DELAYED KINETICS OF SQUID AXON POTASSIUM CHANNELS DO NOT ALWAYS SUPERPOSE AFTER TIME TRANSLATION

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ABSTRACT The activation of potassium ion conductance in squid axons by voltage-clamp depolarization is delayed when the depolarizing step is preceded by a conditioning hyperpolarization of the axonal membrane. Moreover, the control conductance kinetics superpose with the delayed kinetics when they are translated along the time axis by an amount equal to the delay. We have found that the degree of superposition with internally perfused axons depends upon voltage-clamp protocol. The kinetics superpose almost exactly for modest test depolarizations, whereas they clearly fail to superpose completely for more positive levels of membrane depolarization. We have modeled these results by incorporating a time dependence into the rate constant of activation of potassium channel gates in the Hodgkin and Huxley model of potassium ionic conductance.

INTRODUCTION

Recent measurements from frog nodes of Ranvier (Palti et al., 1976; Begenesich, 1979; Ilyin et al., 1980) of the delays induced in the activation of potassium ion conductance kinetics by conditioning hyperpolarizing prepulses have demonstrated a lack of complete superposition of the kinetics after time translation. This is contrary to the original observations on squid giant axons by Cole and Moore (1960). We have reinvestigated this effect using internally perfused squid axons. We have found that the degree of superposition depends upon voltage-clamp protocol. The kinetics superpose almost exactly after time translation for modest levels of depolarizing test potential, whereas they clearly fail to superpose for larger depolarizing levels.

We have modeled these results by incorporating a time dependence into the voltage-dependent rate constants of the potassium channel gating particles. The Hodgkin and Huxley (1952) model of the gating mechanism with this modification is sufficient to describe our results.

METHODS

The experimental results described in this paper were obtained from giant axons of *Loligo pealei* under internal perfusion and conventional axial wire voltage-clamp conditions. The voltage clamp used series resistance compensation. Axons were perfused by means of a cannula that was concentric with the axial wire. Membrane currents were digitized after appropriate filtering. The temperature of all experiments ranged between 5° and 8°C. In any single experiment the temperature was maintained constant to within ±0.1°C.

The internal perfusate consisted of 50 mM KF, 200 mM K-glutamate, 25 mM $K_2H_2PO_4$, and 505 mM sucrose. The external solution consisted of artificial seawater (ASW) containing 440 mM NaCl and 10, 50, or 300 mM KCl; 50 mM $MgCl_2$; 10 mM $CaCl_2$; 10 mM Tris-HCl; and 1 μM tetrodotoxin (TTX). Internal and external solutions each had a pH of 7.2. The liquid junction potentials between the internal and external solutions were measured with the experimental electrodes and a salt bridge of 3 M KCl in 3% agar, which was used to connect the solutions. The results of this procedure were −4.5, −4, and −3 mV for the internal perfusate and the 10, 50, and 300 mM K ASW, respectively. The potentials reported in the text and in Fig. 1 and 2 have been corrected accordingly.

During these experiments the axons were held at −80 mV (inside negative) between each sequence of test potential steps. The voltage-clamp protocol consisted of a 5-ms duration prepulse to a conditioning hyperpolarized level (−100 to −240 mV in 20-mV increments) followed by a step to a depolarizing test potential (0, +50, or +100 mV).

Best fits of the theory described below (Results) to the experimental data were obtained using the mathematical modeling program MLAB (Knott and Shrager, 1972) implemented on the DEC-PDP-10 digital computer (Digital Equipment Corp., Maynard, MA) at the Division of Computing Research and Technology, National Institutes of Health, Bethesda, MD.

RESULTS

Membrane currents recorded for test potentials of +4, +54, and +104 mV and prepulse potentials of either −96 or −236 mV are shown in Fig. 1. The current obtained from the −96 mV prepulse has been translated along the time axis to give maximum superposition with the current obtained with the −236 prepulse for each test depolarization. These results describe our primary observation; the currents superpose almost exactly for the +4 mV test potential and all levels of hyperpolarizing prepulse, whereas they clearly fail to superpose for test levels of +54 and +104 mV with hyperpolarizing prepulses more negative than ≃ −160 mV. Similar results were obtained in all experiments (n = 7). The lack of superposition at the relatively large test potentials of +54 and +104 mV was not related to voltage-dependent block of outward potassium current such as is produced either by internal Cs or Na ions (Bezanilla and Armstrong, 1972; French and Wells, 1977). The internal perfusate in these experiments did not contain potassium channel blockers. Moreover, the instantaneous current-voltage relation was approximately linear in the outward current direction in all experiments. Furthermore, our measurements were not influenced by the level of external potassium ion concentration. Similar kinetics were obtained for 10, 50, or 300 mM potassium TTX-ASW, which suggests that potassium ion accumulation in the periaxonal space did not significantly influence our results.

The relationship of delay amplitude to conditioning hyperpolarization and test depolarization was similar to the results reported by Begenesich (1979). The delay increased monotonically with increasingly negative hyperpolarization. No saturation of the effect was observed, although the rate of increase of delay was smaller for conditioning potentials negative to ≃ −200 mV.

We have modeled our results by incorporating a time dependence into the activation rate constant, α_n, in the Hodgkin and Huxley (1952) model (hereafter referred to as HH) of potassium conductance so that after a voltage jump occurs

$$\alpha_n(t) = \alpha_n(V_2) + [\alpha_n(V_1) - \alpha_n(V_2)]e^{-t/t_D}, \qquad (1)$$

where $\alpha_n(V_1)$ is the value of α_n corresponding to the prepulse potential [$\alpha_n(V_1) \simeq 0$ for the conditions of our measurements], $\alpha_n(V_2)$ is the value of α_n corresponding to the test potential, and t_D is the time constant for the change of α_n from its initial to its final steady-state value. We let t_D be a function of both V_1 and V_2. The kinetics for potassium gate molecules in this model are given by

$$dn(t)/dt = -[\alpha_n(t) + \beta_n]n(t) + \alpha_n(t) \qquad (2)$$

where $n(t)$ and β_n imply the usual HH notation. The solution to Eq. 2 is

$$\begin{aligned} n(t) = {} & \exp[-t/\tau_n(V_2)] \\ & \exp[\alpha_n(V_2)t_D(1 - \exp(-t/t_D))]\,\alpha_n(V_2) \\ & \cdot \left(\int_0^t dt' \exp[t'/\tau_n(V_2)] \exp(-\alpha_n(V_2)t_D \right. \\ & \left. \cdot [1 - \exp(-t'/t_D)])\,[1 - \exp(-t'/t_D)]\right). \end{aligned} \qquad (3)$$

When $t_D = 0$, Eq. 3 reduces to the usual HH expression.

We have fitted the results of Fig. 1 (after correction for linear leakage and capacitative currents) to $I_{ss}(V)$ [$n^4(t)/n^4(\infty)$] as in the HH model where $I_{ss}(V)$ is the steady-state current of the potassium channels and $n(t)$ is given by Eq. 3, rather than by the usual HH expression for $n(t)$. The best-fit theoretical curves are shown in Fig. 2, along with the experimental results represented by the symbols (●). The parameters of the theory for each curve are given in the legend of Fig. 2.

We note that the standard HH n^4 model satisfactorily describes the experimental results for +4 mV with

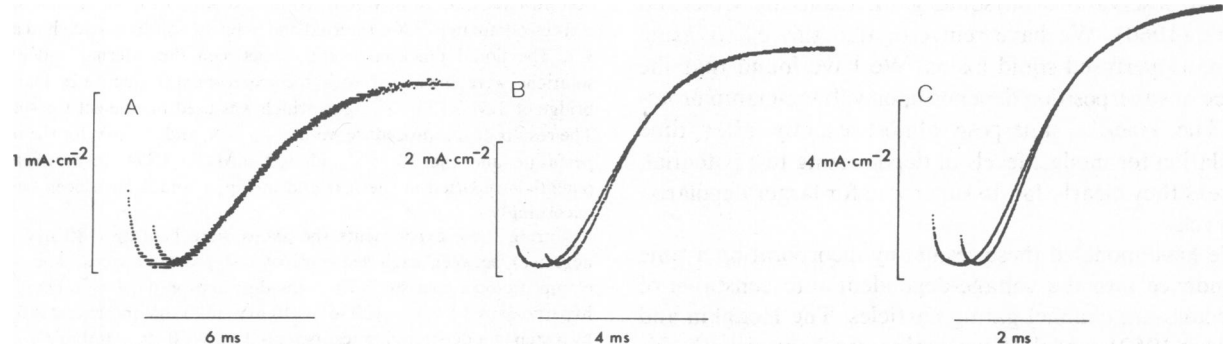

FIGURE 1 Potassium currents from an internally perfused axon bathed in TTX-ASW containing 50 mM K. Currents correspond to a test potential of +4 mV (*A*), +54 mV (*B*), or +104 mV (*C*) with a conditioning hyperpolarization to either −96 or −236 mV. For each pair of records the current with the −96 mV prepulse was shifted along the time axis until maximum degree of superposition was obtained with the record from the −236 mV prepulse. The amplitude of time translation was 0.88 ms for *A*, 0.70 ms for *B*, and 0.46 for *C*. T = 5°C.

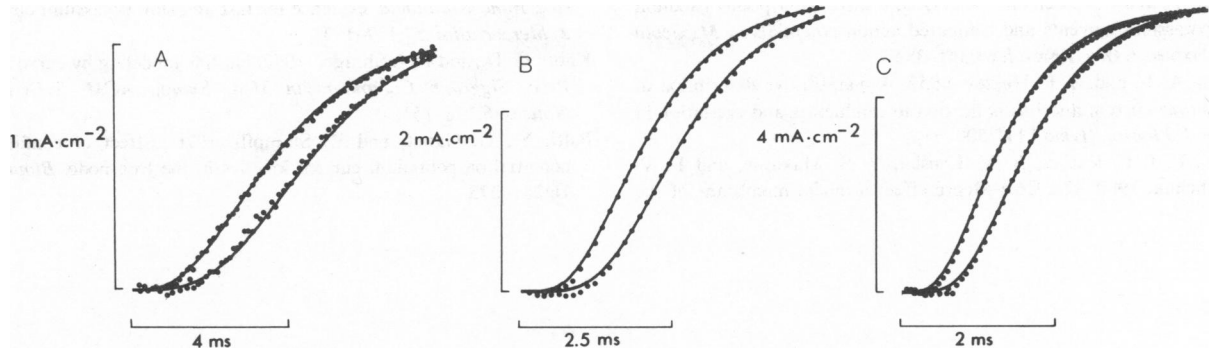

FIGURE 2 Best fit of the model (solid lines) described in the text to the data of Fig. 1. Data are represented by the symbols (●), without time translation and after subtraction of linear leakage and capacitative currents. Parameter values of the model are as follows. *A:* $\tau_n(V_2) = 1.96$ ms; $\alpha_n(V_2) = 0.54$ ms^{-1} for both curves; $I_{ss} = 1.06$ mA · cm^{-2}, $t_D = 0$ for the −96-mV prepulse curve; and $I_{ss} = 1.19$ mA · cm^{-2}, $t_D = 0.88$ ms for the −236-mV prepulse curve. *B:* $\tau_n(V_2) \simeq \alpha_n(V_2)^{-1} = 0.98$ ms for both curves; $I_{ss} = 3.28$ mV · cm^{-2}, $t_D = 0.21$ ms for the −96 − mV prepulse curve, and $I_{ss} = 3.12$ mA · cm^{-2}, $t_D = 0.511$ ms for the −236-mV prepulse curve. *C:* $\tau_n(V_2) \simeq \alpha_n(V_2) = 0.64$ ms; $I_{ss} = 5.86$ mA · cm^{-2}, $t_D = 0.082$ ms for the −96-mV prepulse curve; and $\tau_n(V_2) \simeq \alpha_n(V_2) = 0.71$ ms, $I_{ss} = 5.98$ mA · cm^{-2}, $t_D = 0.24$ ms for the −240-mV prepulse curve.

prepulse to −96 mV, whereas n^4, or n^x, where x is any positive real number, fails to describe the corresponding results for test potentials of +54 and +104 mV. The model with the delay parameter was sufficient to describe all of our activation kinetics. Moreover, it also satisfactorily described our deactivation, or tail, kinetics following return of the membrane potential to holding level after activation of the kinetics by modest test depolarizations. These results closely approximated a single exponential time course. We did not observe a sigmoidal time dependence in the tail currents. This result forced us to delete a time dependence from β_n, the rate constant for closing of the gating particles.

DISCUSSION

We have shown that delayed kinetics of squid axon potassium channels do not always superpose after time translation, in contrast to the original findings of Cole and Moore (1960). However, our results are not directly comparable to theirs because of differences in experimental conditions. The most notable difference is that we used internally perfused axons, whereas they used intact axons. The difference in results could well be attributable to differences between the ionic constituents of our internal perfusate and axoplasm, since internal ions have been shown to influence gating of potassium channels in squid axons (Adams and Oxford, 1981).

Results similar to ours have been reported for frog nodes of Ranvier (Palti et al., 1976; Begenesich, 1979; Ilyin et al., 1980), but not for *Myxicola* axons, which show simple superposition (Goldman and Schauf, 1973). Ilyin et al. (1980) attributed some of their findings to two types of potassium channels, as evidenced by two exponential components in their tail-current recordings. It appears that this mechanism does not apply to our results, because we observed tail currents that closely approximated a single exponential time course. If squid axon potassium channels do, in fact, comprise a homogeneous population, then a model with at least one kinetic feature additional to the Hodgkin and Huxley (1952) equations for potassium channel kinetics is required to describe our results. One way to describe the lack of sperposition is a precursor state that is coupled to the HH, or a similar model, in the traditional way by voltage-dependent rate constants. We have shown that a nontraditional approach involving time-dependent rate constants may provide an alternative model. However, we regard this approach as a tentative description of the data, which may have to be modified after further experimentation.

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