LETTER TO THE EDITOR

On the Relationship between Resting Potential and the Delayed Rectifier in Squid Axons

Dear Sir:

The relationship between specific membrane currents and the resting potential, V_R, is an important issue in neurobiology. Chang (1986) has recently concluded that the delayed rectifier potassium current, I_K, is not a factor in this process based on the relative lack of effect of various potassium channel blockers on V_R. I have drawn the opposite conclusion from his results based on voltage clamp measurements of I_K and a consideration of the voltage dependence of K channel blockers.

The resting potential, V_R, corresponds to the condition in which the net ionic current, I, is zero in steady state. For squid axons at rest,

$$I(V) = I_K(V) + I_{bg}(V), \quad (1)$$

where V is membrane potential, $I_K(V)$ is the steady-state amplitude of the delayed rectifier, and $I_{bg}(V)$ is the background current. The latter component corresponds to the "leak" current in the Hodgkin and Huxley (1952) analysis of squid axon currents. I have written this component as I_{bg} to emphasize that it is intrinsic to the membrane and not an injury or leakage artefact. The sodium current, I_{Na}, is left out of Eq. 1 because it plays a minor role at rest based on the relatively small effects of TTX on V_R. Consequently, the key issue here is the relative contributions of I_K and I_{bg} to the net current. My experiments demonstrate that the amplitude of I_{bg} is remarkably small, as illustrated by the *uncorrected* membrane currents in Fig. 1 for voltage steps to -160, -20, and 0 mV (holding potential, -80 mV). The -20- and 0-mV results illustrate the I_K component. The -160-mV record, as well as the -20- and 0-mV results, illustrate a lack of a significant I_{bg}. In fact, I_{bg} was too small to be measured, although an upper estimate of 20 $\mu S \cdot cm^{-2}$ could be obtained for its conductance. These results are representative of axons having a V_R more negative that -60 mV in normal seawater ($n = 6$).

The steady-state amplitude of I_K in the Hodgkin and Huxley (1952) model is given by $I_K = n_\infty^4 g_K (V - E_K)$, where $n_\infty = [\alpha/(\alpha + \beta)]$ with $\alpha = -0.01 (V + 50)/\{\exp[-0.1 (V + 50)] - 1\}$ and $\beta = 0.125 \exp[-(V + 60)/80]$, $g_K = 36$ mS $\cdot$ cm^{-2}, and E_K, the potassium equilibrium potential, $= -72$ mV. However, measurements of the fully activated current-voltage relation over a broader voltage range than that used by Hodgkin and Huxley (1952) have demonstrated that this relation is, in general, a nonlinear, rather than a linear function of the driving force, which can be approximately described by the Goldman-Hodgkin-Katz equation (Binstock and Goldman, 1971; Clay and Shlesinger, 1983). In particular, I have found that $I_K = 0.92 (V/25) [K_o - K_i \exp(V/25)]/[1 - \exp(V/25)]$ mA $\cdot$ cm^{-2}, with V in millivolts, is a good, phenomenological description of my measurements of the fully activated current-voltage relation with K_o and K_i equal, in moles, to the external and internal potassium ion concentrations, respectively (Clay, 1984). Consequently, the total current, at rest, is given by

$$I(V) = g_{bg}(V + E_{bg}) + 0.92\, n_\infty^4 (V/25) [K_o - K_i \exp(V/25)]/[1 - \exp(V/25)], \quad (2)$$

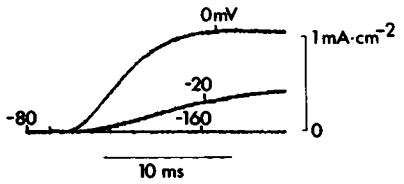

FIGURE 1 Uncorrected membrane current records with voltage steps to -160, -20, and 0 mV (holding potential, -80 mV) showing the relative lack of background current. Internal perfusate contained 250 mM K glutamate, 25 mM K_2HPO_4, and 400 mM sucrose. Methods further described in Clay and Shlesinger (1983).

where $g_{bg} < 20$ $\mu s \cdot cm^{-2}$ and E_{bg} is the reversal potential for the background current. I have used $E_{bg} = -49$ mV, as in the Hodgkin and Huxley (1952) model, although the exact value of this parameter is not of critical significance in the following analysis, provided that it is positive to ~ -50 mV.

Chang (1986) has measured the effect of three modifiers of I_K on V_R: internal application of 20 mM TEA^+, internal application of 2 mM 4-aminopyridine (4-AP) and 100 mM Cs^+, and long term exposure of the membrane both internally and externally to K^+-free media, which has been shown to cause an irreversible loss of I_K (Almers and Armstrong, 1980). Chang (1986) reported a reduction of I_K by 97.6, 99.2, and 91.7%, respectively, for these three conditions. I do not object to the latter two results. However, Chang (1986) has ignored the voltage dependence of the TEA^+ effect by extrapolating the degree of block at $+50$ to -70 mV. Blockade of outward current with TEA^+ is more potent than blockade of inward current (Armstrong, 1966). Specifically, I have found that 20 mM TEA^+ blocks ~70% of I_K at -70 mV (Clay, 1985) rather than the value of 97.6% reported by Chang (1986). This reinterpretation of Chang's work is the critical part of the following analysis. He reported relatively little depolarization of V_R with TEA^+ (Table I), which would argue against a role for I_K in determining V_R, if blockade of I_K at rest were as significant under these conditions as he reported. A similar objection might be raised against his interpretation of results with Cs^+, which is also a voltage-dependent blocker. However, he used 4-AP in these axons, as well, which is a potent blocker of I_K at rest (Yeh et al., 1976). Consequently, the I_K component probably is blocked almost completely under these conditions (Table I). The third condition used by Chang (1986), removal of I_K in K^+-free media, is voltage independent. Therefore, extrapolation of current reduction at $+50$ to -70 mV is clearly appropriate for these conditions.

The experimental results of V_R from Chang (1986) are reproduced in Table I along with the theoretical results calculated from Eq. 2 with $K_o = 0$ and $K_i = 0.25$ M, which corresponds to Chang's experimental conditions. (The parameters V and E_{bg} in Eq. 2 are in millivolts; g_{bg} is in S $\cdot$ cm^{-2}). The respective reductions of I_K are also given in Table I. The value of $g_{bg} = 2.2$ $\mu S \cdot cm^{-2}$ was chosen so as to give the best fit to these results. The

TABLE I
EFFECTS OF VARIOUS MODIFIERS OF I_K ON RESTING POTENTIAL

Treatment	Channels blocked	Experiment	Theory
		V_R	V_R
	%	mV	mV
Control	0	−69.2 ± 1.6	−69.9
TEA^+	68	−68.4 ± 1.3	−66.5
K^+-free	91.7	−61.0 ± 1.7	−62.1
4-AP Cs^+	99.2	−57.8 ± 4.5	−55.0

model agrees, within experimental error, with all four measurements of V_R. Specifically, the model predicts increasing depolarization of V_R with increasing blockade of I_K. A rather significant blockade of I_K is required to produce significant depolarization of V_R precisely because of the relatively small amplitude of I_{bg}. This analysis is not markedly sensitive to the exact value of g_{bg}, if this parameter is relatively small. For example, $V_R = -63.1$ mV with $g_{bg} = 20\ \mu S \cdot cm^{-2}$, as compared with −69.9 mV with $g_{bg} = 2.2\ \mu S \cdot cm^{-2}$. The model also can reproduce the depolarization of V_R with an increase in Ca_o^{+2}, reported earlier by Chang (1983), with a shift of the steady-state I_K activation curve by 12.5 mV in the depolarizing direction per 10-fold increase in Ca_o^{+2} (Frankenhauser and Hodgkin, 1957; Gilbert and Ehrenstein, 1969; Armstrong and Matteson, 1986). A permeability of the membrane to Ca_o^{+2}, proposed by Chang (1983), is not required to explain this result.

The implication of Chang's work is that a new interpretation of membrane current is required to explain his measurements of resting potential. However, his results can be described by the Hodgkin and Huxley (1952) model with two modifications: a significant reduction in the background conductance; and a change of the fully activated current-voltage relation from a linear to a nonlinear function of driving force. The latter result is based on experimental observations. The former result, in particular the value of $2.2\ \mu S \cdot cm^{-2}$ for g_{bg}, is hypothesis, although this result is consistent with the upper limit of $20\ \mu S \cdot cm^{-2}$ from the results in Fig. 1. I have, quite often, observed larger values of g_{bg} from axons having resting potentials more positive than −60 mV. In fact, the amplitude of g_{bg} appears to be correlated with V_R. Because V_R is outside of the physiological range in these axons, I have concluded that these larger values of g_{bg} are artefactual. The value of g_{bg} given above ($2–3\ \mu S \cdot cm^{-2}$) may seem unrealistically low, although this result is not without precedent. For example, Moore and Cole (1960) deduced from their in vivo recordings of resting and action potentials from squid axons that g_{bg} might be as low as $1\ \mu S \cdot cm^{-2}$, based on the rate of repolarization from the foot of the action potential to the resting potential. The ionic nature of I_{bg} is unknown, although it appears to be carried, in part, by sodium ions, based on the hyperpolarization of V_R produced by removal of Na_o (Chang, 1983 and 1986).

The resting potential of intact squid axons under normal physiological conditions ($K_o = 10$ mM) is −67.2 ± 2.9 mV ($n = 10 \pm$ SD). Eq. 2 predicts −66.8 mV. Furthermore, the analysis reveals that the slope conductance of I_K at −66.8 mV is six times greater than g_{bg}. This result together with the above description of changes in V_R produced by K channel blockers suggests that I_K is indeed a significant factor in determining V_R in nerve membrane.

Received for publication 8 February 1988 and in final form 5 July 1988.

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