\Delta\phi - \Delta\phi_h]}$$

Eq. (34) is similar to that obtained experimentally by Hagiwara and Takahashi (1974), and would actually be identical to it if $\Delta\phi$ were multiplied by 3 and g^* were proportional to the square root of C^e . As we have mentioned already, these features could be accounted for, assuming the presence of three gating sites and using Eyring's theory to describe permeation in the channel (see Ciani *et al.*, 1978). The reasons for considering here the case of only one gating site are, as we

mentioned previously, mathematical simplicity, as well as a certain confidence in the fact that this simplification will not affect the direction of the dependence of the time constant on the membrane potential and on the ion concentration. Let us now go back to eq. (29) for the time constant and note that, if the rate constants are expressed explicitly in terms of the potential, we find

$$(35) \quad \tau(\phi) = \frac{1}{k_b C^e e^{-\alpha\phi} + k_u e^{(1-\alpha)\phi}}$$

If α is between 0 and 1, which means that the peak of the activation energy barrier in the 'gating' pathway is somewhere between the external and the internal surface, eq. (35) shows that τ decreases for highly negative, as well as for highly positive potentials, and attains a maximum at

$$(36) \quad \phi^M = \ln \left\{ \frac{\alpha}{1-\alpha} \frac{k_b C^e}{k_u} \right\}$$

However, if the peak of the barrier is so close to the internal surface that $\alpha \approx 1$, τ has no more maximum, and decreases monotonically with increasing hyperpolarization, as well as with increasing the external potassium concentration. In the limit of $\alpha = 1$, eq. (35) can be rewritten in the two following equivalent forms:

$$(37) \quad \tau(\phi) = \frac{1}{k_u} \cdot \frac{1}{1 + \frac{k_b}{k_u} C^e e^{-\phi}}$$

or, recalling the definitions (33)

$$(38) \quad \tau(\Delta\phi) = \frac{1}{k_u} \cdot \frac{1}{1 + \exp[\Delta\phi_h - \Delta\phi]}$$

Eqs (35), (37) and (38) are probably the most important results of this paper, since they show that 'Electrochemical-Gating' can predict dependences of the time constant, τ , on the variables, C^e , ϕ and $\Delta\phi$, which are in agreement with those observed experimentally both in muscle and in egg cells. In agreement with the data is also the fact that such expressions are independent of the internal potassium concentration, C^i . Although eqs (37) and (38) are totally equivalent, eq. (37) has the advantage, similarly to eq. (35), of being expressed in terms of the parameters that affect 'gating' most directly, namely C^e and ϕ . Eq. (38) is appealingly simple, but may give the false impression that τ is also a function of the internal concentration, C^i , since C^i appears in the definition of both $\Delta\phi$ and $\Delta\phi_h$, and it may not be immediately obvious that it cancels out in the difference, $\Delta\phi_h - \Delta\phi$. Eq. (35) has been used to fit certain data on muscle, selected from Fig. 7 of the paper by Hestrin (1981); and Fig. 3 of the present paper, in which those data are reproduced, illustrates the quality of such fit. The fact that the calculated value of α is very close to unity implies that the same data could have also been fitted by the simpler equations (37) or (38). Also the data on starfish egg cells, shown in Fig. 3 of

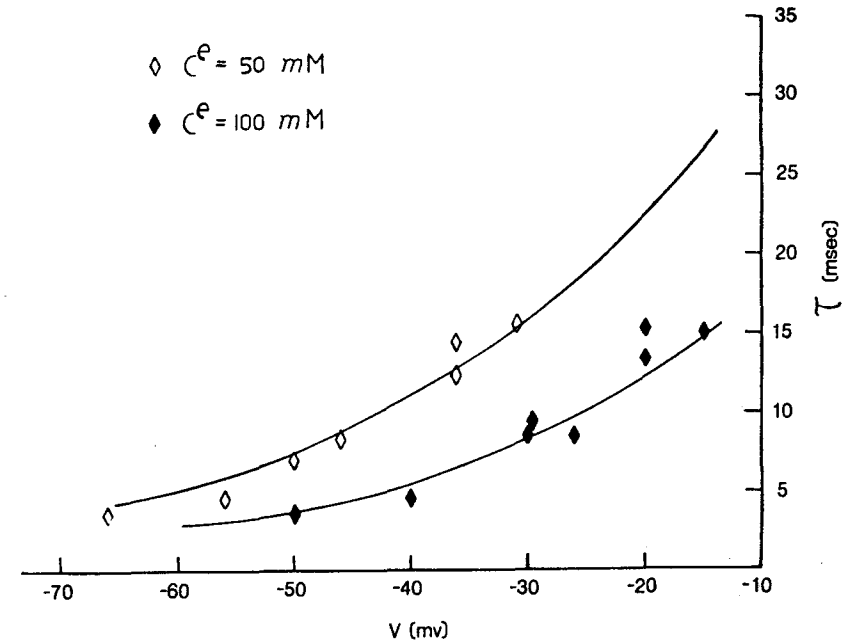


Fig. 3. Fit of data on muscle fibers, taken from Fig. 7 of Hestrin (1981), with eq. (35). The two sets of points refer to the two external concentrations of potassium indicated in the figure. The continuous curves correspond to the following values of the fitting parameters: $k_b = 372 \text{ s}^{-1} \text{ M}^{-1}$; $k_u = 4 \text{ s}^{-1}$; $\alpha = 0.93$.

Hagiwara *et al.* (1976), and indicating that $\log \tau$ is practically a linear function of $\Delta\phi$, with slope of 1, in the range $-40 \text{ mV} < RT\Delta\phi/F < -7 \text{ mV}$, can be fitted easily with eq. (38) if $\Delta\phi_h$ is so chosen that the quantity, $\exp[\Delta\phi_h - \Delta\phi]$, remains greater than unity in that whole potential range. In fact, if this condition is not satisfied, eq. (38) predicts, at low hyperpolarizing potentials, a saturation that is not shown by the data. (The requirement that $\exp[\Delta\phi_h - \Delta\phi]$ be greater than 1 in the range $-40 \text{ mV} < \Delta V < -7 \text{ mV}$, is not satisfied if one chooses for $\Delta V_h (= RT\Delta\phi_h/F)$ the value that was deduced by fitting the steady-state conductance of starfish egg cells with eq. (37) of Ciani *et al.* (1978): namely, $\Delta V_h = -13.5 \text{ mV}$. It should be noted, however, that it would be logically unjustified to compare the ΔV_h of that treatment with the ΔV_h of the present one, since, due to the different assumptions used (e.g. three gating sites instead of only one), the physical meanings of the parameter are not quite the same in the two cases.)

3. Discussion

Some considerations about the instantaneous conductance

In the previous sections we dealt with two models of 'anomalous rectification', referred to as the 'Blocking Particle' and the 'Electrochemical-Gating' hypotheses,

and showed that the predictions of the latter for the time constants of relaxation are consistent with the existing data. However, there is an important expectation of the simple version of the 'Electrochemical-Gating' picture given here that contradicts the experiments: it concerns the behavior of the instantaneous conductance. In fact, according to eqs (28) and (30), the number of open pores immediately after the application of the step of voltage, ($e^{-t/\tau} \simeq 1$), is equal to the number of pores that were open prior to the pulse, and is thus a quantity that is expected to be independent of the pulse size. Consequently, if the conductance of the single channel is approximately ohmic, as we assume to be the case, also the macroscopic (multi-channel) instantaneous conductance should be so. Most experimental results do not agree with this expectation, clearly indicating that the instantaneous conductance rectifies and is, like the steady-state one, a function of $\Delta\phi$ (e.g. see Figs 2 and 3 of Hagiwara *et al.*, 1976). As a matter of fact, the curves for the instantaneous conductance versus $\Delta\phi$ are very similar to those for the steady-state one, the main difference being that the absolute values of the latter are larger in the hyperpolarizing region. These findings are susceptible to various interpretations. One possibility is that the $\Delta\phi$ -dependent, instantaneous rectification is a property of the single channel, due, for example, to asymmetries in the profile of the energy barriers in the conduction pathway. If so, it is clear that the rectification should manifest itself 'instantaneously', since the relaxation times for ionic redistribution in the pore are expected to be far too short to be resolvable. The relaxation observed experimentally would thus be due to an independent mechanism, regulating the opening and the closing of the channels with a relatively slow kinetics. Although this interpretation is plausible, it seems somehow circumstantial that two phenomena, the conductance of the single channel and the gating of such channel, would be totally independent of each other and, at the same time, both functions of $\Delta\phi$. Moreover, as we mentioned already in discussing the 'Blocking Particle' model, it is not easy and, in the case of a single site, actually impossible, to find suitable arrangement of energy barriers in the conduction pathway that would give a conductance dependent on $\Delta\phi$ and also proportional to the geometric mean of the external and internal ion concentrations (as is found experimentally).

We shall suggest here an alternative way of accounting for the non-ohmic behavior of the instantaneous conductance, without postulating rectification of the single channel. It implies a minor modification to the version of the 'Electrochemical-Gating' model presented in the previous section, and is based on the assumption that an additional conductive state of the channel exists between the closed and the fully open one. More precisely, we shall make the following three assumptions. (a) The gating mechanism consists of two steps, the first of which implies the binding of an ion to a gating site (as in the previous version of the model), while the second is a voltage-independent conformational change of the channel structure. As illustrated in Fig. 4, the pore now has three states, denoted by A_0 , A_1 and A_1^* , respectively. (b) The state A_1 is conductive as well as A_1^* . (c) The kinetics of the transition from A_0 to A_1 (and reverse) is much faster

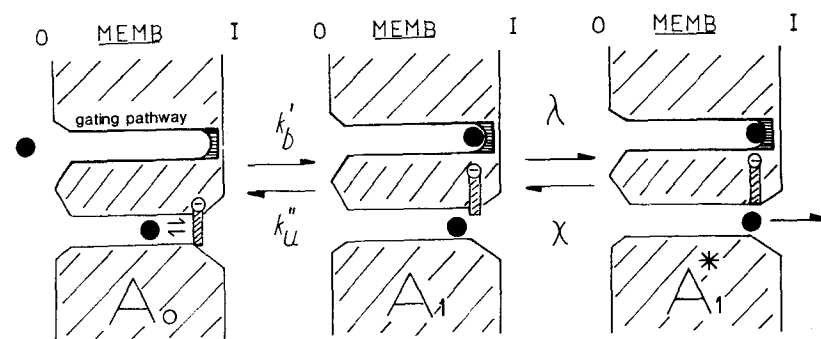
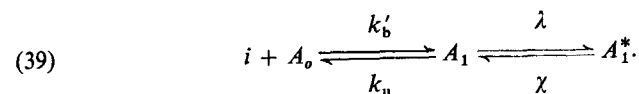


Fig. 4. Diagram for the version of the 'Electrochemical-Gating' model treated in the Discussion. It differs from the model of Fig 2 in that it postulates two kinetic steps instead of one.

than the conformational change between A_1 and A_1^* . As a result of assumption (a), the gating kinetics can now be described by the following sequence of reactions



Since we assume that also the state A_1 is conductive, the total conductance at the time, t , per unit area, will be

$$(40) \quad G_t = g \cdot [A_1]_t + g^* \cdot [A_1^*]_t.$$

Now, if the potential is suddenly changed, say from ϕ_0 to ϕ , it is still true that the 'theoretical instantaneous' conductance will be determined by the number of pores that were in the states A_1 and A_1^* prior to the application of the voltage; so that, if g and g^* are ohmic, it should be independent of the amplitude of the applied voltage. However, if the transition from A_0 to A_1 is too fast to be resolved, it is clear that the 'measured instantaneous' conductance will not coincide with the theoretical one, since, by the time the measurement is taken, the current will already include the contributions of newly formed A_1 pores. In order to derive quantitative results, let us recall that the total number of channels per unit area, $[A^T]$, is now given by

$$(41) \quad [A^T] = [A_0] + [A_1] + [A_1^*].$$

If the transition between A_0 and A_1 is so fast that we can assume equilibrium between these two states, we shall have

$$(42) \quad [A_1]_t = KC^*e^{-\phi}[A_0]_t; \quad K = k_b/k_u,$$

whereas the second transition between A_1 and A_1^* will be described by the rate equation

$$(43) \quad \frac{d[A_1^*]}{dt} = \lambda[A_1] - \chi[A_1^*].$$

Combining eqs (41), (42) and (43) and integrating, explicit results can be obtained for $[A_1]_t$ and $[A_1^*]_t$. Inserting the result in eq. (40), the time dependent conductance can be expressed in the form

$$(44) \quad G_t = G_\infty + [G_o - G_\infty]e^{-t/\tau}$$

where

$$(45) \quad \tau = \frac{1}{\lambda + \chi} \cdot \left\{ 1 + \frac{\lambda/\chi}{1 + \exp[\Delta\bar{\phi}_h - \Delta\phi]} \right\}$$

with

$$(46) \quad \Delta\phi = \phi - \phi_R \quad \text{and} \quad \exp[\Delta\bar{\phi}_h] = KC^i \left(1 + \frac{\lambda}{\chi} \right)$$

C^i being the internal concentration of permeant ion and K the equilibrium constant defined in eq. (42). The steady-state and the instantaneous conductance, G_∞ and G_o , introduced in eq. (44), are

$$(47) \quad G_\infty = \frac{g + \lambda g^*/\chi}{1 + \lambda/\chi} \cdot \frac{[A^T]}{1 + \exp[\Delta\phi - \Delta\bar{\phi}_h]}$$

and

$$(48) \quad G_o = g^* \cdot [A_1^*]_o + g \cdot \{ [A^T] - [A_1^*]_o \} \frac{1}{1 + \exp[\Delta\phi - \Delta\bar{\phi}_h]}$$

with

$$(49) \quad \exp[\Delta\bar{\phi}_h] = KC^i.$$

Note that eq. (47) for the steady-state conductance is similar to eq. (34), showing thus that one of the most important predictions of the previous simpler version of the model is not altered in the present one. Analogous considerations apply to the expression for the time constant, eq. (45), which shows that τ decreases with $\Delta\phi$, as in the case of eq. (38). From eq. (45) we deduce

$$(50) \quad \lim_{\Delta\phi \rightarrow -\infty} \tau = \frac{1}{\lambda + \chi} \quad \text{and} \quad \lim_{\Delta\phi \rightarrow \infty} \tau = \frac{1}{\chi}.$$

The main difference between eqs (38) and (45) is that, for $\Delta\phi \rightarrow -\infty$, the former vanishes, while the latter approaches to the finite level $(\lambda + \chi)^{-1}$. However, the two functions may become experimentally indistinguishable if λ is sufficiently large.

More interesting to the objectives of this section is eq. (48), from which it is apparent that the 'measured instantaneous' conductance is, like the steady-state

one, a decreasing function of $\Delta\phi$, in agreement with the experimental data often referred to previously. It should be clearly realized that this result does not require rectification of the single channel, but is due to the hypothesis of the existence of the intermediate, conductive state, A_1 , as well as to the assumption that the first kinetic step in eq. (39) is undetectably fast.

However, a limitation of eq. (48) in its ability to account for certain aspects of the experiments should be acknowledged. For example, in the case of egg cells, Hagiwara *et al.* (1976) give evidence that the instantaneous conductance is independent of the 'previous history of the membrane potential', which means that it depends on the final value of the voltage step, but not on the potential from which the pulse is started (holding potential). This requirement is not satisfied by eq. (48) in general, since the right hand side depends on $[A_1^*]_o$, which is, in fact, the density of open channels at the holding potential, and will certainly vary with it. Nevertheless, if the pulse starts from potentials sufficiently high that $[A_1^*]_o \ll [A^T]$, and if the additional condition, $g^* \cdot [A_1^*]_o < g \cdot [A^T] / (1 + e^{t[\Delta\phi - \Delta\bar{\phi}_h]})$, is satisfied in the experimental range of $\Delta\phi$, eq. (48) reduces then to

$$(51) \quad G_o = \frac{g \cdot [A^T]}{1 + \exp[\Delta\phi - \Delta\bar{\phi}_h]}$$

which is independent of the holding potential. Incidentally, these considerations suggest that it may be interesting to investigate more thoroughly whether G_o is really independent of the holding potential, particularly when this is low enough that eq. (51) is not expected to be a good approximation of eq. (48).

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APPENDIX

Outline of the derivation of the equations for the time dependent current in the the 'Blocking-Particle' model: eqs (9) to (17)

In the derivation of the equations for the time-dependent current density, attention must be given to the fact that, in order to comply with the requirement of continuity of the electric current, the 'displacement' current must be added to the ion fluxes. Fig. 1 shows schematically the profile of the energy barrier in the pore for the two ionic species, the permeant ion, i , and the blocking ion, B . It

should be emphasized that those profiles are not meant to represent two physically distinct channels, but rather the different free-energy profiles resulting from the interaction of the two ionic species with the same channel. Since Eyring's formalism implies that the ions reside in the energy wells (sites) much longer than at, or near, the peaks, the ions within the membrane can be schematically viewed as distributed over two planes, one of which contains the permeant ions, i , and the other the ions, B . As is shown in Fig. 5, the former is located at the distance, $p + q$, from

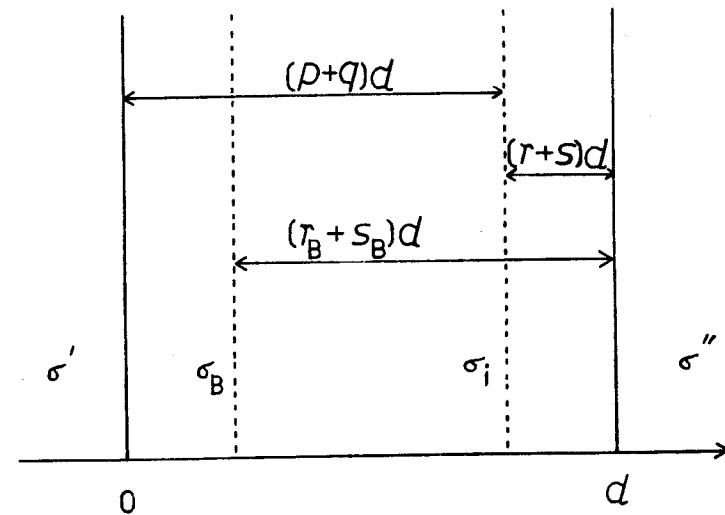


Fig. 5. Approximate location of the charges within and at the surface of the membrane. The σ_B -plane corresponds to the position of the sites for the blocking cations, and the σ_i -plane to that of the sites for the permeant ions.

the outer surface, and the latter at the distance, $r_B + s_B$, from the internal surface. We shall denote by σ_i and σ_B the surface charge densities on the two planes. As it is evident from the same figure, the membrane can be subdivided in three compartments, in each of which the electric field will be generally different and may change with time, even though the total membrane voltage is 'clamped' at a constant value. Denoting by σ' and σ'' the net charge densities of the two aqueous diffuse double layers, the requirement of electroneutrality gives

$$(A1) \quad \sigma + \sigma_B + \sigma_i + \sigma'' = 0.$$

If we now make the simplifying assumption that the dielectric constant, ϵ , is the same in the three compartments of Fig. 5, and if we regard the two aqueous ionic phases as conductors, a simple application of Gauss' theorem allows us to deduce the electric fields, E_1 , E_2 , and E_3 in such compartments. If σ_B is closer to the external surface than σ_i , we find

$$(A2) \quad \varepsilon E_1 = \sigma' - \frac{\sigma_B}{2} - \frac{\sigma_i}{2} - \sigma'',$$

$$(A3) \quad \varepsilon E_2 = \sigma' + \frac{\sigma_B}{2} - \frac{\sigma_i}{2} - \sigma'',$$

$$(A4) \quad \varepsilon E_3 = \sigma' + \frac{\sigma_B}{2} - \frac{\sigma_i}{2} - \sigma''.$$

(It is easy to realize that the signs in front of σ_B and σ_i would have to be changed in eq. (A3) if the σ_i layer were the closest to the external surface.) It is now appropriate to recall that the total membrane voltage, $V = V_d - V_o$, is related to the above electric fields, by

$$(A5) \quad -\frac{V}{d} = (1 - r_B - s_B)E_1 + (r_B + s_B - r - s)E_2 + (r + s)E_3,$$

where d is the thickness of the membrane and the quantities, r , s , r_B and s_B are fractional distances, whose meaning is illustrated in Fig. 5 as well as in Fig. 1. The five relations, (A1) to (A5), can be considered as five equations in the unknown σ' , σ'' , E_1 , E_2 , and E_3 . Eliminating σ' and σ'' , the electric fields in the three compartments are given by

$$(A6) \quad \varepsilon E_1 = -\varepsilon \frac{V}{d} - (r_B + s_B)\sigma_B - (r + s)\sigma_i,$$

$$(A7) \quad \varepsilon E_2 = -\varepsilon \frac{V}{d} + (1 - r_B - s_B)\sigma_B - (r + s)\sigma_i,$$

$$(A8) \quad \varepsilon E_3 = -\varepsilon \frac{V}{d} + (1 - r_B - s_B)\sigma_B + (1 - r - s)\sigma_i.$$

Adding the displacement currents to the ionic fluxes, the total current densities in the three compartments are given by

$$(A9) \quad FJ' + \varepsilon \frac{dE_1}{dt}; \quad F(J' + J_B) + \varepsilon \frac{dE_2}{dt}; \quad F(J'' + J_B) + \varepsilon \frac{dE_3}{dt}.$$

If we now substitute the right hand sides of eqs (A6), (A7) and (A8) in the respective terms of eq. (A9), and recall that

$$(A10) \quad \frac{d\sigma_B}{dt} = -FJ_B; \quad \frac{d\sigma_i}{dt} = F(J' - J''); \quad p + q + r + s = 1,$$

we find that the three quantities in eq. (A9) are all equal (as they should be because of the continuity of the electric current) and are given by eq. (9), namely

$$(A11) \quad \frac{I}{F} = (p + q)J' + (r + s)J'' + (r_B + s_B)J_B.$$

An explicit expression for the current density, I , requires thus the derivation of explicit equations for the fluxes, J' , J'' and J_B . The procedure we have used can be summarized in the following way: Eliminating N_e from eqs (1), (2) and (3), we obtain a system of differential equations of the type:

$$(A12) \quad \frac{dN}{dt} = aN + bN_B + G$$

$$(A13) \quad \frac{dN_B}{dt} = cN + dN_B + H$$

where

$$(A14) \quad \begin{aligned} -a &= \bar{\mu}'C^e + \bar{\mu}''C^i + \bar{v}' + \bar{v}''; & -b &= \bar{\mu}'C^e + \bar{\mu}''C^i; & G &= -bN^T; \\ -c &= \bar{\mu}_B C_B; & -d &= \bar{\mu}_B C_B + \bar{v}_B; & H &= -cN^T. \end{aligned}$$

Note that the system (A12) and (A13) is linear only if the terms defined in (A14) are independent of N and N_B . Rigorously speaking, this is not the case, because the coefficients defined in (A14) depend on potential differences between barriers and wells, and such potential differences are, on account of eqs (A6), (A7) and (A8), functions of N and N_B . However, if we make the reasonable assumption that the first term on the right-hand side of eqs (A6), (A7) and (A8), $-\varepsilon V/d$, is much greater than the others, we can then assume that the quantities in the definitions (A14) are practically determined by the voltage step, V , and are thus constant during the pulse. In such case, the system of eqs (A12) and (A13) can be integrated with standard methods. Although the exact solution can be obtained in an analytical form, the expressions are rather unwieldy and, at any rate (as we discussed in the text), we are mainly interested in the approximation corresponding to the case in which the kinetics of the blocking particle is much slower than that of the permeant ion. In terms of the quantities defined in eq. (A14), this hypothesis amounts to postulating the following inequalities:

$$(A15) \quad |c| \text{ and } |d| \ll \text{both } |a| \text{ and } |b|,$$

from which it follows that

$$(A16) \quad H \ll G.$$

In order to illustrate briefly how the approximated equations were derived from the general solution, we shall consider in detail here only the case of the time constants, τ_1 and τ_2 : the exact integration of eqs (A12) and (A13) yields expressions that are proportional to the exponentials e^{-t/τ_1} and e^{-t/τ_2} , where

$$(A17) \quad -\frac{1}{\tau_{1(2)}} = \frac{a+d}{2} \left\{ 1 (\mp) \sqrt{1 - 4 \frac{ad-bc}{(a+d)^2}} \right\}.$$

As a result of the conditions (A15), it is easy to realize that the second term in the square root is much smaller than unity, so that, simplifying the square root according to the formula, $\sqrt{1+x} \simeq 1 + x/2$, eq. (A17) gives for τ_1

$$(A18) \quad -\frac{1}{\tau_1} \simeq \frac{ad - bc}{a(1 + d/a)},$$

or, neglecting terms of the order of d/a on account of the assumptions (A15),

$$(A19) \quad -\frac{1}{\tau_1} \simeq d - \frac{bc}{a}.$$

Using the same order of approximation, we find for τ_2

$$(A20) \quad -\frac{1}{\tau_2} \simeq a\left(1 + \frac{d}{a}\right) \simeq a.$$

(Recalling (A14), it is easy to see that eq. (A19) coincides with eq. (15) of the text.) Little consideration is required to realize, from the conditions (A15) and the definitions (A14), that

$$\tau_1 \gg \tau_2$$

and that τ_2 is determined by the very fast kinetics of the permeant ion. Neglecting thus the terms proportional to e^{-t/τ_2} , and approximating all the terms of the exact solution of the system (A12) and (A13) according to the same criterion as that used to deduce eqs (A19) and (A20) from eq. (A17), one finds

$$(A21) \quad N(t) = \frac{b}{a} \left\{ N_B(\infty) - N_B(0) \right\} e^{-t/\tau_1} + \frac{bH - dG}{ad - bc}$$

and

$$(A22) \quad N_B(t) = \left\{ N_B(0) + \frac{aH - cG}{ad - bc} \right\} e^{-t/\tau_1} + \frac{cG - aH}{ad - bc}.$$

Recalling that

$$(A23) \quad N_c(t) = N^T - N(t) - N_B(t),$$

the explicit equation for $N_c(t)$ can be obtained by substituting (A21) and (A22) into (A23). Finally, substituting (A21), (A22) and (A23) into eqs (6), (7), (8) and (9), one finds the explicit expressions given in the text [see eqs (11) to (17)]. Note, however, that eq. (14) contains the additional approximation, whereby the transient flux of the blocking cation, J_B , is considered negligibly small compared to J' and J'' , and is thus omitted from the total current, eq. (9).