Currents, Models and Definitions of Membrane Potassium Channels

Ionic channels in excitable membranes have many similarities, including homologous-subunit structures, potential thresholds for activation, and selectivities for certain ionic species. We usually think of the phenotype of a channel as its function, best described by an accurate model.

Outward currents in molluscan and other types of neurons differ mainly in the range of membrane potentials over which activation and inactivation occur, and sensitivity to blocking agents such as 4-aminopyridine and tetraethylammonium. At present, separate molecular channels are assumed to exist in the membrane for each type of potassium current. This paper indicates that transient "A-currents" and delayed "K-currents" in mollusc neurons are predicted by a single-channel kinetic model having two open states coupled via two closed states. The probabilities of occupation of particular states are set by the holding potential, and the rate constants are determined by the test potentials. Thus, no difference in phenotype is required to predict both the early and late outward currents. The implications for channel definition and evolution are considered.

Key Words: *potassium, channels, A-current, K-current, model, Aplysia*

INTRODUCTION

Recent developments in biochemical and molecular neurobiology suggest that membrane ionic channels have more similarities than differences: In eukaryotic cells, at least, all known channels act as catalysts, facilitating the flow of available ion species down their

Comments Theoretical Biology
1990, Vol. 2, No. 1, pp. 45–55
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concentration gradients under appropriate conditions of voltage or ligand concentrations.[1] Channels which have been studied at the molecular level appear to have the same plan, built from four or five homologous subunits around an aqueous pore.[2-4] Chemical alteration of even a single amino acid in a subunit may affect the channel conductance.[5,6]

So-called "Na," "K," or "Ca" channels are distinguished primarily by their ionic selectivities, and many channel types share the property of sharp activation once a certain level of potential is reached, followed by slower or very slow inactivation. The A- or transient and K- or delayed outward-current channels have the same high selectivity for potassium over sodium ions. These channels are usually distinguished by (1) the range of potentials in which they activate and inactivate, and (2) sensitivities to blocking agents such as 4-aminopyridine (4-AP) which blocks A-currents and tetraethylammonium (TEA) which reduces both A- and K-currents.[7,8] In conceiving of a channel, then, we tend to use the functional, as opposed to the structural definition, where the functional is more abstract and model-based. We regard the channel phenotype as its function, which is often best described by the parameters of an accurate and hopefully appropriate model (such as peak conductances and time- or rate-constants).

With regard to questions of whether two different types of current such as A- and K-currents may be carried by the same, similar, or different types of channels, we are again brought back to the problem of definition: If two channels have the same amino acid sequence and the same chemical structure in detail, but operate in different environments (say, a different local electrical potential or a different local concentration of a "co-factor" like Ca^{++} because of other accessory proteins), most would say the channels are "the same." If two channels have the same amino acid sequence but different glycosylation, co-factors, or post-translational modifications, the channels could operate differently (e.g., producing A-currents or K-currents), but it is arguable whether they should be called different channels or not. If the two channels have different primary structure, different amino acid sequence and different genes, most would call them different channels. But even in this case the distinction is not altogether clear. The channels

might have several different subunits: only one might differ; and even then the difference might be in only a few amino acids, enough to modify a particular rate constant between states of the channel, just enough to modify the currents through the channel which are its function.

From consideration of a simple four-state, single occupancy model for outward currents in molluscan neurons, we have been struck by the fact that little or no change in phenotype is necessary to produce qualitatively different currents, usually associated with separate ion-channel mechanisms. The implications of this result are considered for the definition of an ion channel, and for the evolutionary processes leading to different phenotypes.

A- AND K-CURRENTS IN *APLYSIA* NEURONS

The techniques of voltage-clamping and intracellular perfusion of *Aplysia* cells were basically those of Lee *et al.*,[9] and have been presented previously.[10] Cells in the visceral ganglion with diameters between 100 and 200 μm were studied at 13–15°C. Tetrodotoxin 10 μg/cc was added to the external solutions to suppress the inward current. Voltage and current data were digitized and stored on diskettes, and compared with predictions from the model.

The top part of Figure 1 shows currents recorded upon stepping from a holding potential of −90 mV to −12, +7, +30, +64 and +102 mV. The transient, or A-current is visible by itself at more negative test potentials, while at more positive potentials the delayed, or K-current is also seen. The bottom part of the figure shows currents upon stepping from a holding potential of −30 mV to approximately the same potentials as in the top part. In this case, the A-current is completely suppressed, and only the K-current is seen. The pure A-current is usually defined as the difference between the top and bottom traces at each test potential.

The A- and K-currents are usually explained in terms of separate channels, that is, separate membrane-spanning proteins with distinct voltage gates and selectivity "filters."[7,8,11–13] However, the separation of the currents is itself artificial, involving an algebraic subtraction as mentioned above. We wished to consider whether

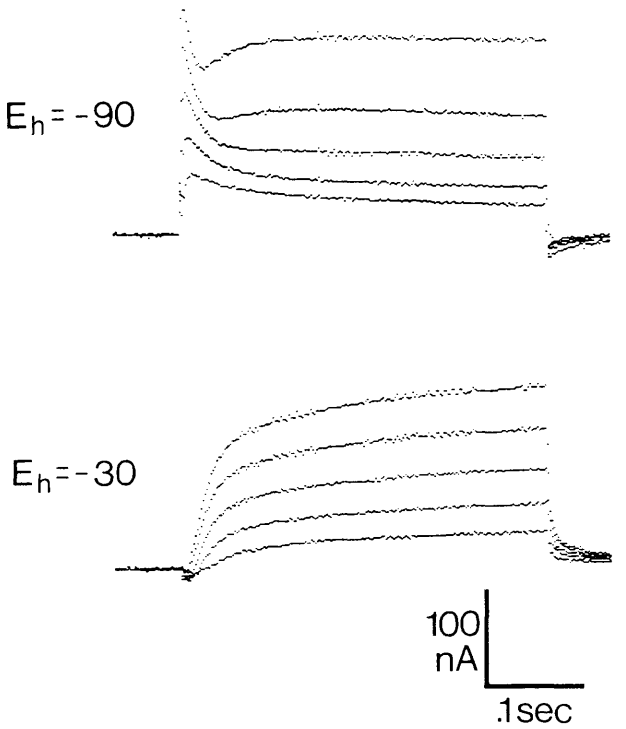
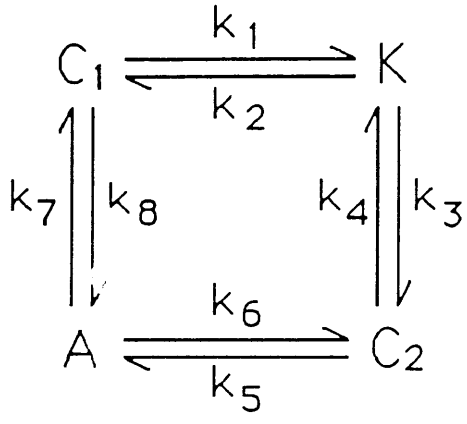


FIGURE 1 Voltage-clamp currents recorded from perfused *Aplysia* neuron. Holding potential (E_h) shown at left. Potential stepped to −12, +7, +30, +64 and +102 mV.

a model with only one type of channel could predict the observed currents equally well.

THE MODEL

It is assumed that channels may have to open states, A and K, and two closed states, C_1 and C_2, as shown below. The A-state produces the transient current and the K-state the delayed current. Previous models of potassium channels have included a greater number of closed states, to account for the slow onset of the K-current.[14,15] However, the outward current in *Aplysia* neurons activates with little inflection, and it was desired to have the least possible number of parameters in the model.



The state equations for this system are

$$dP_{C_1}/dt = k_2P_K - k_1P_{C_1} + k_7P_A - k_8P_{C_1} \tag{1}$$

$$dP_{C_2}/dt = k_3P_K - k_4P_{C_2} + k_6P_A - k_5P_{C_2} \tag{2}$$

$$dP_A/dt = k_5P_{C_2} - k_6P_A + k_8P_{C_1} - k_7P_A \tag{3}$$

$$dP_K/dt = k_1P_{C_1} - k_2P_K + k_4P_{C_2} - k_3P_K \tag{4}$$

where P_i = probability that the channel is in state i
k_i = i-th rate constant

$$P_{C_1} + P_{C_2} + P_A + P_K = 1 \tag{5}$$

In order for detailed balance, or microscopic reversibility[16-18] to hold, it must be true that

$$k_1 \cdot k_3 \cdot k_5 \cdot k_7 = k_2 \cdot k_4 \cdot k_6 \cdot k_8 \tag{6}$$

Detailed balance is thought to be a general property of rate constant models of chemical systems and gating,[19] but this point requires explicit consideration, if not proof, given the counter examples and discussion in Gardiner[20] and Whittle.[21]

If the conductance of the A- and K- states is assumed to be the same, then the membrane current (I) is given by

$$I = g_K (E - E_K)(P_A + P_K) \tag{7}$$

where g_K = maximum potassium conductance
E = membrane potential
E_K = potassium equilibrium potential

To account for the removal of resting inactivation, it is assumed that the initial state distribution at a holding potential of -90 mV is $P_{C_1} = 1$ and at -30 mV, $P_{C_2} = 1$. (Further justification for this assumption is given below.) Current-time relations in the model are found from Equation 7, using Euler integration with a time increment of 0.001 s.

Figure 2 shows fits of the model to the data in Figure 1. The rate constants, except k_2, were empirically chosen to give reasonable fits to the observed currents, and k_2 was found from Equation 6. The rate constants were the same for a given potential in both the top and bottom records; only the starting probability distribution was changed, as shown. The agreement with real-neuron data, while not perfect, indicates that the single-channel model gives a good first approximation to the time-course of both the A- and K-currents over a wide range of membrane potentials.

The variation of the rate constants with membrane potential is shown in Table I. The method of estimating the rate constants at each potential was as follows: At large positive potentials k_8 was large, since it controlled the initial peak of current when the channel suddenly passed from state C_1 to A. Likewise, k_6 was fairly large, as it determined the rapid inactivation of the A-current. The more slowly-developing K-current was regulated by k_1 and k_4, so these were relatively smaller. For simplicity k_3 and k_7 were assumed constant. Some rate constants were reduced at more negative potentials, to give reasonable fits to the observed currents. The rate constants at -30 mV and -90 mV (parentheses) were obtained by extrapolation from the k–V curves at more positive potentials.

The resting distribution of state probabilities at the two different holding potentials can be found as[17]

$$P_{C_1} = (k_2 k_5 k_7 + k_2 k_4 k_6 + k_3 k_5 k_7 + k_2 k_4 k_7)/\Sigma \tag{8}$$

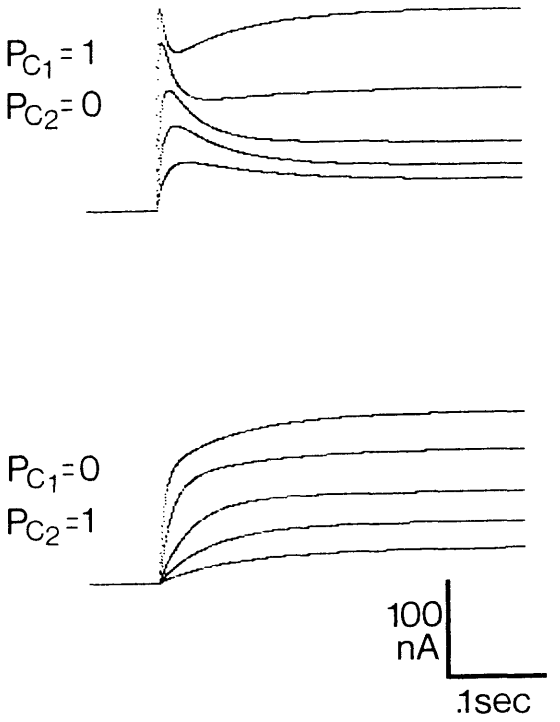


FIGURE 2 Four-state model simulation of currents in Figure 1. Rate constants assumed to vary with test potential. Initial probability distribution between closed states assumed to vary with holding potential.

TABLE I

Variation of rate constants in the model with membrane potential. Values at −90 and −30 mV found by graphic extrapolation. k_2 follows passively from Equation 6.

Potential, mV:	−90	−30	−12	7	30	70	102
k_1, s^{-1}	(4.0)	(7.8)	8.5	11.0	14.0	20.0	30.0
k_2	(1333.0)	(89.1)	39.5	13.2	5.73	1.56	0.72
k_3	(3.0)	(3.0)	3.0	3.0	3.0	3.0	3.0
k_4	(0.015)	(0.075)	0.13	0.25	0.5	1.6	5.0
k_5	(1.0)	(2.0)	2.6	3.5	6.0	16.0	40.0
k_6	(1.2)	(3.5)	4.3	7.0	11.0	24.0	50.0
k_7	(10.0)	(10.0)	10.0	10.0	10.0	10.0	10.0
k_8	(5.0)	(20.0)	30.0	50.0	80.0	160.0	200.0

TABLE II

State probabilities at different holding potentials, calculated from Equations 8–11.

Holding Potential	P_{C_1}	P_{C_2}	P_A	P_K
−90 mV	0.476	0.285	0.238	0.001
−30	0.152	0.531	0.304	0.013

$$P_{C_2} = (k_2 k_6 k_8 + k_1 k_3 k_6 + k_3 k_6 k_8 + k_1 k_3 k_7)/\Sigma \quad (9)$$

$$P_A = (k_2 k_5 k_8 + k_1 k_3 k_5 + k_3 k_5 k_8 + k_2 k_4 k_8)/\Sigma \quad (10)$$

$$P_K = (k_1 k_5 k_7 + k_1 k_4 k_6 + k_4 k_6 k_8 + k_1 k_4 k_7)/\Sigma \quad (11)$$

where $\Sigma = k_2 k_5 k_7 + k_2 k_4 k_6 + k_3 k_5 k_7 + k_2 k_4 k_7 + k_2 k_6 k_8 + k_1 k_3 k_6 k_3 k_6 k_8 + k_1 k_3 k_7 + k_2 k_5 k_8 + k_1 k_3 k_5 + k_3 k_5 k_8 + k_2 k_4 k_8 + k_1 k_5 k_7 + k_1 k_4 k_6 + k_4 k_6 k_8 + k_1 k_4 k_7$

Plugging in the inferred rate constants at −90 and −30 mV gives the state distributions at each holding potential shown in Table II. At −90 mV there is a relatively large probability that the channel is in state C_1, and less that it is in C_2. At −30 mV the highest probability is that the channel is in state C_2. One feature of the model which contributes to this behavior is the inversion of k_7 and k_8 between −30 and −90 mV. While not exactly the same as the ideal cases assumed in Figure 2, these results support the idea that the initial probability distribution shifts from C_1 to C_2 when the holding potential is shifted from −90 mV to −30 mV.

The ability of this or any other model to predict different types or outward currents should not be taken as evidence of uniqueness. It simply shows that a hypothesis involving one type of channel can account for more than one type of outward current, when the only altered parameter is the membrane potential.

DISCUSSION

The principal differences between this model and a parallel two-channel model (with unrelated A- and K-current channels) are

that (1) it does not require the separate evolution of distinct molecular structures in the membrane, having different kinetic and pharmacologic properties, and (2) it is more permissive of the relatively non-specific inhibitory action of tetraethylammonium ions on the two currents. (One might note in passing that in nature the K-current is never subtracted from the total outward current to reveal the A-current, nor are neurons subjected to the action of nonspecific blocking agents.)

Some recent studies have been interpreted to show the existence of separate A- and K-current channels: Muscle cells in *Shaker* mutants of *Drosophila* appear to lose a native A-current while retaining the K-current,[22,23] suggesting that these may be carried by separate channels. However, the currents observed in the mutant could be explained equally well by a modification of the four-state channel such as inactivation of the open A-state or the closed C_2 state. In addition, the *Shaker* locus has been cloned,[24] and an mRNA transcribed from cDNA clones used to express functional A-current channels, without K-current channels, in *Xenopus* oocytes.[25,26] Again, one might interpret the cloning and expression processes as affecting one or more states in the single-channel model. Evidence which apparently supports the sameness of channels is that the mouse brain gene MBK1 which is highly homologous to the Shaker gene[27] expresses not an A-type but a K-channel phenotype in *Xenopus* oocytes.[28]

In dissociated heart cells,[29] application of a sodium-regulating peptide makes the Na^+ channel more permeable to calcium ions and blocks Na^+ currents at the same time. Such a change in selectivity of the permeation site has not been seen for A- and K-currents.

Rapidly and slowly-inactivating Ca-currents in pituitary cells[30] have different threshold voltages for activation and different Ca/Ba selectivity ratios, and a decrease in one type of current is not accompanied by an increase in the other. Thus, they appear to be carried by different channel types. It is interesting to note that Jones and Hartline[31] have observed a transient A-current in lobster neurons which varies inversely with a TEA-dependent outward current (J-current), which could be carried by the same channel types.

It is possible, of course, that the A- and K-currents in *Aplysia*

cells flow through separate proteins, proteins coded by different genes and thus properly called different channels. In that case, our analysis suggests how these genes might be related. It seems likely, at least if our model is roughly correct, that the functionally relevant differences in the two channel molecules would be just those necessary to give the rate constants we suggest (Table I). In this view, the genes of the two channel types would be viewed as siblings, arising from a common ancestor, one with mutations selected to allow A-currents as a phenotype, the other with mutations selected to allow K-currents as a phenotype. Evolution might have chosen the simplest path, with the mutations confined to single subunits (polypeptide chains) coded by single exons, producing simple changes in rate constants and thus the function and phenotype of the channel.

Acknowledgments

Supported by NIH grant RR 05304 and NSF grant DCB-8903795. We thank E. Serrano for suggestions.

DOUGLAS JUNGE
School of Dentistry and Department of Physiology and Brain Research Institute, University of California, Los Angeles, California 90024

ROBERT S. EISENBERG
Department of Physiology, Rush Medical College, 1750 West Harrison Street Chicago, Illinois 60612

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