BRIEF COMMUNICATION

A MODEL FOR THE EFFECTS OF POTENTIAL AND EXTERNAL K^+ CONCENTRATION ON THE Cs^+ BLOCKING OF INWARD RECTIFICATION

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ABSTRACT Inward rectification in starfish egg cell membranes is blocked by external Cs^+, the degree of blocking being an increasing function of hyperpolarizing potentials and of the external concentration of K^+. While the effect of K^+ and the particular functional dependence of blocking on potential are difficult to reconcile with a one-ion pore model, both features can be accounted for assuming that the pore has at least two sites and that a considerable fraction of the blocked pores is simultaneously occupied by a Cs^+ ion in the inner site and by a K^+ in the outer one.

The inward K^+ current of "anomalous rectification" is decreased by small concentrations of Cs^+ both in starfish egg cells (Hagiwara et al., 1976) and in skeletal muscle (Gay and Stanfield, 1977). This communication, which elaborates on preliminary work presented tersely in an abstract (Ciani et al., 1977), suggests a model for the blocking of Cs^+ in egg cells and for the manner in which such blocking is altered by the external K^+ concentration. The results will be used to fit certain data reported previously by Hagiwara et al. (1976). If I_K^{Cs} and I_K are the K^+ currents in the presence and in the absence of Cs^+, respectively, their ratio can be fitted, at the same membrane potential and the same K^+ concentration, by the empirical equation:

$$\frac{I_K^{Cs}}{I_K} = \frac{1}{1 + \frac{C_{Cs}}{\tilde{K}} e^{-\alpha\phi}}, \tag{1}$$

where C_{Cs} is the external Cs concentration, ϕ is the membrane potential (inside minus outside) in units of RT/F, α is a dimensionless constant approximately equal to 1.5 and $\tilde{K}$ is an "apparent" dissociation constant which depends on external potassium. A similar equation (with $\alpha < 1$) was experimentally inferred and theoretically deduced from a single-ion-pore model for the H^+ blocking of the Na^+-channel in nerve, the value of α being interpreted as indicative of the approximate location of the binding site for H^+ in the pore (Woodhull, 1973; cf. Eq. 5). However, in the case of egg cell membranes, two features of Eq. 1: (*a*) the value of

α greater than unity, and (*b*) the dependence of $\tilde{K}$ on the external K^+ concentration, cannot be easily reconciled with a single-ion-pore model, while they are consistent, as we shall show, with the hypothesis that a considerable fraction of the blocked channels is simultaneously occupied by one Cs^+ and one K^+ ion.

Multi-occupancy of a biological pore (>1 ion/channel) was postulated originally by Hodgkin and Keynes (1955) for the K^+-channel in nerve, and is a concept of current interest, since many data on both artificial and biological pores point to its applicability (Neher, 1975; Cahalan and Beganisich, 1976; McBride and Szabo, 1978; Neher et al., 1978). The model presented here is but a particular case of the theories for multi-occupancy developed so far (Hechmann, 1965*a*, 1965*b*, 1968, 1972; Sandblom et al., 1977; Hille and Schwarz, 1978), which, nonetheless, yields manageable equations that fit the data satisfactorily and provides an intuitive understanding of the phenomena.

The Model

Using Eyring's formalism, the pore is viewed as a sequence of free energy wells and barriers, each well (also referred to as a "site") being able to accommodate only one ion at a time. Since the simultaneous presence of two ions in the same pore is allowed for, the simplest model that is compatible with this feature, and thus the one considered here, has two sites and three barriers (see Fig. 1 *a*). The Eyring rate theory is used to describe the ion movements, and the fluxes are calculated in terms of rates of transition between different states of occupancy of the pore. As usual, direct transitions between two states are allowed only when differing by only one ionic jump, so that transitions involving the simultaneous movement of two ions are neglected. The following notation is used for the voltage-independent part of the rate constants: λ, μ, and υ denote rate constants for crossing the external, middle, and internal barriers, respectively (see also Fig. 1 *a*). The subscripts K and Cs specify the ion to which they refer, and the indices ′ and ″ denote inward and outward movement, respectively. As the rate constants for entering and leaving the channel (λ', υ'' and λ'', υ') will generally depend on

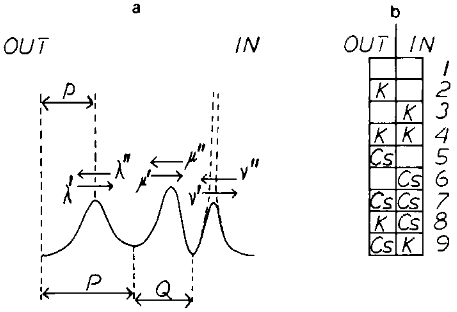

FIGURE 1 (*a*) Generalized potential profile for a two-site, three-barrier model of an ion-permeable pore. The "infinitely" high barrier indicated by dashed lines refers to the impermeant Cs^+. λ, μ, and ν, are rate constants for ion permeation, defined more explicitly in the text. *p*, *P* and *Q* denote fractions of the membrane potential and coincide with the corresponding fractions of the membrane thickness in the approximation of constant field. (*b*) Diagram of the possible states of occupancy of the two-site pore in the presence of K^+ and Cs^+ ions. Eq. 3 of the text corresponds to the case in which only states 1, 6, and 8 are considered, while Eq. 5, which is sufficient to fit the data, corresponds to the even simpler one in which only states 1 and 8 are taken into account.

whether the channel is empty or contains one ion already, the superscripts K and Cs are used as follows to remove ambiguities of notation: When no superscripts are added to λ (υ), it is implied that the inner (outer) site is empty. Alternatively, when either K^+ or Cs^+ is present in the inner (outer) site, the corresponding superscript is added to λ (υ). Thus, to give an example, λ'_K denotes the rate constant for "inward" movement of potassium across the outer barrier when the inner site is empty, while λ'^{Cs}_K denotes the same quantity when the inner site contains a Cs^+ ion. On the other hand, μ' and μ'' can be used unambiguously without subscripts, since, having neglected transitions with simultaneous movements of two ions, a jump from well 1 into well 2 is possible only if 2 is empty, and vice versa. Finally, the complete, voltage-dependent rate constant for an ionic jump from a given site across a given barrier is obtained by multiplying the previously defined constants by the exponential of the normalized voltage difference between that site and the top of that barrier. Thus, the rate constant for the inward movement of K^+ across the outer barrier when the pore is empty will be $\bar{\lambda}'_K = \lambda'_K e^{-p\phi}$, where a bar is used to distinguish the total rate constants from their voltage-independent components, and p is the appropriate fraction of the membrane potential (see Fig. 1 *a*). (Naturally, the relative distances between the peaks and wells shown in Fig. 1 *a* may depend on the type of ion and the state of occupancy of the pore. However, since the data are not sufficient to sort out these details, we shall assume that these distances are independent of the ion and the pore's state.)

Although Cs^+ is virtually impermeant, since it has negligible effects on the membrane potential (e.g., Hagiwara and Takahashi, 1974), one natural inference from the strong voltage dependence of its effects on the K^+ current is that Cs^+ ions can nevertheless move a long way into the pore, say up to the inner site, but are unable to cross the innermost barrier, thereby blocking the flux of potassium (or other permeant ions). Thus, if both K^+ and Cs^+ ions can occupy either site of the pore schematized in Fig. 1 *a*, the pore admits of nine states of occupancy, as shown in Fig. 1 *b*, and the same number of equations is generally required to evaluate the fluxes. Although the impermeability to Cs^+ (viewed here as the inability of this ion to overcome the inner barrier) simplifies the problem somewhat, the result obtained considering all the nine states is too complicated to be testable. Consequently, certain approximations will be made. These, and our rationale for their justification, can be summarized as follows: (*a*) Since a significant occupancy of the outer site by Cs^+ is not compatible with either the steep voltage dependence of the blocking, or with the enhancement of such blocking for increasing external K^+ concentrations, it will be assumed that all the states with Cs^+ in the outer site only (states 5 and 9 of Fig. 1 *b*) can be neglected compared to those with Cs^+ in the inner one (states 6, 7, and 8 of Fig. 1 *b*). Moreover, since the Cs^+ concentration in the external medium is always much smaller than that of K^+, and considering that the "blocking" effect is a function of the first power of the Cs^+ concentration, we shall also neglect the state with double occupancy by Cs^+ (state 7 of Fig. 1 *b*) with respect to that with Cs^+ in the inner site and K^+ in the outer one (state 8 of Fig. 1 *b*). (*b*) Compatible with the finding that in the absence of Cs^+, the conductance increases proportionally to the geometric mean of the external and internal K^+ concentrations (Hagiwara and Yoshii, 1979), there being no evidence of either saturation or blocking, it will be assumed that pores containing K^+ as the sole ionic species (states 2, 3, and 4 of Fig. 1 *b*) are statistically negligible compared to those that are either empty or contain Cs^+ in the inner site only (states 1, 6, and 8

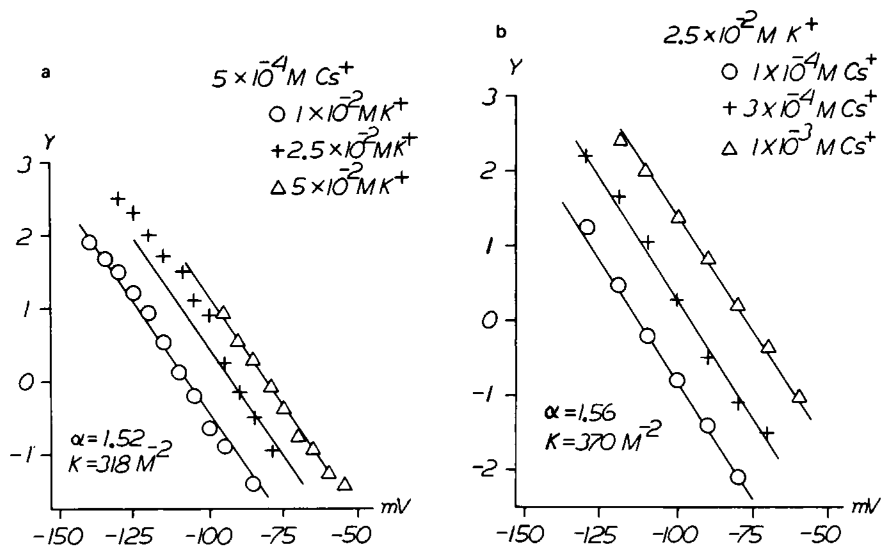

FIGURE 2 Fit with Eq. 6 to data from Hagiwara et al. (1976), using the least squares method for the determination of α and K. (*a*) The three sets of data refer to different external K^+ concentrations, and the continuous straight lines are drawn according to Eq. 6, using the indicated values of α and K. (Because of the irregular behavior of the data at $C_K = 2.5 \times 10^{-2}$ M, only the data of the other two sets have been used in the fitting procedure.) (*b*) The three sets of data, which refer to a different cell than that of *a*, correspond now to $C_K = 2.5 \times 10^{-2}$ M and the different Cs^+ concentrations indicated.

of Fig. 1 *b*). This simplification is made because it is consistent with the data of Fig. 2 and because it is expedient (e.g., at least six additional parameters would be required just to include states 2 and 3 of Fig. 1 *b*). However, we cannot prove that no pores contain K^+ as the sole ionic species; for example Hille and Schwarz (1978; see their Fig. 3) have shown that the conductance vs. symmetrical K^+ concentration curve can be linear for values of K^+ occupancy up to ~ 40% if one choses the heights of the energy barriers and wells inside the pore appropriately.

As a result of assumptions *a* and *b*, states 1, 6, and 8 are in fact the only ones considered to be significantly populated. Using these simplifications, the ratio of the K^+ flux in the presence of Cs^+ to that in the absence of Cs^+ is found to be:

$$\frac{I_K^{Cs}}{I_K} = \frac{1}{1 + \Omega C_{Cs}}, \tag{2}$$

where

$$\Omega = K_0^{Cs}[e^{-(P+Q)\phi} + K_K^{Cs} C_K e^{-(2P+Q)\phi}], \tag{3}$$

C_K is the external K^+ concentration, P and $P + Q$ are the "electrical distances" from the external interface to the outer and the inner sites, respectively (see also Fig. 1 *a*), and K_0^{Cs} and K_K^{Cs}, expressed in terms of the previously defined rate constants, are given by:

$$K_0^{Cs} = \frac{\lambda'_{Cs}\mu'_{Cs}}{\lambda''_{Cs}\mu''_{Cs}};\ K_K^{Cs} = \lambda_K^{\prime Cs}/\lambda_K^{\prime\prime Cs}. \tag{4}$$

Three features of Eqs. 2 and 3 are worth noticing: (*a*) The blocking of the K^+ current by Cs^+ increases with the external concentration of Cs^+, as in the empirical Eq. 1. (*b*) The coefficient of ϕ in the last exponential of Eq. 3 will be greater than unity for a variety of positions of the two sites, P and $P + Q$, although each is necessarily smaller than unity in the approximation of constant field. For example, if the inner site were sufficiently close to the internal medium that $P + Q \simeq 0.9$, and the outer one were near the middle of the pore (e.g., $P \simeq 0.6$), $2P + Q$ would be 1.5, a value very close to that used to fit the data of Fig. 2. (*c*) Finally, Eq. 3 shows that, by increasing the concentration of external K^+, Ω increases too, thereby enhancing the blocking effect of Cs^+, in agreement with the data of Fig. 2 *a*.

Although the expression obtained inserting Eq. 3 into Eq. 2 is certainly adequate to fit the available data on starfish egg cells, an even simpler version of it was found to be sufficient. Namely, Eq. 3 can be reduced to:

$$\Omega = K_0^{Cs} K_K^{Cs} C_K e^{-(2P+Q)\phi}, \tag{5}$$

which corresponds to the limiting physical case in which the fraction of channels occupied solely by Cs^+ is small compared to that of those occupied by both Cs^+ and K^+. Inserting Eq. 5 into Eq. 2 and rearranging, one finds:

$$y = -\alpha\phi + ln\,[K C_K C_{Cs}], \tag{6}$$

where

$$y = ln\,[(I_K/I_K^{Cs}) - 1];\ \alpha = 2P + Q;\ K = K_0^{Cs} K_K^{Cs}. \tag{7}$$

Fig. 2*a* and *b* shows a fit of data taken from Hagiwara et al. (1976), with Eq. 6, the least squares method being used to evaluate α and K. The three sets of data in Fig. 2 *a* correspond to equal Cs^+ and different K^+ concentrations, while Fig. 2 *b*, which refers to a different cell, corresponds to the reciprocal situation of constant K^+ and different Cs^+. It can be seen that the fit is reasonable and that the values deduced for α and K are remarkably close in the two different cells of Fig. 2 *a* and *b*, those of α differing by <10% and those of K by ~20%.

In the above considerations we have shown that various features of the phenomena, some of which are intuitively understandable, such as the increase of blocking for increasing Cs^+ concentrations, and others less so, such as the effects of potential and of external K^+, can nevertheless all be accounted for by assuming double occupancy of the pore. It should be emphasized, however, that whereas the enhancement of blocking by increasing external Cs^+ is a general consequence of the assumption that the impermeant Cs^+ binds to a site inside the channel, the similar effect for increasing concentrations of the permeant K^+, predicted by Eq. 3 and 5, depends more strictly on assumptions *a* and *b* regarding the relative population of the different states of occupancy. Different behaviors for increasing K^+ concentrations, such as either a decrease of the blocking or an initial increase followed by a maximum and subsequent decrease, would certainly be obtained if different assumptions were made (e.g., if the population of the states doubly occupied by K^+ were assumed to be comparable to that of the states with mixed ionic species).

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REFERENCES

CAHALAN, M., and T. BEGENISICH. 1976. Sodium channel selectivity. Dependence on internal permeant ion concentration. *J. Gen. Physiol.* **68:**111–125.

CIANI, S., S. HAGIWARA, and S. MIYAZAKI. 1977. A model for Cs^+ blocking of the K^+ conductance in anomalous rectification. *Biophys. J.* **17:**46 *a.* (Abstr.).

GAY L., and P. R. STANFIELD. 1977. Cs^+ causes a voltage-dependent block of inward K^+ currents in resting skeletal muscle fibers. *Nature (Lond.).* **267:**169–170.

HAGIWARA, S., S. MIYAZAKI, and P. ROSENTHAL. 1976. Potassium current and the effect of cesium on this current during anomalous rectification of the egg cell membrane of a starfish. *J. Gen. Physiol.* **67:**621–638.

HAGIWARA, S., and K. TAKAHASHI 1974. The anomalous rectification and cation selectivity of the membrane of a starfish egg cell. *J. Membr. Biol.* **18:**61–80.

HAGIWARA, S., and M. YOSHII. 1979. Effects of internal potassium and sodium on the anomalous rectification of the starfish egg as examined by internal perfusion. *J. Physiol.* **292:**251–265.

HECKMANN, K. 1965*a*. Zur Theorie der "single file" Diffusion. I. *Z. Phys. Chem.* **44:**184–203.

HECKMANN, K. 1965*b*. Zur Theorie der "single file" Diffusion. II. *Z. Phys. Chem.* **46:**1–25.

HECKMANN, K. 1968. Zur Theorie der "single file" Diffusion. III. Sigmoide Konzentrationsabhängigkeit unidirectionaler Flüsse bei "single file" Diffusion. *Z. Phys. Chem.* **58:**206–219.

HECKMANN, K. 1972. Single file diffusion. *Biomembranes.* **3:**127–153.

HILLE, B. 1975. Ionic selectivity, saturation and block in sodium channels. A four barrier model. *J. Gen. Physiol.* **66:**535-560.

HILLE, B., and W. SCHWARZ 1978. Potassium channels as multi-ion single file pores. *J. Gen. Physiol.* **72:**409-442.

HODGKIN, A. L., and R. D. KEYNES. 1955. The potassium permeability of a giant nerve fiber. *J. Physiol.* (*Lond.*). **116:**61–88.

LÄUGER, P. 1973. Ion transport through pores: a rate theory analysis. *Biochim. Biophys. Acta.* **311:**423–441.

MCBRIDE, D., and G. SZABO. 1978. Blocking of gramicidin channel conductance by Ag^+. *Biophys. J.* **21:**25 *a.* (Abstr.).

NEHER, E. 1975. Ionic specificity of the gramicidin channel and the thallous ion. *Biochim. Biophys. Acta.* **401:**540–544.

NEHER, E., J. SANDBLOM and G. EISENMAN. 1978. Ionic selectivity, saturation, and block in gramicidin A channels. *J. Membr. Biol.* **40:**97–116.

SANDBLOM, J., G. EISENMAN and E. NEHER. 1977. Ionic selectivity, saturation, and block in gramicidin A channels. I. Theory for the electrical properties of ion selective channels having two pairs of binding sites and multiple conductance states. *J. Membr. Biol.* **31:**383–417.

WOODHULL, A. 1973. Ionic blockage of sodium channels in nerve. *J. Gen. Physiol.* **61:**687–708.