

A NEW INTERPRETATION OF THE DYNAMIC CHANGES OF THE POTASSIUM CONDUCTANCE IN THE SQUID GIANT AXON

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ABSTRACT The solutions, $n(t)$, of the differential equation $dn/dt = \alpha (1 - n) n (4 - 6n + 4n^2 - n^3) - \beta n^3 (4 - 6n + 4n^2 - n^3)$ in which α and β are instantaneous functions of membrane potential, are shown to fit with good accuracy the time courses of the rise of potassium conductance during depolarizing steps in clamp potential, found experimentally by Hodgkin and Huxley and by Cole and Moore. The equation is derived by analysing the dynamic behaviour of a system consisting of a square array of interacting pores. The possible role of Ca^{++} ions in this system is discussed.

INTRODUCTION

Johnson and Tille (unpublished work) have used an empirically derived differential equation to describe the rise of the potassium conductance, g_k , in the squid giant axon, under voltage clamp conditions. In this equation,

$$\frac{dn}{dt} = kn(\omega - n)^2 - \beta n \quad (1)$$

k is a constant independent of membrane potential, β and ω are instantaneous functions of membrane potential, and n is related to g_k by

$$g_k = \bar{g}_k n \quad (2)$$

in which \bar{g}_k is a constant. Solutions of equation (1) fit with good accuracy the experimental results both of Hodgkin and Huxley (1952) and of Cole and Moore (1960). A tentative suggestion was made by Johnson and Tille that equation (1) is consistent with a system of dipoles, each of which has a conducting and a non-conducting orientation, but in which a dipole cannot change its orientation unless situated next to a dipole with the *conducting* orientation.

In the present paper an analysis of the dynamic behaviour of such a system is

carried out and this behaviour is shown to agree with the experimental results. The differential equation derived in this paper avoids some of the difficulties of interpretation that are inherent in the Cole and Moore modification of the Hodgkin and Huxley equations and in the system proposed by Hoyt (1963).

METHODS

Equations (8) and (12) were solved on a Donner 3200 analog computer. The multiplications were performed on 28 segment "quarter-square" multipliers. Three multipliers were used for equation (8) and four for equation (12).

Accuracy of the Initial Values. The use of the multipliers imposes a limit to the accuracy of the computation, especially at very low values of the variable. For these reasons the initial value of n given in the legend in Fig. 4 cannot be regarded as very reliable. An accurate value could be best obtained on a digital computer.

Accuracy of the Rate Constants. The use of the multipliers also limits the accuracy close to the equilibrium values of the variable and, because of this, the values of the rate constants could be inaccurate by about 3 per cent. However this is not very significant when compared with the accuracy of the experimental results. The accuracy of the rate constants is also limited by the inability to obtain a unique best fit on an analog computer. For this reason one could adopt the procedure of fitting the experimental results in a way which would make the rate constants vary smoothly with membrane potential. However the results of such a procedure are misleading in that they would appear more accurate than they really are. For this reason I used the less subjective method of simply obtaining a good fit to the experimental points.

RESULTS

Consider a system consisting of a membrane containing a number of conducting pores. Each pore can exist in two states, open and closed. The transition from one state to the other could be accomplished, for example, by the reorientation of an associated electric dipole, or by the movement of a charged blocking particle.

In particular, consider the following system:

- (a) Let the membrane contain N pores per unit area, each pore capable of being blocked by some particle.
- (b) A blocking particle cannot enter the membrane *unless* two vacant adjacent pores are available.
- (c) A blocking particle can leave the membrane *only* from a pore situated next to an open pore.
- (d) A blocking particle can migrate from a blocked pore to an adjacent open pore without leaving the membrane.

In other words, a pore can change from one state to the other only if it is situated next to an open pore.

Let there be n' open pores per unit area at any one time. If, to begin with, the pores are assumed to be distributed over the membrane in a hexagonal array, each pore will have three nearest neighbours. Condition (d) should ensure that the open

and closed pores are randomly distributed. The probability that a given pore will have at least one open pore for a neighbour is given by

$$p_3 = \frac{3n'}{N} - \frac{3(n')^2}{N^2} + \frac{(n')^3}{N^3} \quad (3)$$

in which the subscript is used to signify three nearest neighbours.

Let n be the probability that any one pore is open; *i.e.*,

$$n = \frac{n'}{N} \quad (4)$$

Equation (3) can then be rewritten as

$$p_3 = n(3 - 3n + n^2) \quad (5)$$

The probability that a certain pore is both closed and situated next to an open pore is given by

$$p_3' = (1 - n)n(3 - 3n + n^2) \quad (6)$$

and the probability that a pore is both open and situated next to an open pore is given by

$$p_3'' = n^2(3 - 3n + n^2) \quad (7)$$

If the rates of arrival and departure of the blocking particles are assumed to depend on the membrane potential, the rate of opening of the pores is given by

$$\frac{dn}{dt} = \alpha(V)(1 - n)n(3 - 3n + n^2) - \beta(V)n^2(3 - 3n + n^2) \quad (8)$$

Equation (8) can be rewritten as

$$\frac{dn}{dt} = An(B - n)^2 - Cn - Dn^4 \quad (9)$$

With $A = 4\alpha + 3\beta$, $B = (6\alpha + 3\beta)/(8\alpha + 6\beta)$, $C = (6\alpha + 3\beta)^2/(16\alpha + 12\beta) - 3\alpha$ and $D = \alpha + \beta$ and in this form can be seen to resemble the empirically derived equation (1).

Fig. 1 shows four solutions of equation (8) fitted to four of the experimental results of Hodgkin and Huxley (1952). Although the fit is good over the greater part of each curve, it was found that the initial positive curvature of the computed curves was too small and the fit for low values of n (≤ 0.05) was poor.

A better ratio of the positive and negative curvatures for each curve was obtained by taking the case of a square array of pores, each pore having now four nearest neighbours. The analogs of equations (6) and (7) are now, respectively

$$p_4' = (1 - n)n(4 - 6n + 4n^2 - n^3) \quad (10)$$

and

$$p_4'' = n^2(4 - 6n + 4n^2 - n^3) \quad (11)$$

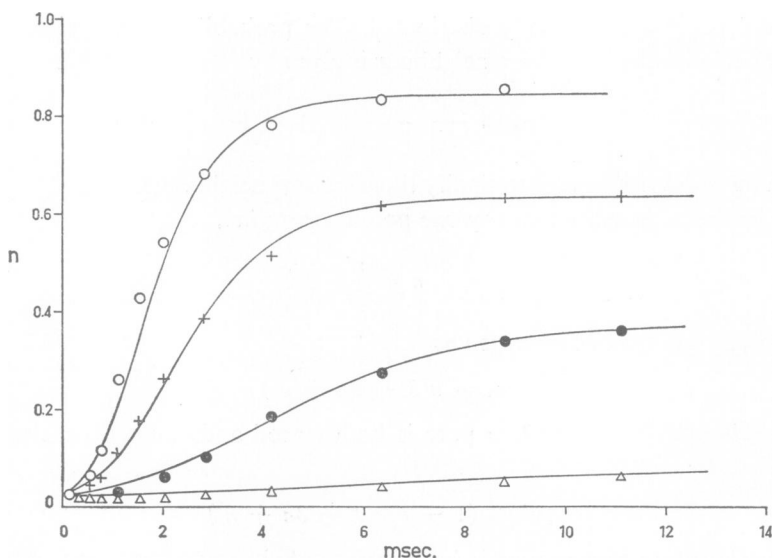


FIGURE 1 Experimental points, replotted from Fig. 3 of Hodgkin and Huxley (1952), showing the rise in g_k during various depolarizing steps in membrane potential, fitted with solutions of equation (8) (solid lines) with $g_k = \bar{g}_k n$ in which $\bar{g}_k = 24 \text{ mmho cm}^{-2}$. The value of n at $t = 0$; i.e., at the resting potential is 2.6×10^{-3} . The magnitudes of the depolarizing steps are (in mv): \circ , 100; $+$, 63; \bullet , 32; \triangle , 10.

The differential equation governing the rate of opening of the pores becomes

$$\frac{dn}{dt} = \alpha(V)(1 - n)n(4 - 6n + 4n^2 - n^3) - \beta(V)n^2(4 - 6n + 4n^2 - n^3) \quad (12)$$

The solutions of equation (12) fitted the experimental results of Hodgkin and Huxley (1952) with good accuracy over the whole range of n . This is shown in Fig. 2. The rate constants α and β used in Fig. 2 are plotted against membrane potential in Fig. 3. Fig. 4 shows a solution of equation (12) fitted to the experimental results of Cole and Moore (1960, Fig. 5). Fig. 5 demonstrates the relative rates of rise and of fall of g_k during two subsequent steps in potential (*cf.* Fig. 2 of Hodgkin and Huxley, 1952).

DISCUSSION

All of the physical mechanisms which have been suggested in the literature as compatible with the behaviour of g_k under voltage clamp can be considered as variants of the following, more general system. In this system a change in the membrane potential causes an instantaneous change in a set of rate constants which determine the equilibrium value of some unknown quantity (X). Such a change is equivalent to shifting the equilibrium value of X , whereupon X approaches its new

equilibrium value in an *exponential* way. The fact that the rise of g_k is not exponential but S-shaped is explained by making g_k proportional, exactly or approximately, to some positive power (>1) of X .

The original results of Hodgkin and Huxley (1952) could be fitted by making g_k proportional to the 4th power of a hypothetical quantity, n , which, during voltage clamp, was assumed to rise or fall exponentially. One possible interpretation of the 4th power is that four identical events, each with a probability n , must occur at the same site to open a pore in the membrane. Since then, however, Frankenhaeuser and Hodgkin (1957) have shown that the slow initial part of the S-shaped rise in g_k can be prolonged by applying a hyperpolarizing step in membrane potential before the depolarizing step. Cole and Moore (1960) have shown, by extending the range of experimental observations, that g_k would have to be proportional to the 25th, and possibly higher, power of n if an exponential rise in n is assumed. This new power of n appears too high to be interpreted in the same way as the 4th power. Hoyt (1963) used the different approach of actually deriving the best functional relationship between the hypothetical quantity, v_k , and g_k rather than using a simple power relationship. Again, v_k is assumed to rise and fall exponentially during voltage clamp. However, Hoyt's subsequent analysis shows that the results of Cole and Moore (1960) require that no less than nine events must be associated with a single pore to open it to the passage of potassium ions.

It is difficult to reconcile this high number of identical events required for the opening of each single pore with the assumed exponential changes in the probability of the single events. An exponential rise and fall in the probability of a single event implies that the events are independent; *i.e.*, that they have no energy of interaction. At the same time all the events associated with a single pore must be well localized if any single one of them can interfere with the passage of the same potassium ion. Thus it is hard to conceive of a situation in which nine or more events can occur close enough for each to be capable of stopping the same potassium ion and yet far enough not to interfere with one another. To take a specific example, if nine dipoles were situated symmetrically on the circumference of a round pore the interdipole distance would be only one-third of the diameter of the pore. The difficulty which arises when the nine events are assumed to be located in one line through the membrane is discussed in Hoyt's paper.

The physical system which was used in deriving equation (12) is different from the above in two ways. First, only one event is required to block or open a single pore. Second, the events at neighbouring pores are not independent; each pore behaves as if a high potential barrier prevented any changes in its configuration while that particular pore is surrounded by four blocked pores. It can be seen from the figures that such a system, with empirically chosen rate constants, accounts well for the experimental results.

If the real mechanism causing the observed changes in g_k were indeed of the kind

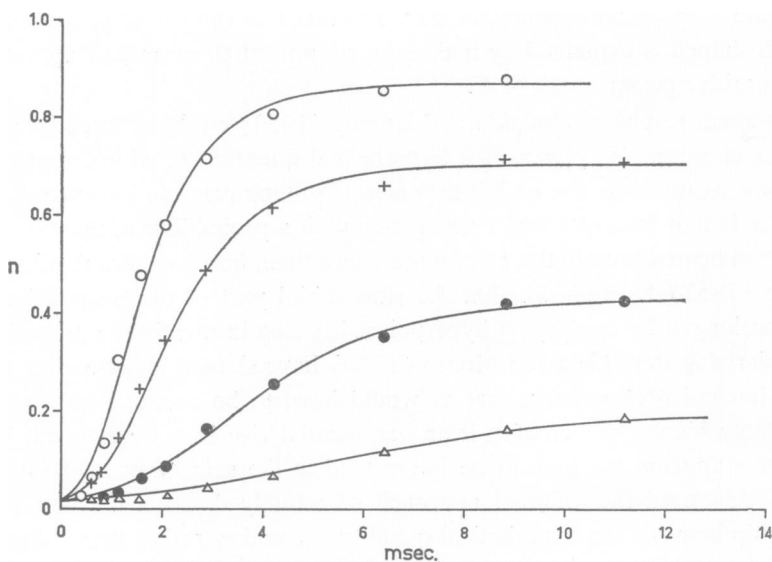


FIGURE 2a

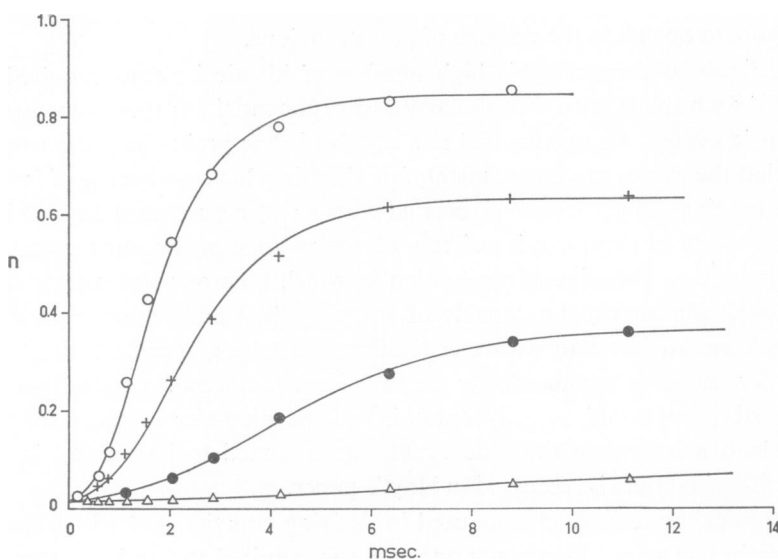


FIGURE 2b

FIGURES 2a, 2b, and 2c. Experimental points, replotted from Fig. 3 of Hodgkin and Huxley (1952) as in Fig. 1, fitted with solutions of equation (12) (solid lines). $\bar{g}_K = 24 \text{ mmho cm}^{-2}$, $n_\infty = 1.7 \times 10^{-2}$. The magnitudes of the depolarizing steps are (in mv): (a) \circ , 109; $+$, 76; \bullet , 38; \triangle , 19. (b) \circ , 100; $+$, 63; \bullet , 32; \triangle , 10. (c) \circ , 88; $+$, 51; \bullet , 26; \triangle , 6. The values of α and β which were used are plotted against membrane potential in Fig. 3.

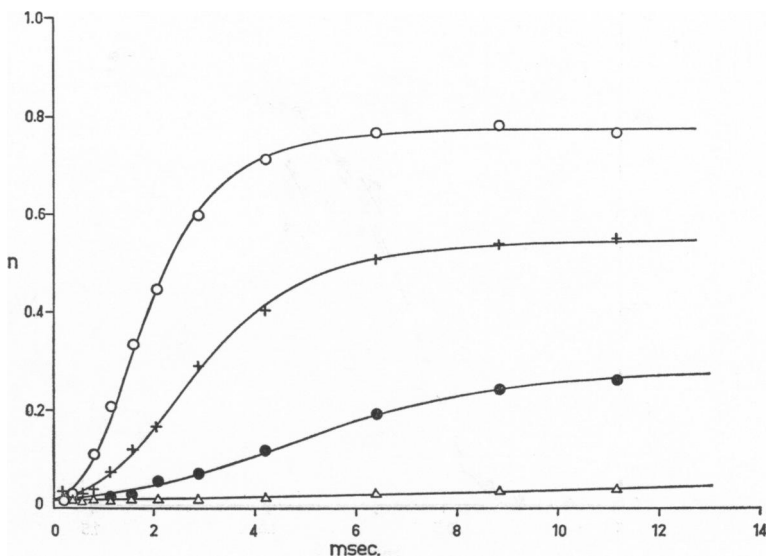


FIGURE 2c

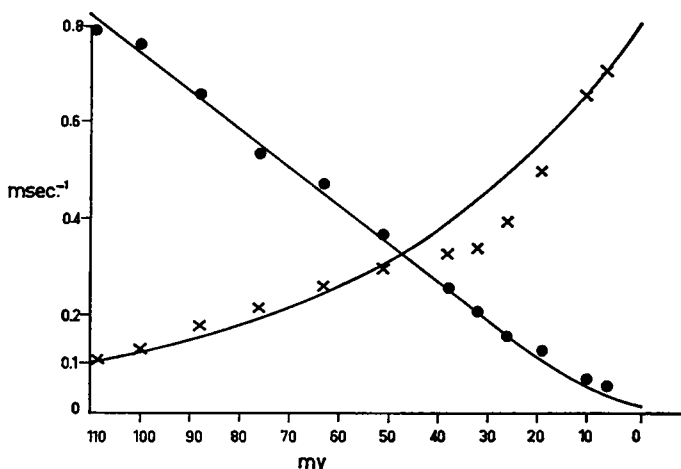


FIGURE 3 The values of α , (\bullet), and β , (\times), used in fitting the points in Figs. 2a, 2b, and 2c, plotted against membrane potential. The scale on the abscissa gives the depolarization of the fibre from the resting potential. The solid lines are given by $\alpha = 0.0079 (V - 5.9) / [1 - \exp (5.9 - V) / 4.0] \text{ msec}^{-1}$ and $\beta = 0.79 \exp (-V/54) \text{ msec}^{-1}$, in which V is the depolarization of the membrane from the resting potential (in mv).

postulated in this paper, then the relationship between the rate constants α and β , and the membrane potential should give some indication of the nature of the changes occurring in the membrane. It is interesting that within the available limits of ac-

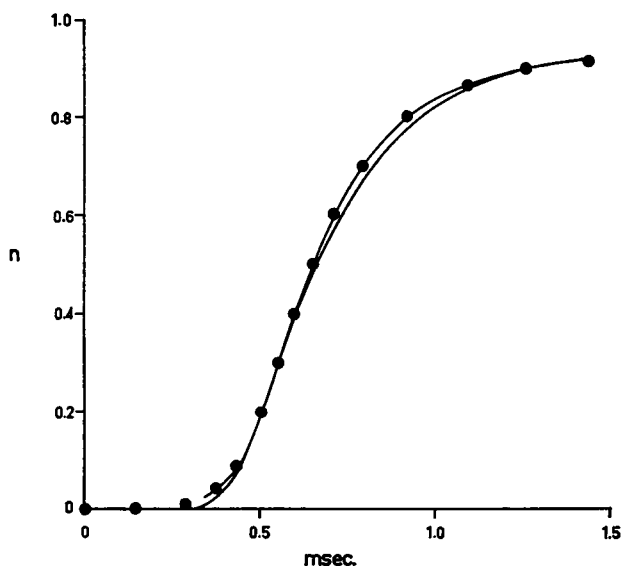


FIGURE 4 The experimental results of Fig. 5b of Cole and Moore (1960) (solid lines) in which the potassium currents obtained near the sodium potential, V_{Na} , after 3 msec. hyperpolarization at various potentials are superimposed by changes of origin of the time scale. The solid circles give a solution of equation (12) with $\alpha = 4.13 \text{ msec}^{-1}$, $\beta = 0.32 \text{ msec}^{-1}$, $n_0 = 8 \times 10^{-5}$, and $I_k = 8.27 \text{ n ma cm}^{-2}$. $\bar{g}_k = 8.27 \text{ ma cm}^{-2}/(V_{Na} - V_k) \approx 55 \text{ mmho cm}^{-2}$.

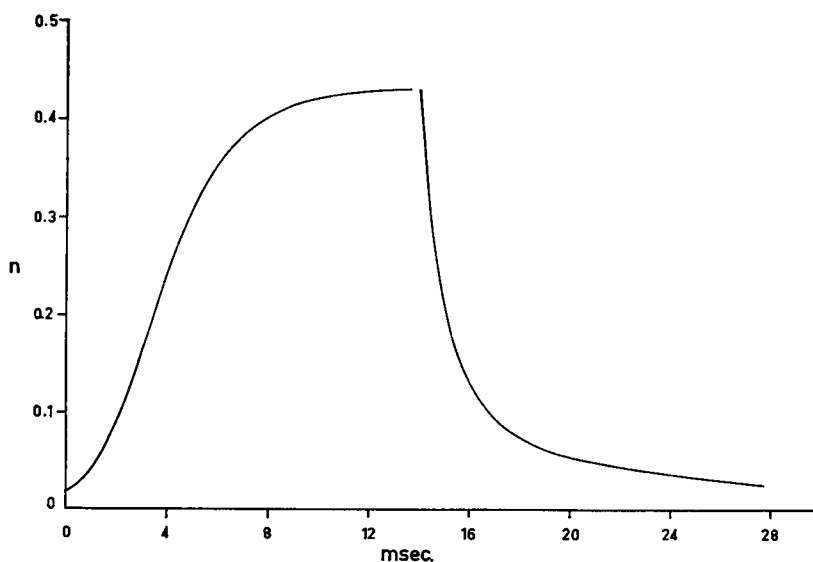


FIGURE 5 The rise and fall of n on depolarization and repolarization back to the resting potential. The solid lines are solutions of equation (12). For the rise $\alpha = 0.253 \text{ msec}^{-1}$ and $\beta = 0.325 \text{ msec}^{-1}$ and the fall $\alpha = 0.02 \text{ msec}^{-1}$ and $\beta = 1.0 \text{ msec}^{-1}$.

curacy the voltage dependence of α and β (see legend to Fig. 3) can be fitted by expressions of the same form as those used by Hodgkin and Huxley to fit the voltage dependence of their rate constants α_n and β_n . The main difference is that unlike α_n and β_n , α and β vary over approximately the same range of values within the experimentally observed range of membrane potential.

Frankenhaeuser and Hodgkin (1957) found that the effect on g_K of a fivefold reduction in the outside calcium concentration was roughly equivalent to a depolarization of 10 to 15 mv. On the basis of this they suggested the possibility that Ca^{++} ions may block selectively permeable pores in the membrane. However, the effect of changing the Ca^{++} concentration was only about one-half of the minimum that could be expected if the Ca^{++} ions were simply displaced by the field in the membrane.

A similar difficulty arises in the present system if Ca^{++} ions are assumed to be the blocking particles. The simple exponential form of the $\beta(V)$ curve might suggest that the process of blocking a pore is one of overcoming a simple potential barrier which is linearly related to the membrane potential. If this were so, then β , the rate constant of blocking of the pores, should be proportional to the Ca^{++} concentration. In this case a fivefold decrease in the Ca^{++} ion concentration should be roughly equivalent to a depolarization of about 30 mv. Thus if Ca^{++} ions were the blocking particles some kind of transport mechanism for these would have to be postulated.

The form of the $\alpha(V)$ curve is more complex than that of $\beta(V)$ and this would indicate that the opening of a pore is accomplished by a more complicated process than its closing. Little would be gained by speculating on the nature of this process before the $\alpha(V)$ relationship can be determined with more accuracy.

Finally, there is no justification for extending the functional relationships derived for α and β beyond the range of experimental observations. In particular, it is unlikely that β would continue to increase in the same way with increasing membrane potential. The reason for this is as follows: If the rate given by β does represent the rate of overcoming a potential barrier which is a linear function of the membrane potential, then the exponential relationship between β and V should hold only up to the potential at which the barrier disappears.

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