

KINETICS OF INWARD RECTIFIER GATING IN THE EGGS OF THE MARINE POLYCHAETE, *NEANTHES ARENACEODENTATA*

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SUMMARY

1. Potassium current through the inward rectifier of *Neanthes arenaceodentata* eggs was studied using a voltage-clamp technique. The instantaneous conductance, steady-state conductance and the time constant of current relaxation were analysed as functions of the membrane potential and the external potassium concentration ($[K]_o$).

2. Both the instantaneous and the steady-state conductances increased sigmoidally with hyperpolarization, reaching saturation values at potentials 40 mV more negative than the potassium equilibrium potential (E_K).

3. The time-dependent change in conductance followed first-order kinetics throughout an 80 mV potential range centred at E_K . The conductances increased with time during hyperpolarizations and decreased with time during depolarizations. The time constant decreased sigmoidally about E_K as the membrane potential (V_m) was made more positive.

4. The results are interpreted in terms of two models. The first model assumes single-channel rectification. Forcing the data to conform with this assumption necessitates that the single-channel conductance, the open-channel probability, and the time constant of current relaxation all be nearly identical functions of $V_m - E_K$. This coincidence is considered implausible.

The second model ascribes the apparent instantaneous rectification to a fast kinetic process. This model correctly predicts the steady-state conductance, the time-constant of current relaxation, and the unexpected proportionality between the ratio of the steady-state and 'instantaneous' conductances and the time constant of current relaxation. This agreement between data and model is obtained even though neither of the rate constants of the slow gating process has the flexibility of voltage or potassium dependence. The results are considered to imply the existence of a fast gating mechanism in the kinetics of inward rectification.

INTRODUCTION

The plasma membranes of a variety of cells, including skeletal and cardiac muscle fibres, neurones, macrophages and oocytes possess a potassium conductance which permits current to pass in through the membrane more easily than out. This inward

rectifying conductance is further distinguished by its dependence on the displacement of the voltage from the potassium equilibrium potential (E_K) when the external potassium concentration ($[K]_o$) is changed (Hodgkin & Horowitz, 1959; Adrian & Freygang, 1962*a, b*; Hagiwara & Takahashi, 1974). Work on starfish eggs (Hagiwara, Miyazaki & Rosenthal, 1976) and skeletal muscle (Almers, 1971; Hestrin, 1981; Leech & Stanfield, 1981) has characterized the time-dependent component of inward rectification in these tissues. In both preparations the time constant decreases as the membrane potential (V_m) is hyperpolarized from the resting potential. As with the conductance, the voltage-dependence of the time constant shifts about E_K when the external potassium concentration is altered.

This paper describes experiments on inward rectification in the eggs of the marine polychaete *Neanthes arenaceodentata* and contrasts the interpretations of these data by two kinetic models. An advantage of this preparation is that current relaxation can be studied at potentials more positive than E_K . As with starfish eggs and skeletal muscle, both the conductance and the time constant in *Neanthes* eggs are functions of $V_m - E_K$ when the external potassium concentration is changed. However, in contrast to those two tissues, the time constant of current relaxation in *Neanthes* eggs increases in a sigmoidal manner with increasing hyperpolarization, indicating some qualitative phylogenetic variation in inward rectification.

The *Neanthes* data is economically described by a kinetic model which postulates that the apparent instantaneous conductance is the result of a fast gating process. The model also possesses the virtue, through an easy extension, of allowing the inward rectifier kinetics observed in *Neanthes* and starfish eggs, as well as in skeletal muscle, to be described by a common model.

METHODS

Materials. Eggs from the segmented marine worm *Neanthes arenaceodentata* were used in these studies. The adult worms were obtained throughout the year from Dr D. J. Reish (California State University, Long Beach, CA U.S.A.). A syringe inserted into the coeloms of the worms was used to sluice eggs into an experimental chamber with a glass bottom to which the eggs adhered. The eggs were typically 400 μm in diameter, though eggs as small as 200 μm were occasionally found.

Voltage clamp. A standard two-micro-electrode voltage-clamp technique was used in all experiments. The electrodes were filled with either 3 M-KCl or 4 M-NaCl, and had resistances of 5–10 M Ω . Current was measured by an *I*-to-*V* converter connected to the bath by an agar bridge. Experiments were done at room temperature, 22 °C.

Solutions. The compositions of the basic solutions used in these experiments were: Na saline—450 mM-NaCl, 10 mM-KCl, 10 mM-Tris HCl, 10 mM-CaCl₂ and 50 mM-MgCl₂; sucrose saline—680 mM-sucrose, 10 mM-KCl, 70 mM-Tris Cl and 10 mM-CaCl₂. To make solutions with greater concentrations of KCl than 10 mM, NaCl and Tris HCl were reduced in Na saline and sucrose saline, respectively, to maintain osmolarity (5100B vapour pressure osmometer, Wescor Inc., Logan, UT U.S.A.). All solutions were buffered to pH 7.6. Most experiments were done in sucrose saline to reduce blocking effects on the inward rectifier by external sodium (Ohmori, 1978). In several experiments 10⁻⁴ M-ouabain (Sigma) was included in the bathing solution without effect.

Experimental procedure. To obtain data for conductance-voltage curves a series of twenty voltage steps from the resting potential was applied by a computer (Nova 3, Data General, Westboro, MA U.S.A.) through a 12-bit D/A converter. These steps covered a range of voltages of 60 mV on each side of the resting potential. Current traces were digitized and stored in the computer. The series was repeated four times and corresponding current traces were averaged. The resulting traces were digitally filtered with a 6-pole sine Butterworth program (Otnes & Enochson, 1978) with a corner frequency of 40 Hz to reduce the background noise. The filtering began with the final point of the

current record and proceeded backwards to a point 10 msec after the beginning of the voltage pulse. In this way the discontinuity of the capacitive transient was avoided. Since time constants less than 80 msec were almost never observed, the 40 Hz cut-off frequency did not appreciably effect the time dependent development of the current.

RESULTS

The dependence of the resting potential, V_0 , on the potassium concentration in the external solution is shown in Fig. 1. From an average value of -71.4 ± 4 mV ($n = 20$) in artificial sea water, the resting potential changed in a Nernstian manner as external potassium was increased. Eliminating NaCl and $MgCl_2$ from the bath by replacement

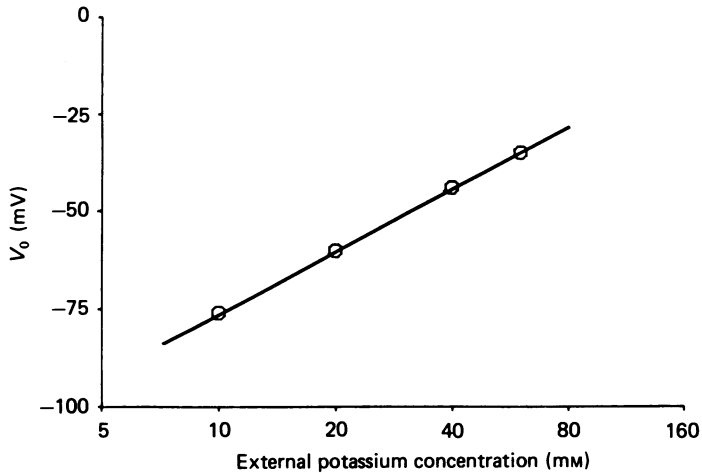


Fig. 1. The dependence of the resting potential on external potassium. The potassium concentration was increased by substitution for sodium in Na saline. The straight line was drawn with a slope of 59 mV for a ten-fold change in the external potassium concentration.

with sucrose, and exchanging $MnCl_2$ for $CaCl_2$ resulted only in a slight hyperpolarization of the resting potential. Since the cell membrane appears to have acted as a potassium electrode in these experiments, V_0 will be identified with E_K in the sequel.

Steady-state conductance. Clamping the membrane potential away from V_0 resulted in a pattern of currents typical of inward rectification (Fig. 2A). A voltage step to a more negative potential elicited an inward current which gradually increased to a maximum. The outward currents resulting from positive voltage steps were of much smaller amplitude than those corresponding to the symmetrical negative voltage steps. The outward currents decreased with time to a minimum. In *Neanthes* eggs, the time-dependent component of the current rarely is as much as one-third of the total current and is commonly of the order of one-tenth of the total.

The steady-state membrane conductance, G_{ss} , is plotted in Fig. 2B as a function of $V_m - E_K$ for several concentrations of external potassium. The steady-state conductance increased as the membrane was hyperpolarized, approaching a limiting value when $V_m - E_K = -40$ mV. In the presence of external sodium, the conductance decreased with further hyperpolarization in agreement with findings on inward

rectification in tunicate eggs (Ohmori, 1978) and skeletal muscle (Almers, 1972*a, b*; Standen & Stanfield, 1979). In the absence of external sodium, the conductance remained at the limiting value. Maximum limiting conductances were often more than fifty times larger than the conductances found in response to large depolarizing voltage pulses.

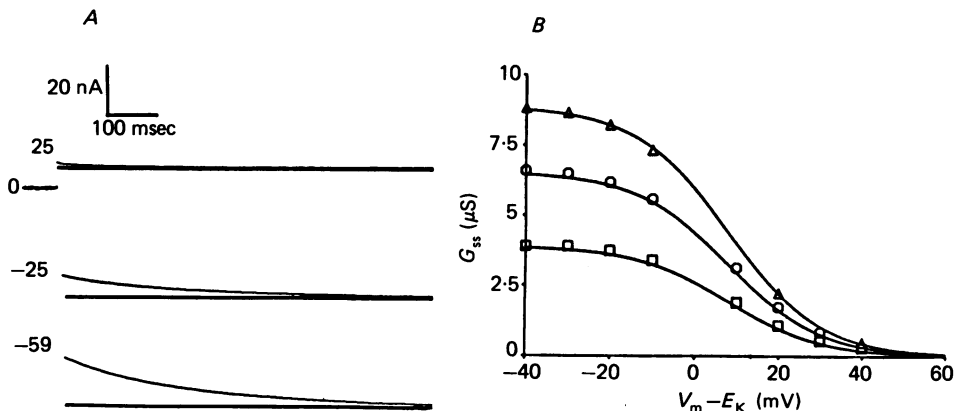


Fig. 2. Steady-state conductance. *A*, membrane currents associated with voltage steps of 25, -25 and -50 mV from a resting potential of -84 mV ($\Delta V = 0$ mV). The heavy lines denote steady-state current levels. 10 mM-potassium sucrose saline. *B*, steady-state conductance as a function of $V_m - E_K$ for three concentrations of potassium in Na-based saline: Δ , 60 mM-K; \circ , 40 mM-K; \square , 20 mM-K. Continuous lines are drawn to eqn. (1):

$$G_{ss} = \frac{0.41[K]_0^{0.75}}{1 + \exp\left(\frac{\Delta V - 5}{11}\right)}$$

Fig. 2*B* also indicates that the conductance increased with increasing external potassium. Altogether, the inward rectifier conductance could be described by the equation of Hagiwara & Takahashi (1974),

$$G_{ss} = \frac{G[K]_0^c}{1 + \exp\left(\frac{\Delta V - V_h}{v}\right)}, \quad (1)$$

where $\Delta V = V_m - E_K$ and, for *Neanthes* eggs, $V_h \doteq +5$ mV, $v \doteq 11$ mV, and $c \doteq 0.7$ ($n = 50$). The maximum steady-state conductance in artificial sea water is generally near 1 μS (0.2 mS/cm²). The steepness factor, v , is close to the value of 11.6 mV found in skeletal muscle (Hestrin, 1981) and somewhat larger than the 7 mV figure found for starfish eggs (Hagiwara & Takahashi, 1974). According to eqn. (1) the conductance is half maximal when $\Delta V = V_h$, so in *Neanthes* eggs, the conductance is slightly more than half activated at the resting potential. This contrasts with the relatively low levels of resting conductance in starfish eggs ($V_h = -15$ mV, Hagiwara & Takahashi, 1974) and in skeletal muscle ($V_h = -25$ mV, Hestrin, 1981). The exponent c is somewhat larger in *Neanthes* than the value of 0.5 found in starfish eggs (Hagiwara & Takahashi, 1974).

Instantaneous conductance. The instantaneous conductance, G_1 , plotted in Fig. 3, also shows rectification and is well described by an equation similar to eqn. (1):

$$G_1 = \frac{G'[K]_0^c}{1 + \exp\left(\frac{\Delta V - V'}{v}\right)}. \quad (2)$$

This 'instantaneous' conductance is actually the conductance 1 msec after the beginning the voltage step, due to the capacitive transient, and may be the result of a time-dependent change in the number of open channels. A change of this nature

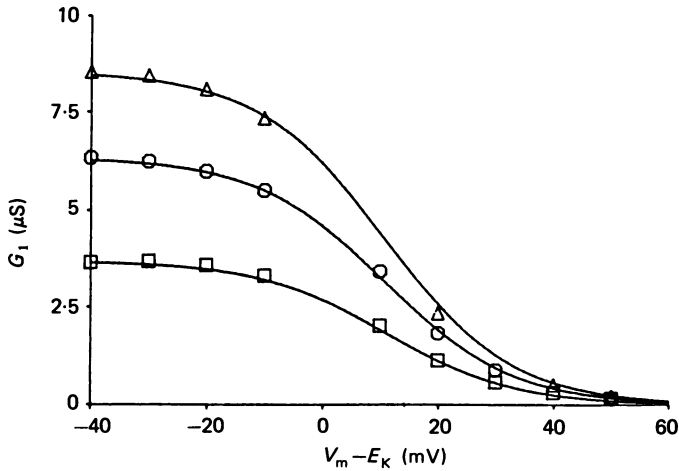


Fig. 3. Instantaneous conductance. Instantaneous conductance as a function of $V_m - E_K$ for three concentrations of potassium in Na-based saline: Δ , 60 mM-K; \circ , 40 mM-K; \square , 20 mM-K. The data are from the same experiment as in Fig. 2B. The continuous lines are drawn to eqn. (2):

$$G_1 = \frac{0.39[K]_0^{0.75}}{1 + \exp\left(\frac{\Delta V - 14}{11}\right)}$$

The instantaneous conductances were obtained by extrapolating currents back to zero time.

would pass unobserved in these experiments if the time constant of activation were of the order of 0.2 msec or less. The values of the steepness factor v and the exponent c are identical in the steady-state and instantaneous conductance equations. The maximum instantaneous conductance is usually 10–25% less than the maximum steady-state conductance and the instantaneous conductance achieves its half maximal value at a more positive potential than does the steady state, with $V' \doteq V_h + 5$ mV ($n = 50$).

Time-dependent potassium current. An investigation of the time-dependent component of the inward rectifier is presented in Fig. 4. When plotted on a semilogarithmic scale, as in Fig. 4A, $I(t) - I_{ss}$ was a linear function of time, where $I(t)$ is the current t seconds after the beginning of the voltage step and I_{ss} is the steady-state current level. The currents, therefore, relaxed to their steady-state values along exponential time courses. This is in agreement with findings in starfish eggs (Hagiwara *et al.* 1976) and skeletal muscle (Almers, 1971; Hestrin, 1981; Leech & Stanfield, 1981).

The time constant, τ , was a sigmoidal function of voltage about E_K , as shown in Fig. 4B. The time constant was somewhat larger under hyperpolarization, but the change in τ with voltage was never large. Values of τ less than 50 msec or greater than 250 msec were seldom seen ($n = 20$). This voltage dependence contrasts with inward rectification in skeletal muscle (Almers, 1971; Hestrin, 1981; Leech & Stanfield, 1981) and starfish eggs (Hagiwara *et al.* 1976) where τ decreases as the membrane is hyperpolarized.

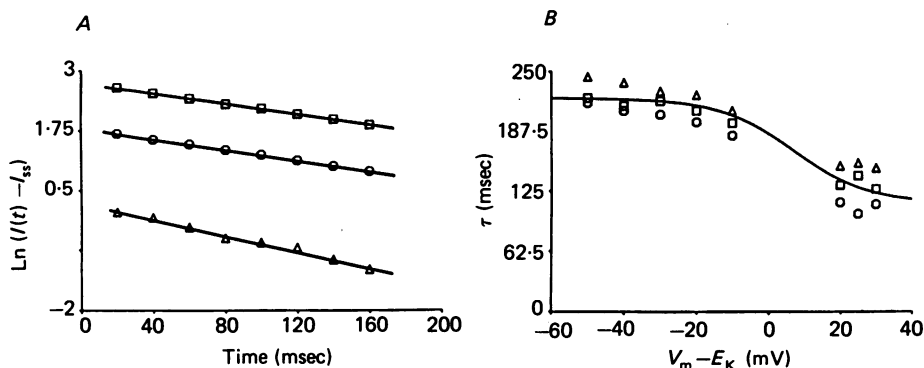


Fig. 4. Time-dependent currents. *A*, linearized currents, $\ln [I(t) - I_{ss}]$, plotted against time for voltage steps of Δ , 25; \circ , -25 and \square , -50 mV from a resting potential of -84 mV. The data are from the current traces in Fig. 2A. The straight lines were fitted by eye. *B*, the time constant of activation plotted as a function of $V_m - E_K$ for three concentrations of potassium in sucrose-based saline: Δ , 40 mM-K; \circ , 20 mM-K; \square , 10 mM-K. The continuous line is drawn to eqn. (3):

$$\tau = 0.223 \left(0.51 + \frac{0.49}{1 + \exp\left(\frac{\Delta V - 5}{11}\right)} \right) \text{sec.}$$

As is the case with the instantaneous and steady-state conductances, τ is best described as a function of $V_m - E_K$ as the external potassium concentration is changed (Fig. 4B):

$$\tau = \left(A + \frac{1 - A}{1 + \exp\left(\frac{\Delta V - V_h}{v}\right)} \right) \tau_{\max}, \quad (3)$$

where A is a constant between 0 and 1 and τ_{\max} is the limiting value of τ under large hyperpolarizations. In eqn. (3), V_h has the same value as the identical parameter in eqn. (1) and v has a common value in eqns. (1), (2) and (3). The constant A is generally a value between 0.75 and 0.9.

As shown in Fig. 4B, τ did not decrease with hyperpolarization of $V_m - E_K$ to -40 mV. In other experiments, voltage steps of -100 mV from the resting potential did not show an indication of further change in τ . Again, when $[K]_o = 5$ mM and $V_o = -101$ mV, stepping to test potentials as negative as -161 mV still elicited only the asymptotic behaviour of τ .

In the discussion section, two kinetic schemes describing these data will be considered. To facilitate this discussion, the steady-state and instantaneous con-

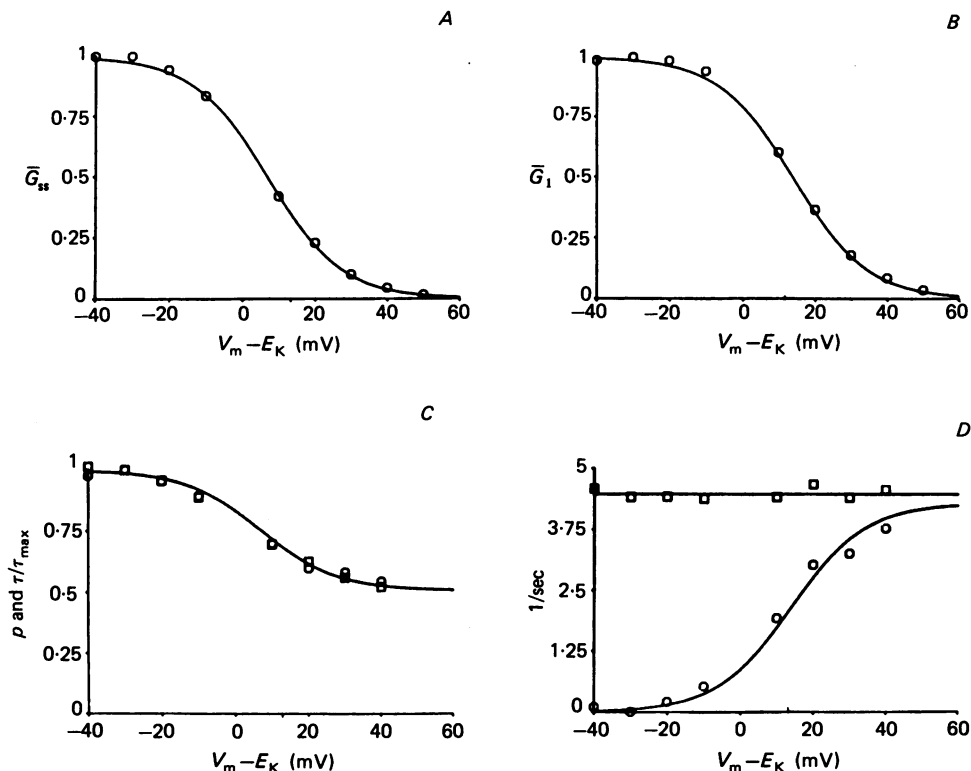


Fig. 5. Interpretation in terms of kinetic models. The four parts of this Figure all relate to the same experiment. 10 mM-potassium in sucrose-based saline. The continuous lines in parts A, B and C are drawn to the three-state model (see Discussion). A, \bar{G}_{ss} , the steady-state conductance normalized to its maximum value as a function of $V_m - E_K$. The continuous line is drawn to eqn. (A 13):

$$\bar{G}_{ss} = \left(1 + \exp\left(\frac{\Delta V - 7}{10.3}\right) \right)^{-1}.$$

B, \bar{G}_1 , the instantaneous conductance normalized to its maximum value as a function of $V_m - E_K$. The continuous line is drawn to eqn. (A 3):

$$\bar{G}_1 = \left(1 + \exp\left(\frac{\Delta V - 14}{10.3}\right) \right)^{-1}.$$

C, $p = \bar{G}_{ss}/\bar{G}_1$ and $\bar{\tau} = \tau/\tau_{max}$ as functions of $V_m - E_K$. The continuous line is drawn to eqns. (A 17) and (A 36):

$$p = \bar{\tau} = 0.51 + \frac{0.49}{1 + \exp\left(\frac{\Delta V - 7}{10.3}\right)}.$$

D, the rate constants of the two-state model (see Discussion): \square , F ; \circ , B . The continuous lines are drawn to eqns. (9) and (10): $F = 4.49/sec$;

$$B = \frac{0.49 \exp\left(\frac{\Delta V - 7}{10.3}\right)}{0.223 \left(1 + 0.51 \exp\left(\frac{\Delta V - 7}{10.3}\right) \right)} \text{ sec}^{-1}.$$

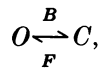
ductances and the time constant of relaxation, normalized to their maximum values, \bar{G}_{ss} , \bar{G}_1 and $\bar{\tau}$ respectively, from one experiment are gathered in Fig. 5. Plotted with $\bar{\tau}$ in Fig. 5C is the ratio of the steady-state and instantaneous conductances, $p = \bar{G}_{ss}/\bar{G}_1$. The dependences of p and $\bar{\tau}$ on $V_m - E_K$ appear to be identical. The agreement between $\bar{\tau}$ and p is predicted by the three-state model developed in the Discussion section. $F = p/\bar{\tau}$ and $B = (1-p)/\bar{\tau}$, the rate constants of the two-state model, are plotted as functions of $V_m - E_K$ in Fig. 5D.

DISCUSSION

Though Katz recognized in 1949 that the resting potassium conductance in skeletal muscle passed inward current more easily than outward, characterization of this conductance has been hindered by the subsequent inability to ascertain whether the rectification is due to a rapid change in the number of open channels or to changes in the single channel conductance. Although no indication of a fast gating process has been found, no study of inward rectification has yet resolved the ionic current at times earlier than 1 msec after the beginning of the potential step. The possibility exists that the apparent instantaneous conductance change is actually the result of a fast transition from an open to a closed state. Recognizing this ambiguity, the data presented here are interpreted according to the two simplest models which incorporate the alternative assumptions: (1) a two-state model where the open channel rectifies, and (2) a three-state model where the open channel conductance is voltage independent and the rectification is caused by a fast transition between the open and closed states.

Two-state model

A two-state model:



where O is the open state, can account for the inward rectifier currents observed in *Neanthes* eggs if the single-channel conductance, γ , rectifies.

Single-channel conductance. In the two-state model the non-linearity of the apparent instantaneous conductance is explained by a voltage-dependent single-channel conductance,

$$\gamma(\Delta V)/\gamma_{\max} = \bar{G}_1(\Delta V).$$

In Fig. 5B, γ is seen to be a sigmoidal function of ΔV , increasing from 0 to γ_{\max} as the cell membrane is hyperpolarized:

$$\gamma(\Delta V) = \frac{\gamma_{\max}}{1 + \exp\left(\frac{\Delta V - V'}{v}\right)}. \quad (5)$$

Open-channel probability. The ratio of the steady-state and instantaneous conductances normalized to their maximum values under large hyperpolarizing voltage steps, yields, up to a multiplicative constant, an expression for $p(\Delta V)$, the open-channel probability.

$$\bar{G}_{ss}(\Delta V)/\bar{G}_1(\Delta V) = [G_{ss}(\Delta V)/G_{ss \max}]/[G_1(\Delta V)/G_1 \max] \quad (6)$$

$$= [Np(\Delta V)\gamma(\Delta V)/(Np_{\max}\gamma_{\max})]/[Np(0)\gamma(\Delta V)/(Np(0)\gamma_{\max})], \quad (7)$$

where N is the number of inward rectifier channels in the cell membrane ($p(0)$ is the open-channel probability at the holding potential). If all of the inward rectifier channels are opened by large hyperpolarizing voltages ($p_{\max} = 1$),

$$p(\Delta V) = \bar{G}_{\text{ss}}(\Delta V) / \bar{G}_1(\Delta V). \quad (8)$$

Comparing eqn. (8) with Fig. 5C, the two-state model interpretation of the open-channel probability is a sigmoidal function of ΔV which asymptotically approaches 1 with large hyperpolarizations and asymptotically approaches a non-zero fraction of 1 with large depolarizations.

Rate constants. The rate constants, F and B , can be calculated as

$$F = p(\Delta V) / \tau(\Delta V) \quad (9)$$

and

$$B = [1 / \tau(\Delta V)] - F. \quad (10)$$

F and B are plotted in Fig. 5D. Although F is a nicely behaved function (indeed a straight line), B is not easily described by any intuitive function, especially as it must react to changes in the external potassium concentration by maintaining its voltage dependence about E_K .

A plausible physical interpretation for this two-state model must account for the unexpected coincidence of the steepness factor v in the voltage dependence of γ , τ and p . That the single-channel conductance, the time constant of current relaxation, and the open-channel probability should all have essentially identical sigmoidal dependence on ΔV is not obvious.

Three-state model

A linear three-state model: $O \xrightleftharpoons[z]{y} C \xrightleftharpoons[x]{w} I$,

where O is the only open state, the single channel conductance, γ , is voltage independent, and the apparent 'instantaneous' rectification is due to a fast transition between states O and C can account for the *Neanthes* data more satisfactorily than the two-state model.

The sigmoidal $\bar{G}_1 - \Delta V$ curve (Fig. 5B) which was interpreted as the instantaneous conductance change in the two-state model must, in the three-state model, be reinterpreted as the result of a rapid equilibrium attained between states O and C :

$$\bar{G}_1(\Delta V) = \frac{1}{1 + z/y} = \frac{1}{1 + \exp\left(\frac{\Delta V - V'}{v}\right)}. \quad (A3)$$

As developed in the Appendix, the linear three-state model with constant values of x and w (both rate constants independent of voltage and external potassium) predicts accurate expressions for both the steady-state conductance, G_{ss} , and the time constant of the slow relaxation process, τ ,

$$\bar{G}_{\text{ss}} = \frac{1}{1 + \frac{z}{y} \left(\frac{x+w}{x}\right)} \quad (A11)$$

$$= \frac{1}{1 + \exp\left(\frac{\Delta V - V_h}{v}\right)}, \quad (A13)$$

where V_h is defined by $V_h = V' - v \ln \left(\frac{x+w}{x} \right)$, and

$$\tau = \frac{y+z}{(y+z)x+zw}, \quad (\text{A } 34)$$

$$\bar{\tau} = A + \frac{1-A}{1 + \exp \left(\frac{\Delta V - V_h}{v} \right)}, \quad (\text{A } 36)$$

where

$$A = \frac{x}{x+w} = \exp \left(\frac{V_h - V'}{v} \right).$$

The curves fitting the data in Fig. 5 *A*, *B* and *C* are drawn to the predictions of the linear three-state model: eqns. (A 13), (A 3), and (A 36) with $x = 4.49/\text{sec}$ and $w = 4.25/\text{sec}$.

Besides the good fit to the data provided by this model, there are two other features worth noting. First, the steepness factors v in the sigmoidal expressions for G_1 , G_{ss} and τ are found experimentally to have the same value. This is a natural consequence of the three-state model where the expressions for each of these variables gain their sigmoidal voltage dependence from a common z/y term. Second, though p , defined as \bar{G}_{ss}/\bar{G}_1 , is no longer interpreted as the open-channel probability in the three-state model, the quotient can still be calculated. The theoretical expression for p , derived in the Appendix, is

$$p = \frac{(y+z)x}{(y+z)x+zw} \quad (\text{A } 14)$$

$$= A + \frac{1-A}{1 + \exp \left(\frac{\Delta V - V_h}{v} \right)}. \quad (\text{A } 17)$$

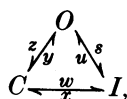
The expression for p is the same as that for $\bar{\tau}$, eqn. (A 36). The unexpected coincidence of the normalized time constant and the ratio of the normalized steady-state and instantaneous conductances (Fig. 5 *C*) is predicted by the linear three-state model.

Comparison with other preparations

The conductance and time-dependent properties of inward rectification have previously been analysed in starfish eggs (Hagiwara & Takahashi, 1974; Hagiwara *et al.* 1976) and in skeletal muscle (Almers, 1971; Hestrin, 1981; Leech & Stanfield, 1981). A comparison of these earlier results with the data reported here indicates that the steady-state and 'instantaneous' conductances are very similar in all three preparations and are well described by the equation of Hagiwara & Takahashi (eqn. (1)). However, the voltage dependence of the time constant is qualitatively different in the *Neanthes* eggs than in skeletal muscle or starfish eggs. In *Neanthes*, as shown in Fig. 4, the time-constant increases sigmoidally with increasing hyperpolarization, whereas, in skeletal muscle and starfish eggs, τ decreases with increasing hyperpolarization. A reconciliation of this difference is possible in the three-state model if direct access to the open state, *O*, is possible from state *I*. The linear three-state model used above to fit the *Neanthes* data will result from this triangular three-state model if the rate constants between states *O* and *I* are negligibly small.

Triangular three-state model

The predictions of the triangular three-state model:



where O is the only open state, s, u, w, x, y, z are rate constants, the single channel conductance, γ , is voltage independent, and the apparent 'instantaneous' rectification is due to a fast transition between states O and C , are developed in the Appendix. The expressions for the steady-state and apparent instantaneous conductances are identical with those of the linear three-state model. The expression for τ is, however, affected by the addition of the pathway between O and I :

$$\tau = \frac{p}{x} \cdot \frac{zw}{zw + ys}, \quad (\text{A } 33)$$

where p is defined as in eqn. (A 17) above.

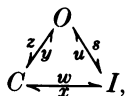
If s is negligibly small, this expression for τ reduces to p/x as in the linear three-state model and the *Neanthes* data. If s is not small, τ may be expected to decrease with increasing hyperpolarization as seen in skeletal muscle and starfish eggs. This expectation is based on the knowledge that y/z increases with increasing hyperpolarization (eqn. (A 3) and Fig. 5B) while w and x are constants in the model and p approaches an asymptotic limit. Consequently, the difference in the voltage-dependent behaviour of τ in *Neanthes* eggs and in skeletal muscle and starfish eggs is reduced to a quantitative difference in the rate constant s in the triangular three-state model.

Conclusion

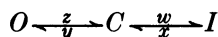
The macroscopic inward rectifier currents in *Neanthes arenaceodentata* can be described by a model with two kinetic states where the single-channel conductance shows voltage dependence, or by a three-kinetic-state model where the apparent instantaneous rectification is the result of a rapid gating mechanism between a closed state and the open state. For the two-state model to consistently reflect the data, the single-channel conductance, the time-constant of current relaxation, and the open-channel probability must all be nearly identical sigmoidal functions of $V_m - E_K$. This coincidence seems implausible. The three-state model, however, correctly expresses the voltage dependence of the steady-state conductance and the time constant, and predicts the unexpected proportionality between the ratio of the steady-state and 'instantaneous' conductances and the time-constant. Moreover, the agreement of this model with the data is obtained even though neither of the rate-constants of the slow gating process has the flexibility of voltage or potassium dependence. The existence of a fast gating mechanism in the inward rectifier kinetics seems to be implied by the *Neanthes* data.

APPENDIX

In this appendix, predictions of the triangular three-state model



are derived. The predictions of the related linear three-state model



will follow by allowing the rate constants u and s to approach zero.

Assumptions. O is the only conducting state. The single channel conductance is independent of voltage. The rate constants w and x are constants, independent of voltage and external potassium. The apparent instantaneous rectification of the channel is due to a fast transition between the open state and the closed state C . Two constraints on the rate constants are:

$$ysx = zwu \quad (\text{microscopic reversibility}), \quad (\text{A } 1)$$

and

$$y + z \gg u + s + w + x \quad (\text{assumption of a fast process}). \quad (\text{A } 2)$$

Initial conductance. The 'instantaneous' number of open channels, following a voltage jump, is the result of an equilibrium condition between O and C . The initial conductance normalized to its maximum value, \bar{G}_1 , is found experimentally to be a sigmoidal function of $V - E_K$,

$$\bar{G}_1 = \frac{1}{1 + z/y} = \frac{1}{1 + \exp\left(\frac{\Delta V - V'}{v}\right)}, \quad (\text{A } 3)$$

where V' and v are constants.

Steady-state conductance. The steady-state open-channel probability can be calculated by solving the simultaneous linear differential equations:

$$0 = \frac{dN_O}{dt} = -(z + s)N_O + yN_C + uN_I, \quad (\text{A } 4)$$

$$0 = \frac{dN_C}{dt} = zN_O - (y + w)N_C + xN_I, \quad (\text{A } 5)$$

$$N = N_O + N_C + N_I, \quad (\text{A } 6)$$

where N_I , N_O , N_C and N represent the number of channels in states I , O and C , and the total number of channels, respectively. The solution for the steady-state conductance normalized to its maximum value is

$$\bar{G}_{ss} = \frac{N_O}{N} = \frac{yu + yx + wu}{yu + yx + wu + z(u + x + w) + s(w + x + y)} \quad (\text{A } 7)$$

$$= \frac{1}{1 + \frac{z}{y} \cdot \frac{u + x + w + (s/z)(w + x + y)}{u + x + (wu/y)}} \quad (\text{A } 8)$$

Using eqn. (A 1) to replace s/z with wu/xy ,

$$\bar{G}_{ss} = \frac{1}{1 + \frac{z}{y} \cdot \frac{u+x+w+(wu/xy)(w+x+y)}{u+x+(uw/y)}} \tag{A 9}$$

$$= \frac{1}{1 + \frac{z}{y} \cdot \frac{u+x+(wu/y)+(w/x)[u+x+(uw/y)]}{u+x+(uw/y)}} \tag{A 10}$$

$$= \frac{1}{1 + \frac{z}{y} \cdot \frac{(x+w)}{x}} \tag{A 11}$$

$$= \frac{1}{1 + \frac{x+w}{x} \exp\left(\frac{\Delta V - V'}{v}\right)} \tag{A 12}$$

$$= \frac{1}{1 + \exp\left(\frac{\Delta V - V_h}{v}\right)}, \tag{A 13}$$

where V_h is defined by $\frac{x+w}{x} \exp(-V'/v) = \exp(-V_h/v)$.

Ratio of steady-state and instantaneous conductances. Using eqns. (A 3), (A 11) and (A 13),

$$p = \bar{G}_{ss}/\bar{G}_1 = \frac{(y+z)x}{(y+z)x+zw} \tag{A 14}$$

$$= \frac{1 + \exp\left(\frac{\Delta V - V'}{v}\right)}{1 + \exp\left(\frac{\Delta V - V_h}{v}\right)}, \tag{A 15}$$

$$= \frac{1 + \left(\frac{x}{x+w}\right) \exp\left(\frac{\Delta V - V_h}{v}\right)}{1 + \exp\left(\frac{\Delta V - V_h}{v}\right)} \tag{A 16}$$

$$= A + \frac{1-A}{1 + \exp\left(\frac{\Delta V - V_h}{v}\right)} \tag{A 17}$$

where $A = x/(x+w)$. The ratio of two sigmoidal functions (such as \bar{G}_{ss} and \bar{G}_1) will be the sum of a constant term plus a sigmoidal term, such as eqn. (A 17), only if the 'v' parameter is identical in both functions. This is a natural consequence of the three-state model.

The time constant. An expression for the time constant of the slow process can be derived from the master equation for the system of linear differential equations (A 4), (A 5), and (A 6),

$$0 = (z+s+u+\lambda)(y+w+x+\lambda) - (z-x)(y-u) \quad (\text{A } 18)$$

$$= \lambda^2 + \lambda(u+s+w+x+y+z) + (zw+zx+sy+sw+sx+uy+uw+zu+xy) \quad (\text{A } 19)$$

$$= \lambda^2 + \lambda S + D, \quad (\text{A } 20)$$

where
and

$$S = u+s+w+x+y+z$$

$$D = zw+zx+sy+sw+sx+uy+uw+zu+xy. \quad (\text{A } 22)$$

$$\lambda = \frac{-S + \sqrt{(S^2 - 4D)}}{2} \quad (\text{A } 23)$$

$$\doteq \frac{-S + S(1 - 2D/S^2)}{2} \quad (\text{since } 1 \gg 4D/S^2) \quad (\text{A } 24)$$

$$= -D/S. \quad (\text{A } 25)$$

The slow time constant τ equals $-1/\lambda$,

$$\tau = S/D. \quad (\text{A } 26)$$

Using eqn. (A 2),

$$S = y+z \quad (\text{A } 27)$$

and

$$D = (y+z)(x+u) + sy + wz, \quad (\text{A } 28)$$

(since $wu, xs \ll (y+z)(x+u)$ and either $y \ll z$, so $s/z \ll 1$ or $y > z$, so $s/z \ll ys/zw$).

$$\tau \doteq \frac{y+z}{(y+z)(x+u) + sy + wz} \quad (\text{A } 29)$$

$$= \frac{y+z}{x(y+z)(1 + ys/zw) + sy + zw} \quad (u = ysx/zw) \quad (\text{A } 30)$$

$$= \frac{(y+z)zw}{x(y+z)(zw + ys) + (zw + ys)zw} \quad (\text{A } 31)$$

$$= \frac{(y+z)}{(y+z)x + zw} \cdot \frac{zw}{zw + ys}. \quad (\text{A } 32)$$

Comparing eqn. (A 32) with eqn. (A 14),

$$\tau = \frac{p}{x} \cdot \frac{zw}{zw + ys}. \quad (\text{A } 33)$$

If s is negligibly small,

$$\tau = \frac{(y+z)}{(y+z)x + zw} \quad (\text{A } 34)$$

$$= p/x, \quad (\text{A } 35)$$

$$\bar{\tau} = A + \frac{1-A}{1 + \exp\left(\frac{\Delta V - V_h}{v}\right)}. \quad (\text{A } 36)$$

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