Recovery from C-Type Inactivation Is Modulated by Extracellular Potassium

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ABSTRACT Extracellular potassium modulates recovery from C-type inactivation of Kv1.3 in human T lymphocytes. The results of whole-cell patch clamp recordings show that there is a linear increase in recovery rate with increasing $[K^+]_o$. An increase from 5 to 150 mM K^+_o causes a sixfold acceleration of recovery rate at a holding potential of −90 mV. Our results suggest that 1) a low-affinity K^+ binding site is involved in recovery, 2) the rate of recovery increases with hyperpolarization, 3) potassium must bind to the channel before inactivation to speed its recovery, and 4) recovery rate depends on external $[K^+]$ but not on the magnitude of the driving force through open channels. We present a model in which a bound K^+ ion destabilizes the inactivated state to increase the rate of recovery of C-type inactivation, thereby providing a mechanism for autoregulation of K^+ channel activity. The ability of K^+ to regulate its own conductance may play a role in modulating voltage-dependent immune function.

INTRODUCTION

Voltage-gated K^+ channels open when depolarized and some, but not all, will inactivate during prolonged depolarization (Jan and Jan, 1992). Two distinct types of inactivation have been identified in *Shaker*-like K^+ channels: N-type and C-type. N-type inactivation involves the binding of a tethered blocking particle, containing positively charged N-terminal amino acids, to the cytoplasmic face of the channel (Hoshi et al., 1990; Demo and Yellen, 1991), and only one inactivation particle, acting independently of the other particles, is sufficient to inactivate the channel (MacKinnon et al., 1993; Gomez-Lagunas and Armstrong, 1995). In contrast, C-type inactivation occurs by a cooperative process in which all subunits participate equally (Panyi et al., 1995). The detailed mechanism of C-type inactivation is unknown, but may involve a constriction of the outer mouth of the pore, preventing conduction (Yellen et al., 1994). While N- and C-type inactivation may be caused by separate mechanisms, when they coexist, they appear to be coupled to each other (Hoshi et al., 1991). N-type and C-type inactivation can be pharmacologically distinguished in that the former is inhibited by internal tetraethylammonium (TEA) whereas the latter is inhibited by external TEA (Choi et al., 1991).

The predominant K^+ channel of T lymphocytes is the *Shaker*-like K^+ channel Kv1.3 (Matteson and Deutsch, 1984; DeCoursey et al., 1984; Attali et al., 1992). This channel inactivates with a time constant of 150–300 ms (Cahalan et al., 1985; Deutsch et al., 1986), and inactivation is slowed by external TEA (Grissmer and Cahalan, 1989a,) but not internal TEA (Levy and Deutsch, unpublished observations). Additionally, an N-terminal deletion mutant of Kv1.3 exhibits inactivation kinetics similar to that of the wild-type channel (Tu et al., 1995). These observations strongly suggest that C-type inactivation is the sole mechanism of inactivation of Kv1.3 in T lymphocytes. The factors that govern entry into and exit from this inactivated state (i.e., inactivation and recovery) have not yet been fully characterized. In this paper, we present evidence that the rate of recovery from C-type inactivation is accelerated specifically by elevated extracellular K^+. The effect of $[K^+]_o$ occurs in the physiological range of elevated K^+ and suggests cation binding to a low-affinity site on the channel. These findings implicate a physiological role for autoregulation of K^+ channels in T cells by extracellular K^+.

MATERIALS AND METHODS

Cell culture and T lymphocyte selection

Human peripheral blood mononuclear cells were isolated from heparinized venous blood collected from healthy human donors, and separated by gradient centrifugation with Ficoll-Hypaque. Cells were cultured in a 5% CO_2 incubator at 37° for 36–80 h in a serum-free, RPMI-based medium (Deutsch et al., 1991). The culture medium also contained 3 μg/ml of the mitogen phytohemagglutinin (Difco, Detroit, MI) to increase K^+-channel expression (Deutsch et al., 1986), and cell stimulation was confirmed on occasion by measuring [^{3}H]thymidine incorporation into DNA during an 8-h period on the third day after isolation of the cells. T lymphocytes were selected by incubation with mouse anti-human CD2 antibody (Becton-Dickinson, San Jose, CA) followed by adhesion to petri dishes coated with goat anti-mouse IgG antibody (Biosource, Camarillo, CA), as previously described by Matteson and Deutsch (1984).

Electrophysiology

Conventional whole-cell patch-clamp procedures were used (Matteson and Deutsch, 1984; Hamill et al., 1981), with pipettes pulled from SG10 glass (Richland Glass, Richland, NJ) and coated with Sylgard 184 (Dow Corning, Midland, MI) to give electrodes of 2–4.5 MΩ. All experiments were carried out at room temperature. Holding potential was maintained at either −70 mV or −90 mV. Current recording did not begin until 8 min after

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achieving the whole-cell configuration to ensure stability of channel kinetics.

A two-pulse protocol was used to measure recovery from inactivation by applying a pair of 800-ms voltage steps to +50 mV, separated by an interpulse interval of varied duration and voltage. The cell voltage was maintained at the holding potential for at least 2 min between each two-pulse protocol. Peak K^+ currents ranged from 0.15 to 6 nA, and therefore 0–70% series resistance compensation, respectively, was used to reduce voltage errors below 15 mV. The driving force was determined for each set of solutions by applying a voltage ramp that passed through the predicted reversal potential, and current traces were subsequently corrected for ohmic leak and voltage error caused by series resistance, as described by Panyi et al. (1995).

Solutions

The normal internal solution contained (in mM) 130 KF, 10 KCl, 11 K_2EGTA, 10 HEPES acid (pH = 7.20 with KOH, 295 mOsm). A low-potassium internal solution contained 118 *N*-methylglucamine-fluoride (NMG-F), 5 NMG-Cl, 5 KCl, 11 EGTA, 22 NMG base, 10 HEPES acid (pH = 7.20 with NMG, 295 mOsm). All external solutions included the following (in mM): 5.5 glucose, 10 HEPES, 2.5 $CaCl_2$, 1 $MgCl_2$ (pH = 7.35, 305 mOsm). The control solution also contained 145 NaCl and 5 KCl. The 150 mM solutions of Na^+, K^+, Rb^+, Cs^+, NH_4^+, choline$^+$, and *N*-methylglucamine$^+$ contained 150 mM of the appropriate chloride salt. The pH was balanced with NaOH for the control and 150 mM Na^+ bath solutions, and KOH was used for the 150 mM K^+ solution. Balance of pH for all of the other extracellular solutions was achieved with *N*-methylglucamine base. The choline-based K^+ solutions were prepared by appropriate mixtures of the 150 mM K^+ and the 150 mM choline$^+$ bath solutions.

RESULTS

To study recovery from inactivation of the endogenous lymphocyte Kv1.3 channel, we used a two-pulse protocol that delivered a pair of depolarizing pulses to +50 mV, with a variable interval between the pulses. Fig. 1 shows the potassium currents elicited from a T cell in response to a pair of 800-ms pulses, from a holding potential of −70 mV. To account for cell-to-cell variability in the extent of inactivation that can occur during a long depolarizing pulse, we defined recovery as follows:

$$\text{Fractional Recovery} = \frac{I_{\text{peak2}} - I_{\text{plateau1}}}{I_{\text{peak1}} - I_{\text{plateau1}}}, \qquad (1)$$

where I_{peak1} and I_{peak2} represent the peak currents elicited by the first and second depolarizing pulses, respectively, and I_{plateau1} represents the minimum outward current observed at the end of the first depolarizing pulse. For the cell shown in Fig. 1 *a*, a 4-s interpulse interval (IPI) allowed 30% of the inactivated channels to recover, whereas a 20-s IPI allowed for 84% recovery. Fig. 1 *b* shows the mean fraction of recovery for the indicated IPI. The experimental values are well fit by a single-exponential function giving a recovery rate of 0.116 s^{-1}, corresponding to a time constant of 8.6 s. The data for each cell were also individually well fit by single exponentials (not shown). The single-exponential time course of recovery suggests that there is a single rate-limiting step for recovery from inactivation. We found that the rate of recovery under control conditions did not change throughout the course of any single experiment, as observed by Kupper et al. (1995).

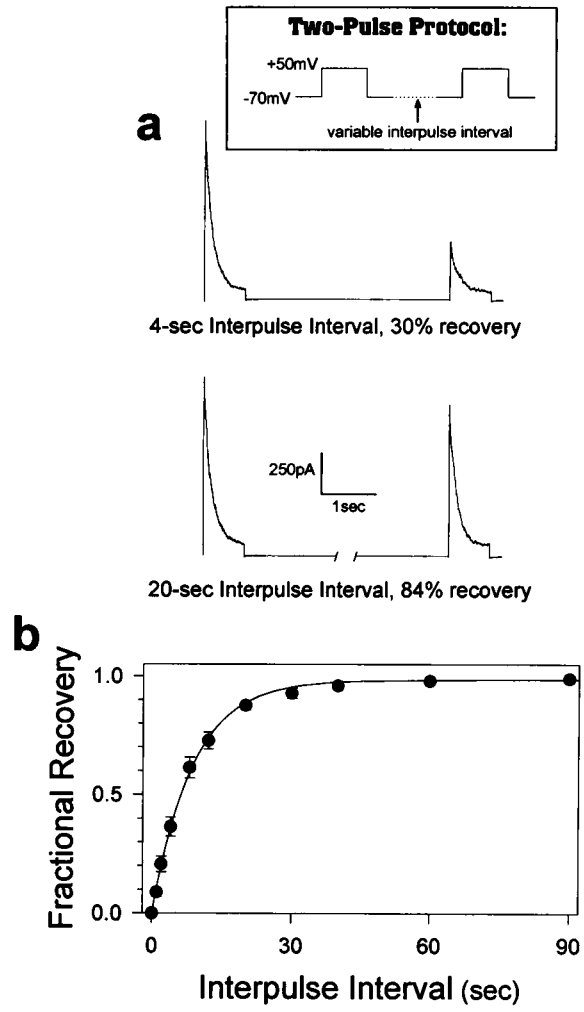

FIGURE 1 Recovery from C-type inactivation. (*A*) Whole-cell currents recorded from a stimulated T lymphocyte. Holding potential was maintained at −70 mV and current for each trace was elicited by a pair of 800-ms voltage steps to +50 mV separated by an interpulse interval (IPI) of 4 s (*above*) or 20 s (*below*) at −70 mV. The 4-s IPI allowed 30% of the inactivated channels to recover, whereas the 20-s IPI allowed for 84% recovery. (*B*) Fraction of recovery as a function of interpulse interval duration. Data points represent mean ± SEM (n = 4). Fractional recovery is defined in the text. The solid line represents a best fit of the function $1 - e^{-(\text{rate})(\text{IPI})}$ to the data.

Extracellular cations regulate recovery from C-type inactivation

Recovery from N-type inactivation can be modulated by extracellular potassium (Demo and Yellen, 1991; Pardo et al., 1992; Gomez-Lagunas and Armstrong, 1994); however, such modulation has not yet been demonstrated for recovery from C-type inactivation. To determine the effect of extracellular K^+ on recovery from C-type inactivation, five cells were perfused with bath solutions containing 5 or 150 mM K^+ and studied using the two-pulse protocol.

Fractional recovery for a representative cell is shown in Fig. 2. The data were well fit by single-exponential functions to give recovery rates of 0.13 s^{-1} and 0.60 s^{-1} for 5 and 150 mM $[K^+]_o$, respectively. The mean recovery rate was 0.11 ± 0.01 s^{-1} for 5 mM and 0.59 ± 0.06 s^{-1} for 150 mM $[K^+]_o$ (±SEM, $n = 5$, $p < 0.001$). Thus, a significant increase in the rate of recovery is seen with the substitution of extracellular K^+ for Na^+.

Raising the $[K^+]_o$ from 5 to 150 mM, we found a 40 ± 5% (±SEM, $n = 8$) increase in maximum whole-cell conductance at +50 mV, similar to that described previously for other voltage-gated K^+ channels (Pardo et al., 1992; Lopez-Barneo et al., 1993). Additionally, 150 mM K^+_o slowed inactivation, increasing the time constant by 58 ± 8%. This effect of K^+ on inactivation rate follows Michaelis-Menten kinetics for a binding site with an apparent K^+ affinity of 12 mM. Similar results have been reported for Kv1.3 in Jurkat cells (Grissmer and Cahalan, 1989b).

To assess the specificity of the enhanced recovery rate seen in 150 mM K^+, recovery from inactivation was measured in cells exposed to bath solutions containing 150 mM of Na^+, K^+, Rb^+, Cs^+, or NH_4^+. As determined by the biionic reversal potentials, the relative permeabilities of these cations are $K^+(1) > Rb^+(0.79) \gg NH_4^+(0.11) \gg Na^+(0.005)$, comparable to those reported by Cahalan et al. (1985). We were unable to observe any inward current with the 150 mM Cs^+ solution, for voltages as low as −140 mV, showing that Cs^+ does not permeate Kv1.3. The recovery rate under each condition was compared to the rate obtained with bath solution containing 145 mM choline and 5 mM K^+. The relative rates of recovery are shown in Fig. 3. Our results indicate that the relative potencies are $K^+ > Rb^+ \gg Cs^+ > NH_4^+ \approx Na^+$. Note that the impermeant Cs^+ ion is more effective than the more permeant NH_4^+ ion. Relative rates of recovery for choline or *N*-methylglucamine were not determined because there was a >95% reduction of the current if either of these molecules were used as the only monovalent cation in the bath. This phenomenon has been noted previously for some other voltage-gated K^+ channels (Pardo et al., 1992; Lopez-Barneo et al., 1993).

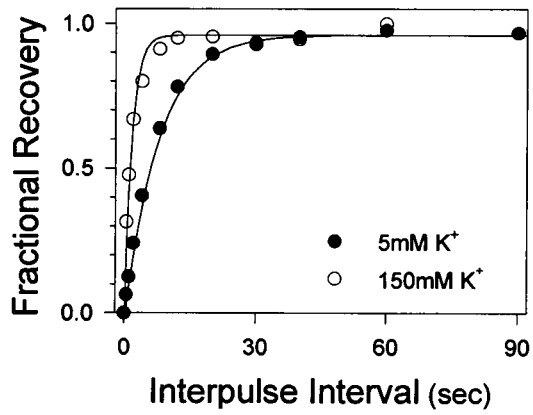

FIGURE 2 The effect of extracellular K^+ on recovery from C-type inactivation. Fractional recovery is shown as a function of interpulse interval for a representative cell perfused with bath solution containing either 5 mM (●) or 150 mM (○) K^+. Holding potential and interpulse voltage were −70 mV. Fractional recovery and recovery rates were determined as described for Fig. 1, giving rates of 0.13 s^{-1} and 0.60 s^{-1} for 5 and 150 mM $[K^+]_o$, respectively.

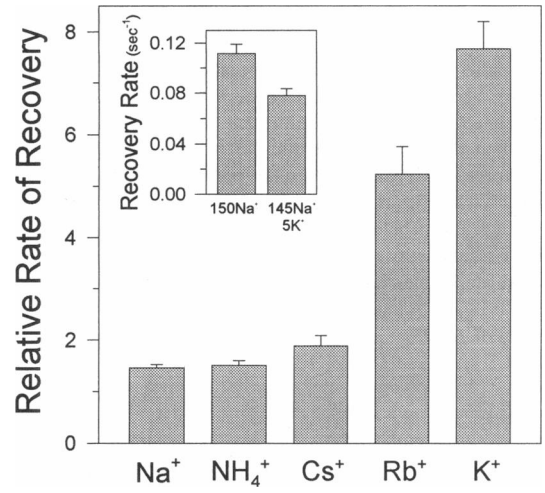

FIGURE 3 The sensitivity of recovery rate to extracellular cations. The relative rate of recovery from inactivation (mean ± SEM, $n = 5$ to 8), normalized to recovery in bath solution containing 145 mM choline and 5 mM K^+, is shown for each test solution. The test solution contained 150 mM of Na^+, NH_4^+, Cs^+, Rb^+, or K^+ as the only permeant cation. Holding potential and interpulse voltage were −90 mV. Absolute recovery rates were estimated from the fractional recovery obtained from each two-pulse protocol (Recovery rate = −ln(1 − fractional recovery)/IPI). For each solution, an interpulse interval was chosen that would allow approximately 35–75% recovery from inactivation. (*Inset*) A low concentration of K^+ interferes with the effect of Na^+_o on recovery from inactivation. Rate of recovery from inactivation (mean ± SEM, $n = 6$) is shown for cells perfused with a 150 mM Na^+ bath or a 145 mM Na^+/5 mM K^+ bath. Replacement of 5 mM of Na^+ with K^+ led to a 30% reduction of recovery rate.

In the course of these experiments, we observed that the recovery rate in a 150 mM Na^+ bath solution exceeded that generally found with a bath solution containing 145 mM Na^+ and 5 mM K^+. We directly compared recovery from inactivation in 150 mM Na^+ bath versus 145 mM Na^+/5 mM K^+ bath solution, and found that addition of 5 mM K^+_o slowed recovery by 30% (inset of Fig. 3, $p < 0.005$, $n = 6$). Therefore, in spite of the enhancement of recovery seen with elevated K^+_o, the presence of a relatively low concentration of K^+ interferes with the effect of Na^+_o.

Potassium binds to a low-affinity modulatory binding site

The variable potency of cations to speed recovery suggests that the inactivated state may be destabilized by selective binding of cations to a site associated with the channel's inactivation machinery. If such a cation binding site exists, then the rate of recovery should correlate with, for example, the concentration of extracellular K^+. Moreover, if this site resides within the membrane electrical field, then hyperpo-

larization during the recovery period should increase cation binding and therefore further speed recovery. To test these hypotheses, we exposed cells to choline-based bath solutions containing various concentrations of potassium. The voltage dependence of this dose response of $[K^+]_o$ on recovery was also measured by adjusting the potential during the interpulse interval. The data are plotted in Fig. 4 *a* as recovery rate versus extracellular potassium concentration for four different interpulse voltages. For each voltage, the data were well fit by linear regressions. The dependence of the recovery rate on $[K^+]_o$ does not saturate and is linear at least until 150 mM K^+, consistent with the involvement of a low-affinity binding site in recovery from C-type inactivation. In spite of this low-affinity binding, the response to $[K^+]_o$ is robust. For example, with an interpulse voltage of −90 mV, there is a sixfold difference in recovery rate between 5 mM and 150 mM K^+_o.

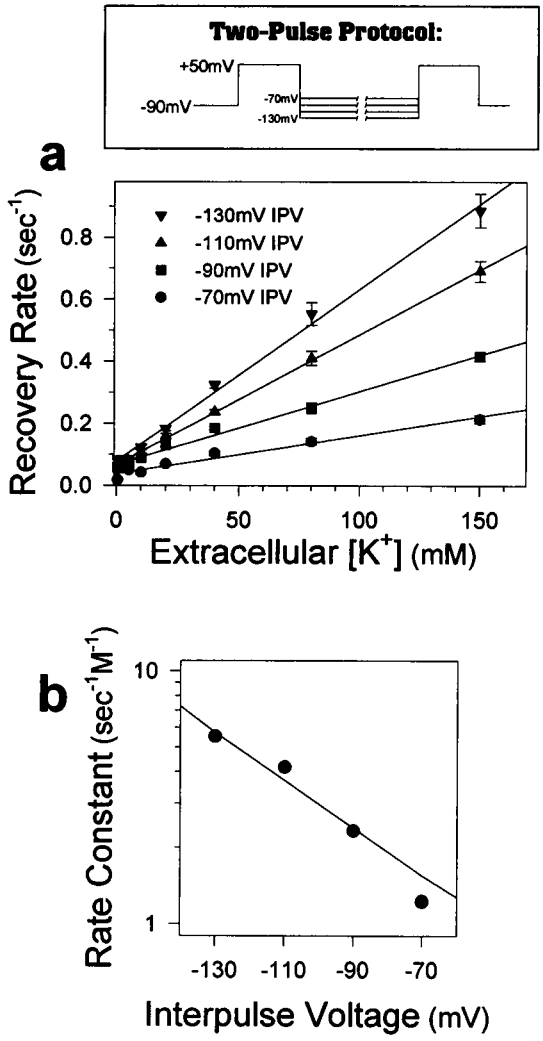

FIGURE 4 The dependence of recovery rate on $[K^+]_o$ and membrane voltage. (*A*) The rate of recovery from C-type inactivation as a function of extracellular $[K^+]$ for four interpulse voltages (IPV). The bath potassium concentration ranged from 2 to 150 mM. Data points represent recovery rates (mean ± SEM, $n = 9$) for IPVs of −70 (●), −90 (■), −110 (▲), and −130 mV (▼). Recovery rates were determined as described for Fig. 3. Holding potential was −90 mV. (*B*) Pseudo-second-order rate constant is plotted for each IPV. Data points represent the slopes of the regressions to the data in *A*. The solid line represents a best fit of the function $Ae^{-\delta EzF/RT}$ to the data, where *A* represents the rate constant extrapolated for 0 mV, and δ is the fraction of the membrane electric field that must be traversed. *E* represents the interpulse voltage, *z* is +1 for the charge of potassium, and *F*, *R*, and *T* have their conventional meanings. In this experiment, $\delta = 0.84$ and $A = 0.34\ s^{-1}$.

The interpulse voltage also influences the recovery rate, causing an effective increase in potency of $[K^+]_o$ at more hyperpolarized potentials. Comparing interpulse voltages of −70 mV and −130 mV, there is approximately a threefold increase in recovery rate for all concentrations of K^+_o tested. Linear regressions of the data obtained for the interpulse voltages of −70 mV, −90 mV, −110 mV, and −130 mV gave respective slope values of 1.2, 2.3, 4.2, and 5.5 s^{-1} M^{-1} ($r^2 > 0.97$). We fit the slopes to an exponential function that describes the voltage sensitivity of a pseudo-first-order reaction between a K^+ ion and a charged site within the transmembrane field (Woodhull, 1973; Yeh and Narahashi, 1977). If the voltage dependence were entirely due to K^+ binding to a single, available site, then the site would be approximately 84% through the membrane electric field (Fig. 4 *b*).

C-type inactivation impedes K^+_o access to the modulatory binding site

C-type inactivation is thought to involve a constriction of the outer mouth of the pore (Choi et al., 1991; Yellen et al., 1994). Therefore inactivation should prevent access of a K^+ ion to a binding site that lies distal to the constriction. To investigate this possibility, we measured the accessibility of the binding site after inactivation by using a two-pulse protocol in which $[K^+]_o$ was elevated after the application of the first 800-ms depolarization. For these experiments, a pulse of 800 ms was sufficient to inactivate >95% of the K^+ channels. If the binding site is accessible after inactivation occurs, then one would expect an increased rate of recovery when the bath $[K^+]$ is increased. On the other hand, if C-type inactivation prevents extracellular cations from entering the K^+-binding site, then there would be no change in recovery rate, despite the increased $[K^+]$ in the bath solution. Results of such an experiment are shown in Fig. 5. The $[K^+]_o$ was raised from 5 mM to 150 mM at the beginning of an 8-s interpulse interval. Complete exchange of bath solution occurred within 2–3 s, as determined from control experiments in which the reversal potential was measured (data not shown). The recovery rate increased by only 15%, from 0.094 ± 0.003 s^{-1} to 0.108 ± 0.004 s^{-1} (mean ± SEM, $p < 0.05$). This small increase is far from the 225% increase predicted for the case in which the cell is exposed to 5 mM K^+_o for 3 full seconds and to 150 mM K^+_o for 5 s. The minimal response to increased K^+_o is not due to a slow association rate constant for potassium binding, because control experiments showed that a 5-s period of

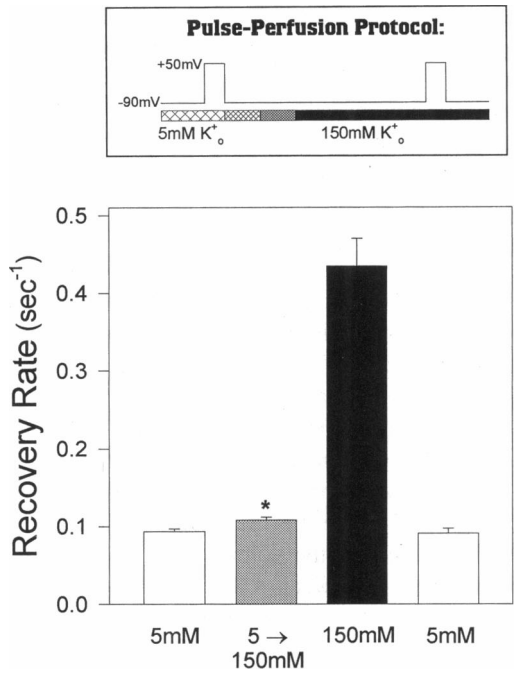

FIGURE 5 The sensitivity of recovery rate to K^+_o exchange after inactivation. Cells were perfused with a 5 mM K^+ bath solution for the first depolarization of a two-pulse protocol (each to +50 mV). Upon termination of the first pulse, after most K^+ channels were inactivated, the bath solution was exchanged for one containing 150 mM K^+. Complete exchange of solutions occurred within the first 2–3 s of an 8-s interpulse interval. Holding potential and interpulse voltage were −90 mV. Data represent mean recovery rates ± SEM ($n = 13$), which were determined as described in Fig. 3. Open bars indicate recovery rates for control protocols in which cells were perfused constantly with 5 mM K^+ solution during the 2 min before and 4 min after the experimental protocol. The gray bar shows the recovery rate for the experimental protocol, which was 15% greater than the initial control ($p < 0.05$). The black bar denotes the recovery rate 2 min after the experimental protocol, during which time the cells were perfused constantly with 150 mM K^+ bath.

perfusion of 150 mM K^+ bath was sufficient to induce rapid recovery from inactivation (not shown). One possible explanation for the slight increase in rate under these conditions is that the recovery process involves an equilibrium between inactivated and noninactivated states. Therefore, a small population of channels that might have reinactivated after recovery in 5 mM K^+_o would have remained in a noninactivated state because of the elevation of $[K^+]_o$. Alternatively, inactivation may lead to a conformational change that reduces but does not eliminate the affinity for potassium at the binding site. The invariance of recovery that accompanies the switch to elevated $[K^+]_o$ indicates that the binding site is deep enough within the channel to be relatively inaccessible to the extracellular solution when the channel is inactivated.

The effect of potassium on recovery is independent of K^+ driving force

The data in Fig. 5 suggest that a K^+ binding site is accessible when the channel is open at depolarized voltages and that the occupancy of this site influences recovery from inactivation at later times and at hyperpolarized voltages. This raises the possibility that occupancy of the K^+ binding site may depend on flux of K^+ through the open channel and even that it may be accessible to internal K^+. To test these possibilities, we lowered internal $[K^+]$ by substituting potassium ions with *N*-methylglucamine. The reversal potential for potassium is changed from −85 mV to 0 mV by either raising $[K^+]_o$ to 150 mM or lowering $[K^+]_i$ to 5 mM. Under both experimental conditions, the driving force for K^+ efflux through the open channel is reduced from 135 mV to 50 mV. The data in Fig. 6 show that recovery rate strongly depends on K^+_o but not on the potassium driving force per se. For example, with an interpulse voltage of −110 mV, the recovery rate was 0.07 s^{-1} for 5 mM K^+_o (*open triangles*) and 0.70 s^{-1} for 150 mM K^+_o (*open circles*), a 10-fold enhancement of rate. In contrast, for an equivalent decrease in driving force, the recovery rate in cells dialyzed with 5 mM K^+_i (*filled triangles*) was unchanged. However, when cells bathed in 150 mM K^+ were dialyzed with 5 mM K^+, the recovery rate was increased by only fourfold to 0.30 s^{-1} (data not shown), indicating that recovery rate is partially sensitive to internal $[K^+]$.

DISCUSSION

Extracellular potassium modulates recovery from C-type inactivation. The characteristics of this modulation are as follows. First, there is a linear relationship between $[K^+]_o$ and recovery rate, with an approximately sixfold difference in recovery rate between 5 mM and 150 mM $[K^+]_o$, for a

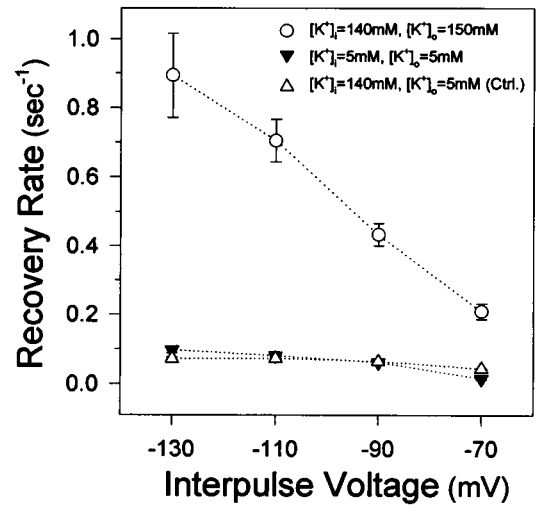

FIGURE 6 The effect of K^+ driving force upon recovery from C-type inactivation. The driving force for potassium was shifted by approximately +85 mV by substitution of internal K^+ with *N*-methylglucamine. The recovery rates were determined as described in Fig. 3 and are shown as a function of interpulse voltage (mean ± SEM) for cells with 140 mM $[K^+]_i$ (○, △, $n = 5$) and with 5 mM $[K^+]_i$ (▼, $n = 5$) under conditions of 5 mM $[K^+]_o$ (▼, △) or 150 mM $[K^+]_o$ (○). The experiment was performed as described for Fig. 4.

holding potential of −90 mV. These results suggest that a low-affinity K^+ binding site participates in destabilizing the inactivated state. Second, the rate of recovery from inactivation is dependent on voltage. Third, enhanced recovery is achieved only if potassium is elevated before channel inactivation, suggesting that K^+ must bind to the modulatory site before inactivation occurs. Most likely, this occurs when the channel is open. Finally, the effect of extracellular K^+ on recovery rate is mediated by the concentration of K^+_o and not the driving force for potassium through the open channel.

Elevated $[K^+]_o$ enhances recovery rates at markedly hyperpolarized voltages. Any of the three following causes may contribute to this voltage dependence: 1) The voltage dependence of recovery is due to the binding of a cation within the membrane field; 2) hyperpolarization leads to a conformational change that increases the affinity for cation binding; or 3) there is a voltage dependence of recovery that is independent of cation binding. If the voltage dependence were a result of cation binding, then we would expect to see a negligible influence of voltage for a case in which the binding site was saturated. Unfortunately, because the modulatory binding site is a low-affinity site and does not saturate within the range of $[K^+]_o$ tested, we have been unable to directly test this possibility. Because the potassium ion appears to be trapped in the binding site before the interpulse hyperpolarization, however, the dependence of recovery rate on the interpulse potential is not due to a voltage-dependent increase of potassium binding. Thus, the voltage dependence of recovery must be independent of cation binding. The most likely explanation is that hyperpolarization increases the likelihood of a conformational change that destabilizes the inactivated state.

Where is the modulatory K^+ binding site located? To speed recovery, extracellular $[K^+]$ must be elevated before the channels are inactivated. This shows that the binding site is somewhat inaccessible to the extracellular solution when the channel is in the inactivated state. The fact that the recovery rate is set by the $[K^+]_o$ before inactivation suggests that the modulatory effect of K^+ involves the entrapment of the cation within the inactivated channel, leading to the destabilization of the inactivated state.

Our model for potassium modulation of recovery is consistent with the model for C-type inactivation initially proposed by Choi et al. (1991) and further characterized by Yellen et al. (1994). According to this model, the channel undergoes C-type inactivation by a constriction of the outer mouth of the pore, which involves the threonine at position 449 (T449) of the *Shaker* channel. This residue is located at the edge of the membrane field in the wild-type *Shaker* channel (Heginbotham and MacKinnon, 1992), and point mutations at this position can change the rate of C-type inactivation (Labarca and MacKinnon, 1992; Lopez-Barneo et al., 1993). External K^+ can slow C-type inactivation in Kv1.3 (this paper; Grissmer and Cahalan, 1989b) and in *Shaker* (Labarca and MacKinnon, 1992; Lopez-Barneo et al., 1993). Although we have found that $[K^+]_o$ can potently enhance the recovery rate of Kv1.3, $[K^+]_o$ has been shown not to affect the kinetics of recovery from C-type inactivation for either the *Shaker* wild type or a number of 449 mutants expressed in oocytes (Labarca and MacKinnon, 1992; Lopez-Barneo et al., 1993). It is unlikely that this discrepancy exists because of differences in expression systems, because the recovery rate of Kv1.3 expressed in oocytes is also increased by elevated extracellular K^+ (Santarelli and Deutsch, unpublished data). Rather, the recovery of Kv1.3 may be sensitive to $[K^+]_o$ because of the histidine (H399) at the position equivalent to *Shaker* T449, and/or other differences between *Shaker* and human Kv1.3 channels. We are currently testing whether mutants of Kv1.3 H399 manifest K^+_o-sensitive recovery.

A "foot-in-the-door" mechanism, whereby potassium must exit the pore before inactivation, has been used to explain how K^+_o can slow C-type inactivation in some *Shaker* mutants (Labarca and MacKinnon, 1992; Lopez-Barneo et al., 1993). The inactivation rate of native Kv1.3 is also slowed by elevated $[K^+]_o$ (Grissmer and Cahalan, 1989b; this paper). The foot-in-the-door mechanism for the influence of K^+_o on C-type inactivation is shown in Scheme 1, with the potassium-bound open state denoted by OK. This model could account for the K^+-enhanced recovery if the open-to-inactivated rate constant is not vanishingly small at negative voltages. Because there is no steady-state inactivation at the recovery potentials and K^+_o concentrations used in this study (Levy and Deutsch, unpublished data), the open-to-inactivated rate constant must be very small. Thus, Scheme 1 is insufficient to explain the ability of extracellular K^+ to speed recovery. An alternative explanation, shown in Scheme 2, accounts for the effects of elevated $[K^+]_o$ on inactivation and recovery, and for the data shown in Fig. 5, which require K^+ to be bound to an inactivated state. In this scheme, K^+ is bound to an open state (OK) and to an inactivated state (IK). Because K^+ is assumed to be trapped in IK at depolarized voltages, the rate constants connecting I and IK at +50mV must be extremely small. However, at the negative recovery voltages, these rates may be appreciable and contribute to the overall recovery rate. When K^+ is bound to the open channel, the open state is favored more than the inactivated state, slowing the inactivation rate. Moreover, exit from IK is faster than from I, thereby accounting for the faster recovery in high $[K^+]_o$. For clarity, we have left out the possibilities of closed-state inactivation (Marom and Levitan, 1994) and K^+-bound closed states from both schemes.

$$\begin{array}{ccc} C \rightleftharpoons O \rightleftharpoons I \\ \quad \downarrow\uparrow \\ \quad OK \end{array}$$

SCHEME 1

$$\begin{array}{ccc} C \rightleftharpoons O \rightleftharpoons I \\ \quad \downarrow\uparrow \\ \quad OK \rightleftharpoons IK \end{array}$$

SCHEME 2

Scheme 2 is capable of accounting for a $[K^+]_o$ dependence of both single-pulse inactivation and recovery. Further modification of this scheme may be necessary to account for the

higher-affinity binding site (this paper; Grissmer and Cahalan, 1989b) underlying single-pulse inactivation.

The rate of recovery from N-type inactivation can be accelerated by extracellular K^+ (Demo and Yellen, 1991; Pardo et al., 1992; Gomez-Lagunas and Armstrong, 1994), and a "knock-off" mechanism, similar to that described by Armstrong (1971), has been invoked to explain this observation. For the case of N-type inactivation, Demo and Yellen (1991) have proposed that a K^+-binding site exists within the membrane field that is located near a positive charge on the surface of the inactivation particle. Binding of the inactivation particle is therefore destabilized by electrostatic repulsion from the K^+ ion. N-type inactivation is not voltage sensitive at positive potentials (Zagotta and Aldrich, 1990), but the rate of recovery at negative potentials is voltage dependent. Although this may be due, in part, to the putative location of the K^+-binding site within the membrane field (Demo and Yellen, 1991), Gomez-Lagunas and Armstrong (1994) have shown that some of the voltage dependence of recovery from N-type inactivation exists independently of cation binding. For Kv1.3, we have also observed an intrinsic voltage dependence of recovery from C-type inactivation, even though movement into the inactivated state is voltage independent at positive potentials (Cahalan et al., 1985; Deutsch et al., 1986). This could be explained by assuming that the voltage-dependent transitions affecting the inactivated state occur exclusively at negative potentials. Elevated $[K^+]_o$ speeds recovery from both C-type and N-type inactivation. In both cases, regardless of the different gating mechanisms, a permeant cation regulates its own conductance.

In T lymphocytes, a rise in extracellular $[K^+]$ slows inactivation and speeds recovery of Kv1.3, both of which lead to a greater K^+ conductance. The T lymphocyte membrane potential is maintained between E_K and E_{Cl} (-87 and -35 mV; Grinstein and Dixon, 1989), and that balance is determined by a weighted sum of the K^+ and Cl^- conductances (G_K and G_{Cl}, respectively). Because high $[K^+]_o$ leads to an increased G_K, E_K will contribute more significantly to the membrane potential when $[K^+]_o$ is elevated. Thus, recovery will influence the T cell resting membrane potential, which in turn modulates mitogen-stimulated lymphokine production and cell proliferation in vitro (Freedman et al., 1992; Lin et al., 1993). Under what circumstances would $[K^+]_o$ increase in vivo? Elevated extracellular K^+ has been found at sites of inflammation, where cell death may lead to potassium release (Silver, 1975). At these sites, autoregulation of K^+ channel activity would affect voltage-dependent immune function. Although we do not know the extent of K^+ modulation in the intact cell at 37°C, we anticipate altered kinetics (Lee and Deutsch, 1990; Oleson et al., 1993), but not a qualitative difference in the conclusion that extracellular K^+ modulates the rate of recovery from C-type inactivation. Destabilization of the inactivated state by elevated K^+_o may also play a physiological role in excitable tissues. For example, an ischemia-related rise of $[K^+]_o$ in neural tissue (Hansen and Zeuthen, 1981) may increase the K^+ conductance and clamp the membrane potential to reduce cellular excitability and consequently cellular metabolic activity.

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