POTASSIUM ION ACCUMULATION SLOWS THE CLOSING RATE OF POTASSIUM CHANNELS IN SQUID AXONS

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ABSTRACT Potassium ion accumulation in the periaxonal space between squid axonal membrane and the Schwann cell surrounding the axon slows the rate of potassium channel closing to a degree that is consistent with the effect on channel closing of an equivalent change in the bulk external potassium concentration. The alteration of channel gating is independent of membrane potential, V, for $V \leq -60$ mV, which suggests that the effect is mediated at a site on the outer surface of the membrane, rather than a site within the channel.

INTRODUCTION

A few years ago, Swenson and Armstrong (1981) demonstrated that an increase in the external potassium ion concentration, K_o, slowed the rate of potassium channel closing in squid axons. They attributed their observations to an interaction between the channel gating mechanism and the occupancy of a site within the channel by a potassium ion (the "foot-in-the-door" model). The effects of potassium ion accumulation on channel closing provide an important test of this hypothesis, since a decrease in the closing rate should occur regardless of the manner in which the increase in K_o is achieved, be it by a change in the bulk concentration or by a change in the local concentration outside the membrane produced by ion accumulation during a depolarizing voltage-clamp step. Alternatively, the results of Swenson and Armstrong (1981) could be attributable to a slowly developing effect of K_o, as deBruin (1982) has suggested, in which case potassium ion accumulation might not alter channel gating. The results in this report demonstrate that ion accumulation does, indeed, slow channel closing to a degree that is consistent with the effect of an equivalent change in the bulk, external potassium ion concentration. However, the effect of K_o on channel gating is independent of voltage for $V < -60$ mV, which suggests that K_o alters the closing rate constant at the surface of the membrane rather than at a site within the channel.

METHODS

Experiments were carried out on internally perfused squid giant axons (*Loligo pealei*) using standard axial wire voltage clamp techniques with series resistance compensation (Clay and Shlesinger, 1983). Internal perfusion was implemented by means of a cannula concentric with the axial wire. The temperature in these experiments ranged between 7° and 9°C. It was maintained constant to with ± 0.1°C during any single experiment. The internal perfusate contained 200 mM K Glutamate, 50 mM KF, 25 mM K_2HPO_4, and 505 mM sucrose. The external solution contained $(440 - x)$ mM NaCl, x mM KCl, where $x = 50$, 100, or 300, 10 mM $CaCl_2$, 50 mM $MgCl_2$, 10 mM Tris-HCl, and 0.5 μM tetrodotoxin (TTX). Experiments were also performed with 500 mM KCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$, 10 mM Tris-HCl, and 0.5 μM TTX as the external solution. Glass pipettes of 50–80 μm tip diameter filled with 0.5 M KCl in 1–2% agar were used as internal and external voltage sensing electrodes. Liquid junction potentials were ≤3 mV. The potentials used here were not corrected for these relatively small voltage offsets. Corrections for leakage and transient capacitance currents were carried out in some but not all of these experiments. Tail current time constants were determined from a single exponential fit to the initial 5 ms of the tail current records. The initial 0.25 ms of each record was deleted in this analysis from uncorrected records.

RESULTS

The primary observations in this report concerning the effect of K_o on channel closing (tail), kinetics are illustrated in Fig. 1. In this experiment the external solution was either 50 mM K^+ seawater (SW), or 300 mM K^+ SW. The membrane potential was stepped to 20 mV with 50 mM K^+ SW as the external solution (Fig. 1 *A*) for either 4, 10, 20, or 60 ms, followed by a return to the holding potential (−100 mV). The membrane current during the depolarizing step increased in the usual sigmoidal manner throughout the first few milliseconds of the step, followed by a slight decline in outward current, which is attributable to potassium ion accumulation in the periaxonal space between the axonal membrane and the Schwann cell surrounding the axon (Adelman et al., 1973; Clay, 1984). The tail currents upon return to the holding potential are also shown in Fig. 1 *A*. The effective potassium ion concentration at the end of each step in Fig. 1 *A* was determined

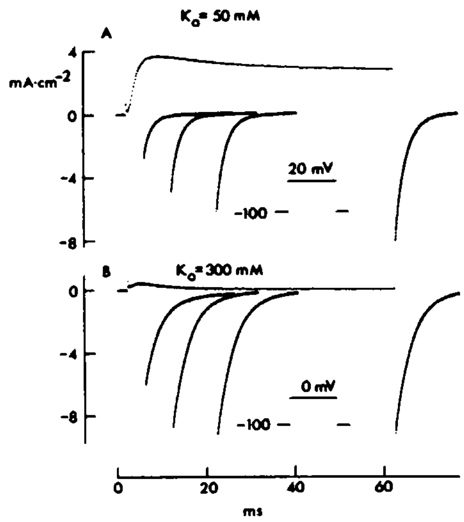

FIGURE 1 (*A*) Superimposed records of membrane current in response to membrane depolarization to +20 mV lasting 4, 10, 20, or 60 ms. External solution was 50 mM K^+ SW. Holding potential was −100 mV. Uncorrected records. Axon C83.13. (*B*) Same protocol as in *A* with depolarization to 0 mV. External solution was 300 K^+ SW. Same preparation as in *A*. T = 8°C.

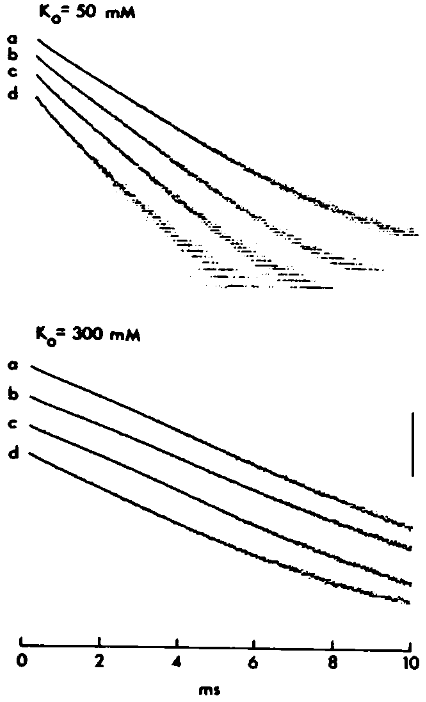

FIGURE 2 (*A*) Semilogarithmic plot of tail currents in Fig. 1 *A*. Record *a*, *b*, *c*, or *d* corresponds to the tail current after depolarization to 20 mV lasting 60, 20, 10, or 4 ms. The time constants determined from the initial 5 ms of each record are (*a*) 4.05 ms; (*b*) 2.56 ms; (*c*) 2.22 ms; (*d*) 1.82 ms. (*B*) Semilogarithmic plots of tail currents in Fig. 1 *B*. Record *a*, *b*, *c*, or *d* corresponds to the tail current after depolarization to 0 mV lasting 60, 20, 10, or 4 ms. The average time constant of all four records is 4.35 ms. The vertical bar represents one natural log unit. T = 8°C.

from the currents (not shown) at the beginning of 5-ms steps to +10, 0, −10, and −20 mV. These results together with the current at the end of the step to +20 mV and the initial tail current amplitude at −100 mV were fitted by the Goldman-Hodgkin-Katz equation (Goldman, 1943; Hodgkin and Katz, 1949), which is given by

$$I_K = A\,V(K_0 - K_i \exp(qV/kT))/(1 - \exp(qV/kT)), \quad (1)$$

where K_o and K_i are the external and internal potassium ion concentrations, respectively, V is the membrane potential, q is the electronic charge, k is the Boltzmann constant, T is the absolute temperature ($q/kT \sim 25$ mV), and A is a constant. The best fit values of K_o for the experiment in Fig. 1 *A* were 60, 110, 160, and 235 mM for the 4, 10, 20, and 60-ms duration steps, respectively. These results are consistent with a time constant of ~45 ms for ion accumulation in the periaxonal space during the prepulse to +20 mV. The external potassium concentration for the results in Fig. 1 *B* was 300 mM. The membrane potential was stepped to 0 mV in this experiment for 4, 10, 20, or 60 ms followed by a return to the holding potential. Under these conditions, relatively little current flowed during the depolarizing step, because the membrane potential was approximately equal to the potassium equilibrium potential, E_K.

The tail currents in Fig. 1 are replotted in a semilogarithmic manner in Fig. 2 on an expanded time scale. These results are approximately described by single exponential functions of time, although the 50 K^+ SW records clearly deviate from single exponentials at small current amplitudes, probably because of an electrode polarization artifact. The results in Fig. 1 *A* demonstrate an effect of pulse duration and, by implication, an effect of K_o on the tail current time constant, τ_{tail}. Alternatively, the effect could, perhaps, be attributable to activation of different populations of K^+ channels for step durations in the 10–60-ms range that deactivate at −100 mV with a time course that is dependent upon pulse duration. The results in Fig. 2 *A* argue against this possibility. The tail current time constants in this experiment with 300 K^+ SW were independent of pulse duration. Moreover, the external potassium concentration did not change significantly during the step to 0 mV. These results are consistent with the membrane having a single, homogeneous population of K^+ channels that are activated with traditional Hodgkin and Huxley (1952) kinetics (Clay, 1984).

The tail currents with 300 K^+ SW in Fig. 2 *B* compared with the tail current following the 4 ms duration depolarization to 20 mV in Fig. 2 *A* with 50 K^+ SW illustrate the increase in τ_{tail} produced by a change in the bulk, external potassium ion concentration, whereas the 10, 20, and 60-ms records in Fig. 2 *A* illustrate the effect of ion accumulation on τ_{tail}. The relationship between τ_{tail} and K_o at −100 mV is further illustrated in Fig. 3, which contains the results of three experiments similar to the one described in Fig. 1 *A*. In two of these experiments τ_{tail} was also determined in 300 K^+ SW, as in Fig. 1 *B*. The effective K_o at the end of the 60-ms pulse to +20 mV with 50 K^+ SW in experiment C83.14 was also 300 mM, and

τ_{tail} under these conditions was virtually identical to τ_{tail} following the change to 300 K^+ SW, as indicated by the arrow in Fig. 3. Fig. 3 also contains results from two preparations in either 50 or 100 K^+ SW followed by a change to 500 K^+ SW. In these experiments a 20-ms pulse to 0 mV was used to activate the potassium channel conductance. The solid line in Fig. 3 is the best fit to these results of $\tau_{\text{tail}}^{-1} = \tau_o^{-1}(1 + K_o/K_D)^{-1}$ with $\tau_o^{-1} = 0.83$ ms^{-1} and $K_D = 122$ mM (Discussion).

The effect of external potassium concentration on τ_{tail} is independent of membrane potential for $V \leq -60$ mV, as illustrated by the results in Fig. 4. Fig. 4 *A* contains tail current records plotted on a semilogarithmic scale for $V = -60, -80, -100$, or -120 mV in 100 K^+ SW (labeled *b*) or 300 K^+ SW (labeled *a*). The ratios of τ_{tail} in 100 K^+ SW and τ_{tail} in 300 K^+ SW are shown in Fig. 4 *B* along with similar results from a second preparation with τ_{tail} in 50 K^+ compared with τ_{tail} in 500 K^+ in the -60 to -200 mV range.

The above analysis is based on the assumption that the external potassium ion concentration in the periaxonal space does not change significantly during the time course of the tail current. The time constant of accumulation in 50 KSW during a voltage step to +20 mV was ~45 ms, as noted above for an outward current amplitude of 3–4 $\text{mA} \cdot \text{cm}^{-2}$ (Fig. 1 *A*). The initial inward current following return to holding potential was 5–8 $\text{mA} \cdot \text{cm}^{-2}$ (Fig. 1 *A*), which would result in a time constant, τ_r, for removal of accumulated ions at the beginning of the tail of ~20 ms. This is a lower bound estimate of τ_r, since channels rapidly close at -100 mV. Consequently, the assumption of a constant potassium ion concentration during the initial 5 ms of the tail current seems justified.

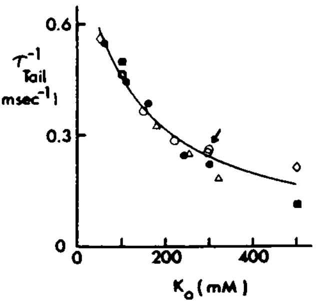

FIGURE 3 Dependence of τ_{tail}^{-1} on K_o for $V = -100$ mV. Each symbol represents the inverse time constant of a single exponential fit to the initial 5-ms portion of the corresponding tail current record. (△) C83.12; (●) C83.13; (○) C83.14. $T = 8$°C for all three experiments. The results in these experiments correspond to tail currents measured after depolarizing pulses to +20 mV in 50 K^+ SW, as in Fig. 1 *A*. The effective K_o for these records was determined from Eq. 1 using the procedure described in the text. The tail current time constant was also determined in experiments C83.13 and C83.14 in 300 K^+ SW, as in Fig. 1 *B*. The effective K_o following the 60-ms pulse to +20 mV with 50 K^+ SW in experiment C83.14 was 298 mM. The value of τ_{tail} under these conditions was virtually the same as in 300 K^+ SW, as indicated by the arrow. In experiment C81.19 (■) τ_{tail} was determined in 100 K^+ SW and 500 K^+ SW after a 20-ms pulse to 0 mV. In experiment C81.67 (◇) τ_{tail} was determined in 50 K^+ SW and 500 K^+ SW after a 20-ms pulse to 0 mV. The results from these different preparations have been scaled as follows: C83.12, 1:0.92; C83.13, 1:1; C83.14, 1:1.2; C81.19 ($T = 9$°C), 1:0.59, C81.67 ($T = 7$°C), 1:1.4.

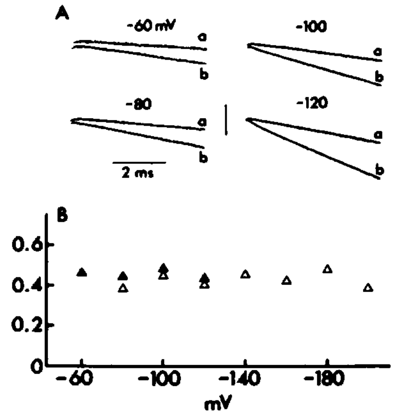

FIGURE 4 Lack of voltage dependence of the effect of K_o on τ_{tail}. (*A*) Semilogarthmic plots of tail currents in 100 K^+ SW (labeled *b*) and 300 K^+ SW (labeled *a*) for -60, -80, -100, and -120 mV. Records corrected for capacitance transients. Experiment C81.18. The vertical bar represents one-half natural log unit. (*B*) Ratio of τ_{tail} $(K_{o,1})$ and τ_{tail} $(K_{o,2})$ as a function of potential. (▲) Experiment C81.18. $T = 8$°C. $K_{o,1} = 100$ mM, $K_{o,2} = 300$ mM. (△) Experiment C81.67. $T = 7$°C. $K_{o,1} = 50$ mM, $K_{o,2} = 500$ mM.

DISCUSSION

The results in this report concerning the effect on channel closing by a change in K_o are consistent with the original observation of this effect by Swenson and Armstrong (1981). They also demonstrate that potassium ion accumulation slows channel closing to a degree that is consistent with an equivalent change in the bulk external concentration. That is, the effect appears to be mediated by rapid binding of a potassium ion to the membrane instead of a slowly developing process, such as sensitization of the membrane to a change in K_o. However, the lack of voltage dependence of the effect, illustrated in Fig. 4, suggests that the binding site is located outside of the membrane electric field, rather than within the channel, as suggested by Swenson and Armstrong (1981). This result can be illustrated by considering the following description of their model:

$$[C] \underset{\beta}{\overset{4\alpha}{\rightleftharpoons}} [C] \underset{2\beta}{\overset{3\alpha}{\rightleftharpoons}} [C] \underset{3\beta}{\overset{2\alpha}{\rightleftharpoons}} [C] \underset{4\beta}{\overset{\alpha}{\rightleftharpoons}} [O] \underset{\ell}{\overset{\kappa}{\rightleftharpoons}} [O'],$$

in which [O] and [O′] are open states of the channel having equivalent conductances, [C] represents closed states of the channel, α and β are traditional Hodgkin and Huxley (1952) rate constants for the potassium channel, $\kappa = \kappa_o K_o \exp(-dqV/2kT)$ and $\ell = \ell_o \exp(dqV/2kT)$, where κ_o and ℓ_o are constants of the order of 1 μs^{-1}, and d is the relative distance within the channel electric field of a binding site for a potassium ion that must leave the channel before the channel can close ($d = 0$ corresponds to the external membrane surface). The forward rate constant, α, for channel activation is virtually zero for $V \leq -60$ mV. Under these conditions, and with $4\beta << \ell_o, \kappa_o$, the tail current time constant is given by ${\tau_{tail}}^{-1} = 4\beta\ell/(\ell + \kappa) = {\tau_o}^{-1}\ell/(\ell + \kappa)$, where τ_o is the time constant with $K_o = 0$ and

$$\ell/(\ell + \kappa) = (1 + K_0 K_D^{-1} \exp{(qdV/kT)})^{-1}, \qquad (2)$$

where $K_D = \kappa_o/\ell_o$. The results in Fig. 4 are consistent with $d = 0$, in which case the above analysis is reduced to ${\tau_{tail}}^{-1} = {\tau_o}^{-1}/(1 + K_o/K_D)$ for all membrane potentials less than -60 mV. The theoretical curve in Fig. 3 is the best fit of this expression to the data with ${\tau_o}^{-1} = 0.83$ ms and $K_D = 122$ mM. This model places the potassium ion binding site outside of the electric field of the channel, whereas closure of channel gates probably occurs within the electric field. An alternative hypothesis, which circumvents this problem, is that a potassium ion binds to a site on the outer surface of the membrane, thereby directly altering the rate constant, β, for channel closing. The forward rate constant, α, does not appear to be altered, since K_o has relatively little effect on channel activation (Clay, 1984). Ion permeation and channel gating are not directly linked in this model. A similar mechanism has been proposed by Cahalan and Pappone (1983) for the effects of trinitrobenzene sulphonic acid (TNBS), which also slows potassium channel closing without altering channel activation. This mechanism is probably not common to all agents which alter K^+ channel gating. For example, zinc and other divalent ions significantly alter K^+ channel activation with only a minor effect on tail kinetics (Gilly and Armstrong, 1982), which suggests a different site of action for these ions than the one suggested here.

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