# A Voltage-Dependent Role for $K^+$ in Recovery from C-Type Inactivation

Daniel I. Levy and Carol Deutsch
Department of Physiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6085 USA

ABSTRACT Recovery from C-type inactivation of Kv1.3 can be accelerated by the binding of extracellular potassium to the channel in a voltage-dependent fashion. Whole-cell patch-clamp recordings of human T lymphocytes show that $K_o^+$ can bind to open or inactivated channels. Recovery is biphasic with time constants that depend on the holding potential. Recovery is also dependent on the voltage of the depolarizing pulse that induces the inactivation, consistent with a modulatory binding site for $K^+$ located at an effective membrane electrical field distance of 30%. This $K^+$-enhanced recovery can be further potentiated by the binding of extracellular tetraethylammonium to the inactivated channel, although the tetraethylammonium does not interact directly with the $K^+$-binding site. Our findings are consistent with a model in which $K^+$ can bind and unbind slowly from a channel in the inactivated state, and inactivated channels that are bound by $K^+$ will recover with a rate that is fast relative to unbound channels. Our data suggest that the kinetics of $K^+$ binding to the modulatory site are slower than these recovery rates, especially at hyperpolarized voltages.

## INTRODUCTION

The inactivation properties of voltage-gated $K^+$ channels can influence the resting membrane potential of cells, as well as the frequency and duration of action potentials in excitable tissue (Hille, 1992). Two distinct molecular mechanisms of inactivation of voltage-gated $K^+$ channels have been identified: N-type inactivation involves a single subunit acting as a tethered blocking particle by binding of its amino-terminus to the cytoplasmic face of the channel (Hoshi et al, 1990; MacKinnon et al., 1993; Gomez-Lagunas and Armstrong, 1995), and C-type inactivation involves four subunits participating equally in a conformational change at the extracellular mouth of the pore (Choi et al., 1991; Panyi et al., 1995; Ogielska et al., 1995). Although these two forms of inactivation can be coupled to each other (Hoshi et al., 1991; Baukrowitz and Yellen, 1995), both entry into and exit from the C-type inactivated state usually occur on a time scale much longer (100–1000 ms) than that seen for N-type inactivation (1–100 ms; Hoshi et al., 1991; Lopez-Barneo et al., 1993; Hoshi et al., 1990; for exceptions, see Smith et al., 1996; Seoh and Papazian, 1995). Although $K^+$-channel inactivation has been studied extensively, there is a paucity of information on the mechanisms underlying recovery from C-type inactivation.

The $K^+$ conductance of a cell is influenced not only by the rate at which channels inactivate, but also by the rate at which they recover from inactivation. Recovery from both N-type and C-type inactivation is modulated by extracellular potassium ($K_o^+$), such that elevated $[K^+]_o$ speeds the rate of recovery (Demo and Yellen, 1991; Pardo et al., 1992; Gomez-Lagunas and Armstrong, 1994; Levy and Deutsch, 1996). Extracellular $K^+$ speeds recovery from N-type inactivation by a "knockoff" mechanism (Demo and Yellen, 1991), which involves the binding of $K^+$ to an already inactivated state of the channel to destabilize the inactivation particle. Similarly, $K_o^+$ speeds recovery from C-type inactivation by binding to a low-affinity modulatory site, which leads to the destabilization of the inactivated state (Levy and Deutsch, 1996). However, it is unclear which conditions allow $K_o^+$ to bind to this modulatory site. To address this issue, we studied recovery from C-type inactivation in Kv1.3, a *Shaker*-like $K^+$ channel that lacks N-type inactivation and is found in immune and neuronal tissue (for review see Chandy and Gutman, 1994). In this paper, we investigate the voltage dependence of $K_o^+$ modulation of recovery. We also show that extracellular tetraethylammonium (TEA) can potentiate $K^+$-enhanced recovery by binding to the inactivated channel. These results suggest that a modulatory $K^+$-binding site is located within the outer mouth of the pore and its accessibility is voltage dependent.

## 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, using gradient centrifugation through Ficoll-Hypaque, and cultured (5% $CO_2$ incubator at 37°) in a serum-free, RPMI-based medium (Deutsch et al., 1991). Cells were stimulated to proliferate by the continuous presence of 3 $\mu$g/ml mitogen phytohemagglutinin (Difco, Detroit, MI). The extent of 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. For electrophysiological studies, T lymphocytes were selected 36–84 h after mitogenic stimulation 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

*Received for publication 1 July 1996 and in final form 10 September 1996.*
Address reprint requests to Dr. Carol Deutsch, Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104-6085. Tel.: 215-898-8014; Fax: 215-573-5851.

0006-3495/96/12/3157/10 $2.00

(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 −90 mV or −130 mV. Current recording did not begin until 8 min after achieving the whole-cell configuration to ensure the 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 at the holding potential. To preserve baseline conditions, the cell voltage was maintained at the holding potential for at least 2 min between each two-pulse protocol. For each cell, fractional recovery was periodically measured under control conditions, and was usually stable for greater than 1 h of recording, as has been previously reported for Kv1.3 (Kupper et al., 1995). In cases where recovery did become unstable, data acquisition was terminated. This made measurement of the complete time course of recovery somewhat difficult, as instability of recovery (as well as sudden loss of the seal) often occurred after a few hours of recording from a cell.

Peak $K^+$ currents ranged from 0.2nA to 6nA, and 0–75% series resistance compensation was used to reduce voltage errors below 15 mV. Current traces for all experiments were corrected for ohmic leak and voltage error caused by series resistance, as described by Panyi et al. (1995). For precise correction of the current traces, the driving force was determined for all sets of solutions; the reversal potential was measured from tail currents induced by hyperpolarizing steps that followed a brief depolarizing pulse.

## Solutions

The internal solution contained (in mM) 130 KF, 10 KCl, 11 $K_2$EGTA, 10 HEPES; (pH = 7.20 with KOH; 295 mOsm). Stock external solutions contained 150 mM of either NaCl, KCl, or choline chloride; as well as the following (in mM): 5.5 Glucose, 10 HEPES, 2.5 $CaCl_2$, 1 $MgCl_2$; (pH = 7.35 with NaOH, KOH, or *N*-methylglucamine base, respectively; 305 mOsm). The control bath solution contained 145 mM NaCl and 5 mM KCl, and other bath solutions were prepared by appropriate mixing of the stock solutions. Low pH solutions were prepared by adding less base. Solutions containing tetraethylammonium (TEA) were prepared by substitution of the primary bath salt with the appropriate concentration of TEA·Cl (Kodak, Rochester, NY).

Cells were perfused continuously during the data acquisition. A multiport manifold with a single output tube (PE-10) was positioned ~250 μm from the cell so that any one of six bath solutions would perfuse the cell. The absence of contamination from surrounding bath solution was confirmed either by measuring reversal potential or by confirming complete recovery from channel block. The bath solution was exchanged by manually turning valves or by computer synchronized switching of solenoid valves. Using this manifold, solutions could be completely exchanged within 250 ms of the valve switching, as measured by shifts in reversal potential. Use of this perfusion system had no substantial effect on peak current or the kinetics of inactivation and recovery from C-type inactivation. For the experiments shown in Fig. 2, a more rapid exchange of two solutions was achieved with the use of a U-tube perfusion system, partially based on the design of Brett et al. (1986). Cells were lifted from the dish and brought to within 20 μm (about twice the cell diameter) of a circular opening, produced by cutting a 75-μm notch in the U-tube (PE-10). As determined by monitoring the reversal potential, exchange began within 50 ms of valve switching and was completed within the subsequent 20 ms. Figure legends show the exposure to bath solutions after accounting for the 70-ms delay in complete exchange. Use of this perfusion system led to a threefold increase of inactivation rate ($\tau$ = 40–50 ms) and caused a progressive rundown of the peak current (typically half of the initial peak, after 90 min), which may have been caused by the substantially faster flow rate. Recovery from inactivation, however, was stable after the first 10 min of perfusion and occurred with rates typically measured in 5 mM or 150 mM $K^+$ bath solutions.

## Statistical testing

Comparisons of recovery from inactivation under various test conditions were made by performing the paired *t*-test or the Student-Newman-Keuls test, when appropriate, on the fractional recovery measured at equivalent interpulse intervals (ipi). However, to highlight the differences between recovery in (for example) high versus low $[K^+]_o$, we show recovery rates for Figs. 4–6. A weighted sum of recovery-rate constants was approximated by the following function: Recovery rate = −ln(1 − fractional recovery)/ipi. Our method for determining the fractional recovery is described in the Results.

## RESULTS

We have previously shown that recovery from C-type inactivation of Kv1.3 is specifically enhanced by $K_o^+$ in a dose-dependent fashion and that there is a voltage dependence to the recovery process (Levy and Deutsch, 1996). We explained this behavior using a model in which $K_o^+$ binds to a channel and destabilizes the inactivated state, thereby speeding recovery. This model predicts that recovery occurs from either a $K^+$-bound or an unbound inactivated state and that the recovery of a population of channels should therefore follow biexponential kinetics. Fig. 1 shows such a biphasic time course of recovery from C-type inactivation of Kv1.3 channels, recorded from a human T lymphocyte. Potassium currents were recorded in response to pairs of 800-ms depolarizing pulses to +50 mV, with interpulse intervals of variable duration at −90 and −130 mV, respectively. For each interpulse voltage, recovery was measured during perfusion with 5, 75, or 150 mM $K_o^+$. The fraction of recovery was quantified as follows:

$$\text{Fractional Recovery} = (I_{peak2} - I_{min1})/(I_{peak1} - I_{min1}), \quad (1)$$

where $I_{peak1}$ and $I_{peak2}$ represent the peak currents elicited by the first and second depolarizing pulse, respectively, and $I_{min1}$ represents the minimal outward current observed at the end of the first depolarizing pulse.

We fit the data with the sum of two exponential functions:

$$1 - \text{Fractional Recovery} = Ae^{-ipi/\tau_f} + (1 - A)e^{-ipi/\tau_s} \quad (2)$$

where for any ipi duration, a fraction of channels ($A$) recovers with a fast time constant ($\tau_f$), and the remainder recovers with a slower time constant ($\tau_s$). If the two populations of channels do not interconvert during recovery, then $\tau_f$ and $\tau_s$ will be reciprocals of the corresponding rate constants of recovery. If they do interconvert on the time scale of recovery, then $\tau_f$ and $\tau_s$ will vary with $[K^+]_o$ and each will be a function of the rate constants connecting the channel populations and a recovered state (see scheme in Discussion). For each recovery, voltage data from the three concentrations of $K_o^+$ were simultaneously fit such that the time constants did not change with $[K^+]_o$ but that the weighting of the two components ($A$) could vary. For all cells measured at −90 mV, $\tau_f$ and $\tau_s$ were 4.8 ± 1.8 s and 16.5 ± 3.5 s (mean ± SEM, $n$ = 4), and at −130 mV, the time constants were 0.49 ± 0.02 s and 12.7 ± 4.3 s,

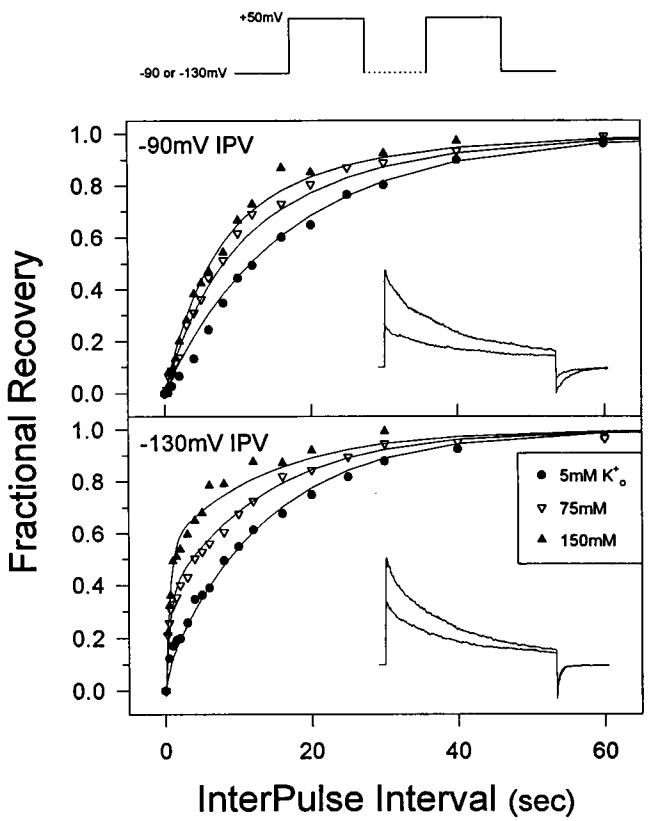



FIGURE 1 Recovery from C-type inactivation. Whole-cell currents were elicited from a T lymphocyte by a pair of 800-ms voltage steps to +50 mV from a holding potential of either −90 mV (*top panel*) or −130 mV (*bottom panel*). Fractional recovery was calculated, as defined in the text, for a series of interpulse intervals (ipi) with durations between 0.3 and 90 s at the indicated interpulse voltage (IPV). The cell was perfused with choline-based bath solutions containing 5 mM (●), 75 mM (▽), or 150 mM (▲) $K^+$. The time courses for recovery were simultaneously fit using the equation: 1 − Fractional Recovery = $Ae^{-ipi/\tau_f} + (1 - A)e^{-ipi/\tau_s}$. At −90 mV, the fit yielded: $\tau_f$ = 7.8 s, $\tau_s$ = 18.5 s, $A_{5\ mM} = 10^{-5}$, $A_{75\ mM}$ = 0.58, and $A_{150\ mM}$ = 0.80. At −130 mV, the fitted values were: $\tau_f$ = 0.59 s, $\tau_s$ = 14.3 s, $A_{5\ mM}$ = 0.10, $A_{75\ mM}$ = 0.34, and $A_{150\ mM}$ = 0.55. For this cell and all cells measured ($n$ = 4), the fit to the data for each voltage passed the runs test ($p < .025$; Wolfowitz, 1943), and $r^2$ was always greater than 0.98. *Insets*: Current recordings (superimposed) for paired 800-ms depolarizing pulses with interpulse intervals of 4 s, in the presence of 75 mM $K^+$. Peak currents from the first pulse were ~2 nA, and fractional recovery was 31.1% at −90 mV and 45.5% at −130 mV.

respectively. Note that $\tau_f$ was smaller for the more hyperpolarized holding potential ($p < .05$). The weight of the fast component of recovery increased as $[K^+]_o$ was raised from 5 to 150 mM (0.04 ± 0.08 vs. 0.58 ± 0.27 at −90 mV and 0.16 ± 0.07 vs. 0.52 ± 0.16 at −130 mV, $p < .05$). Because the weight of the fast component increases with increasing $[K^+]_o$ without a change in the recovery time constant, it appears that the population of quickly recovering channels is increased by the binding of $K_o^+$. This biphasic behavior is consistent with a model in which 1) a $K^+$-bound inactivated channel is destabilized relative to an unbound inactivated channel, and thereby recovers faster; and 2) the $K^+$-bound and unbound inactivated channels do not interconvert rapidly during the interpulse interval under the experimental conditions of these measurements.

Until now, we have not been able to observe such biphasic behavior of the recovery process (Levy and Deutsch, 1996). Multiple exponential processes can appear to be monoexponential if the time constants are similar and/or if weighting factors effectively yield only one major component. In the current work, however, we were able to unmask a double exponential process by using more hyperpolarized recovery potentials. Under these conditions, the time constants of the two components are sufficiently different and the weighting factors for each component are sufficiently comparable to enable observation of two recovery phases.

## Voltage-dependent recovery

We have previously observed that exposure of the channel to elevated $[K^+]_o$ during the interpulse interval was insufficient to maximally enhance the recovery from inactivation. Specifically, we saw only a 15% enhancement of recovery rate when the $[K^+]_o$ was raised from 5 to 150 mM for the last 6 s of an 8-s interpulse interval. This was substantially lower than the 400% faster recovery of cells perfused continuously with 150 mM $K^+$ (Levy and Deutsch, 1996). From this finding we suggested that there must be a greatly increased accessibility of $K_o^+$ to a modulatory site before inactivation occurs, presumably to either the open or closed state of the channel. This binding would subsequently destabilize the inactivated state and speed recovery. To test this hypothesis, we used a faster method of solution exchange to assess the sensitivity of recovery to $[K^+]_o$ at selected periods during the two-pulse protocol. Complete exchange was achieved within 20 ms, as determined by reversal potentials.

Fig. 2 *A* shows data from cells perfused continuously with a control solution containing 5 mM $K^+$ and 145 mM $Na^+$ except for a 1-s period in which the perfusate contained 150 mM $K^+$. In all cases using 5 mM $K^+$ throughout the first depolarizing pulse, >96% of the channels were inactivated by the end of the first pulse (see sample current trace derived from protocol A). Using 150 mM $K^+$ throughout the first depolarizing pulse, >92% of the channels were inactivated by the end of the first pulse (see sample current trace derived from protocol A*). Protocol A yields the fractional recovery for continuous perfusion with the 5 mM $K^+$ solution and protocol I provides the fractional recovery for continuous exposure to 150 mM $K^+$. The fractional recovery for protocol I was not statistically different from the recovery using continuous perfusion with 150 mM $K^+$ for >30 s (not shown).

Our previous results suggested that a $K^+$ ion is trapped at the modulatory site in an inactivating channel. We were therefore surprised to find that exposure of the channel to elevated $K^+$ during the entire depolarizing pulse was insufficient to cause maximal recovery (compare protocol D with I). This observation indicates that $K^+$ must also be present

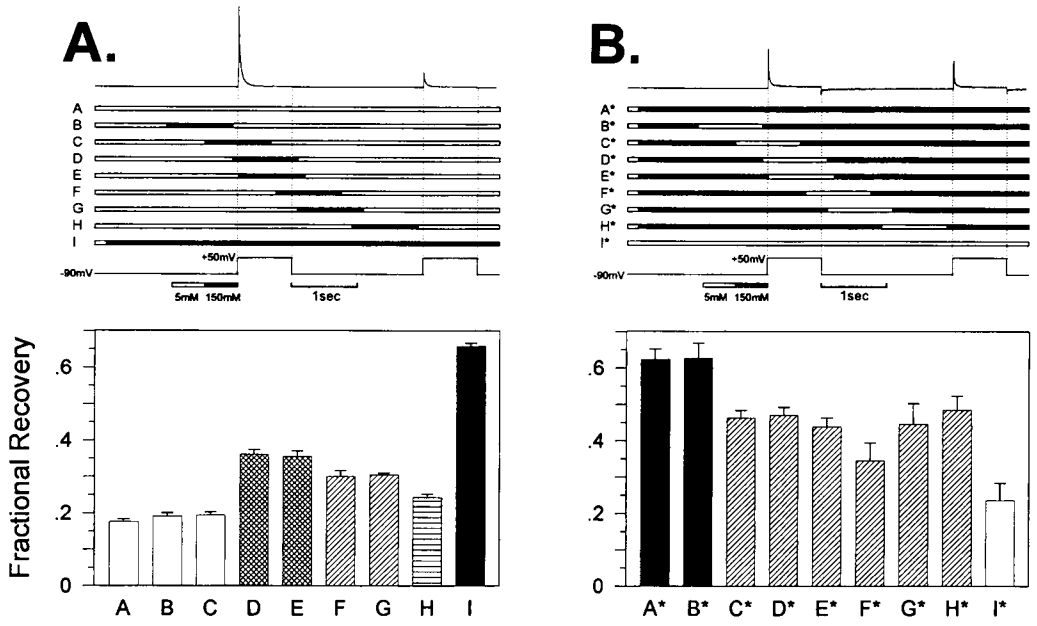



FIGURE 2 Effect of pulsatile $[K^+]_o$ on recovery. (*A*) Fractional recovery is shown for each of the perfusion protocols depicted above the graph (mean ± SEM, $n = 6$). Cells were continuously perfused with a 5 mM $K^+$/145 mM $Na^+$ bath solution (denoted by the *open portions* of the horizontal bars), except for a 1-s period during which cells were exposed to 150 mM $K^+$ (*filled portion* of the horizontal bars). Solution exchange was complete within 20–25 ms, using a rapid perfusion system (see Methods). The current trace above the protocols shows the recovery under control conditions (protocol A). Fractional recovery elicited by each perfusion protocol was compared by the Student-Newman-Keuls pairwise method ($p < .05$), leading to segregation into five significantly different groups (*vertical bars*: *open* < *flat-hatched* < *left-hatched* < *cross-hatched* < *filled*). (*B*) Fractional recovery is shown for protocols complementary to those in *A* ($n = 3$). Cells were perfused with a 150 mM $K^+$ bath except for a 1-s period during which the bath was replaced with a 5 mM $K^+$ solution. The current trace above the protocols shows recovery during perfusion with 150 mM $K^+$, as in protocol A*. Pairwise comparison yielded three significantly different groups (*vertical bars*: *open* < *left-hatched* < *filled*). Sequential perfusions were done in the order shown, and control protocols were performed every 10 min to confirm the stability of recovery kinetics. Because of the differences in $[K^+]_o$ during measurement of the two peak currents for protocols C, D, C*, and D*, recovery was calculated after correcting for changes in driving force and whole-cell conductance. Specifically, the test pulse current was scaled by the ratio of control peak currents under the 5 mM vs. 150 mM $K_o^+$ conditions.

during the hyperpolarized interpulse interval to speed recovery maximally and that $K^+$ bound during depolarization can subsequently dissociate from the channel at negative voltages. Dissociation of bound $K^+$ also occurs at positive voltages, as shown by the results obtained using protocol C. In this case, $[K^+]_o$ was lowered to 5 mM before the end of the depolarizing pulse, during which time $K^+$ dissociates rapidly enough to yield no enhanced recovery (compare recovery for protocols A and C). Conversely, the increase of fractional recovery caused by brief exposure to increased $[K^+]_o$ during the hyperpolarized interpulse interval shows that $K_o^+$ can bind to the inactivated channel (compare protocols G and H with A). These data also show that the modulatory effect of high $[K^+]_o$ is greater at the depolarized voltage (compare protocols D and E with F, G, and H), consistent with the predictions of Levy and Deutsch (1996). Protocols D and E do not differ in recovery despite the 28% decrease in open channels (based on a $\tau_{inact}$ of 45 ms in 150 mM $K^+$ (see Methods) and a 15-ms elapsed time before the change in perfusion and the start of the depolarizing pulse in protocol E). This suggests that there is no preferential binding of $K^+$ to the open state over the inactivated state. To confirm that cells treated according to protocols D and E truly differ with respect to the number of open channels exposed to 150 mM $K^+$, we integrated the current traces for the period during which the cells were both depolarized and exposed to 150 mM $K^+$. The ratio of these current integrals for protocol E to protocol D was 0.74 ± 0.05 ($n = 4$, mean ± SEM), indicating a 26% lower open probability for exposure to 150 mM $K^+$ in protocol E. Thus, the open channel does not appear to play a larger role in recovery than the inactivated state.

Fig. 2 *B* shows the fractional recovery for protocols that are complementary to those in Fig. 2 *A*, i.e., cells were continuously perfused with 150 mM $K^+$ except for a 1-s period in which the perfusate contained 5 mM $K^+$. Perfusion with 5 mM $K_o^+$ before the depolarizing pulse had no effect on recovery (protocol B*), suggesting (along with B, Fig. 2 *A*) that neither $K^+$ binding nor unbinding to the closed channel influences the rate of recovery from C-type inactivation. On the other hand, slowed recovery from protocols C*, D*, and E* confirms that $[K^+]_o$ during the depolarizing period is important and that the binding of $K^+$ at the hyperpolarized recovery voltages is not fast enough to yield maximal recovery. In each case, the fractional recovery was reduced, consistent with less $K^+$ bound to the channel. This could arise from a decreased association rate of $K^+$ for either the open or the inactivated states of the

channel during the sustained depolarization and/or an increased dissociation rate as discussed above (protocol C). These results eliminate the possibility that a $K^+$ ion occupying a modulatory binding site remains trapped within an inactivated channel until it recovers. There was also a significant decline of fractional recovery when $[K^+]_o$ was reduced during the hyperpolarized interpulse interval (protocols G* and H*), which supports the conclusion that $K^+$ can unbind from the inactivated channel during this interval.

In summary, the experiments shown in Fig. 2 suggest that $K_o^+$ can bind to and unbind from its modulatory site when the channel is open or inactivated, both at depolarized and hyperpolarized voltages. The specific association and dissociation rates are dependent on the membrane voltage and $[K^+]_o$. During depolarization, binding of $K^+$ is slow and unbinding is relatively fast; whereas during the subsequent hyperpolarization, both binding and unbinding are slow.

Why would a channel promote $K^+$ dissociation more when the membrane is depolarized than when it is hyperpolarized (best demonstrated by the lack of enhanced recovery for protocol C)? The simplest explanation for the differences in affinity is that $K^+$ dissociation is voltage dependent due to the location of a modulatory site within the membrane electrical field. We have already demonstrated a dependence of recovery on the degree of hyperpolarization during the interpulse interval (Levy and Deutsch, 1996). However, this dependence could reflect an intrinsic voltage dependence of the recovery process (e.g., a voltage-dependent conformational change). Therefore, we chose to measure the dependence of recovery on the voltage of the depolarizing pulse. Because an extracellular cation binding site within the electrical field would experience greater repulsion as the membrane is depolarized, we expected recovery to be slowed as the pulse voltage was made more positive.

Recovery was measured using a two-pulse protocol in which the pair of depolarizing pulses was +10, +30, +50, or +70 mV, and the holding potential was −90 mV (*inset*, Fig. 3 *A*). In the continuous presence of 150 mM $K_o^+$, the fractional recovery was clearly dependent on the voltage of the depolarizing pulse, as each 20-mV increment of depolarization produced successively less recovery with an ipi of 3 s. In contrast, no such differences were observed when the cells were perfused with the 5 mM $K^+$ bath solution, using an ipi of either 3 s (not shown) or 12 s. There was no significant influence of voltage on the inactivation rate in either 5 mM (ANOVA-repeated measures, $p = 0.31$) or 150 mM bath ($p = 0.40$), consistent with previous results (Deutsch et al., 1986), which suggests that the pulse voltage did not affect entry into the inactivated state. To quantify the degree to which the depolarizing voltage influences the probability of $K^+$ binding, we measured the time course of recovery that followed depolarizations to either +10 or +70 mV. Biexponential fits to the data shown in Fig. 3 *B* yield a greater contribution of the fast component to recovery at +10 mV in 150 mM $K_o^+$, which indicates that an increased fraction of channels exhibits enhanced recovery, consistent

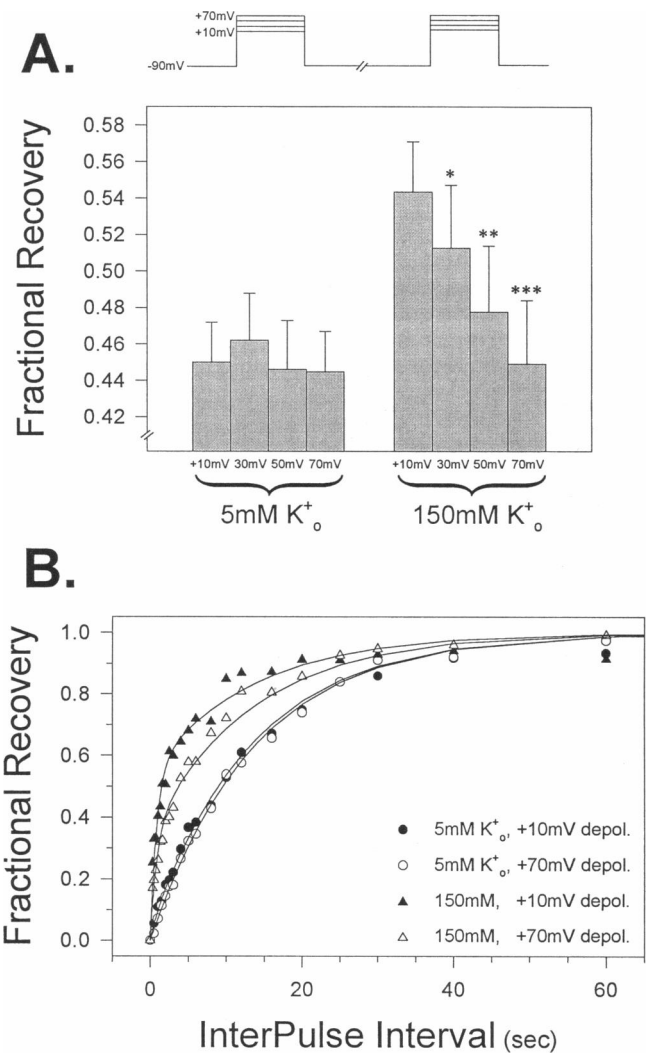



FIGURE 3 Dependence of $K^+$-modulated recovery on depolarizing voltage. (*A*) Recovery was measured for pairs of depolarizations to +10, +30, +50, or +70 mV from a holding potential of −90 mV. In a 5 mM $K^+$/145 mM choline$^+$ bath there was no significant effect of depolarizing voltage on fractional recovery (45.0 ± 2.2% for +10 mV, 46.2 ± 2.6% for +30 mV, 44.6 ± 2.7% for +50 mV, and 44.5 ± 2.2% for +70 mV; ANOVA-repeated measures, $p = 0.61$, $n = 11$) following a 12-s interpulse interval. In 150 mM $K_o^+$, each incremental depolarization elicited successively less fractional recovery (54.3 ± 2.8% for +10 mV, 51.3 ± 3.4% for +30 mV, 47.8 ± 3.6% for +50 mV, and 44.9 ± 3.5% for +70 mV; Student-Newman-Keuls all pairwise comparison, $p < .05$) following a 3-s interpulse interval, as indicated by the asterisks. (*B*) Time course of recovery for depolarizations from −90 mV to either +10 mV (*filled*) or +70 mV (*open*) in bath solutions containing either 5 mM (*circles*) or 150 mM $K^+$ (*triangles*). The data for this cell were fit as in Fig. 1, yielding the following values: $\tau_f = 0.84$ s, $\tau_s = 13.7$ s, $A_{5\ mM,\ +10\ mV} = 0.044$, $A_{5\ mM,\ +70\ mV} = 0.0025$, $A_{150\ mM,\ +10\ mV} = 0.548$, $A_{150\ mM,\ +70\ mV} = 0.351$. This is one of two such experiments.

with reduced $K^+$ binding when the membrane is more strongly depolarized. If the putative $K^+$-binding site is located within the membrane electric field, these data may be used to estimate the effective electrical distance of this site with a Boltzmann function where the fraction of bound $K^+$ is proportional to $e^{-VzF\delta/RT}$, and $V$ represents the depo

larizing voltage, $z$ is +1 for the charge of potassium, and F, R, and T have their conventional meanings. In 150 mM $K^+$, the effective electrical field distance, $\delta$, was 0.31 (Data from an additional cell yielded a distance of 0.29). Taken together, these results suggest that $K^+$ binds to a modulatory site that lies within the membrane electrical field.

## Involvement of the TEA-binding site

Although our results are consistent with a modulatory site located within the outer mouth of the $K^+$-channel pore, we do not know the precise location of this site. Among the possible candidates is the TEA-binding site. When a *Shaker* $K^+$ channel is open, the binding site for TEA (which involves residue T449) lies at the outer mouth of the channel, 20% within the membrane electrical field (Heginbotham and MacKinnon, 1992). Residue 449 also mediates $K^+$-sensitive entry into the C-type inactivated state (Lopez-Barneo et al, 1993). These observations indicate that residue 449 is accessible to extracellular ions. After entry into the inactivated state, this residue becomes more exposed to the extracellular solution (Yellen et al., 1994; Liu et al., 1996). Thus, the equivalent residue in Kv1.3, histidine (H399), seemed a plausible candidate contributing to the modulatory $K^+$-binding site. Because histidine is titratable in the physiological pH range (see below), we tested this possibility by varying extracellular pH and measuring the $K^+$-enhanced recovery. If H399 were part of the modulatory $K^+$-binding site, then a decrease in $pH_o$ might inhibit $K^+$ binding through protonation of H399, thereby reducing the ability of $K_o^+$ to speed recovery. As shown in Fig. 4, decreasing $pH_o$ did not inhibit the $K^+$-enhanced recovery. Whereas acidification to pH 6.85 reversibly reduced the fractional recovery by 15 ± 2% (mean ± SEM, $n = 10, p < .01$) in a 5 mM $K^+$ bath solution, there was actually a 12 ± 3% increase of recovery with the pH 6.85 bath containing 150 mM $K^+$ ($p < .01$). Protonation does not reduce the effect of $K_o^+$ on recovery, yet dramatically increases the inhibition constant for TEA block of the channel as expected (see below; Kavanaugh et al., 1991). Therefore, H399 is not likely to be contributing directly to the modulatory $K^+$-binding site. If this is true, then $K^+$ binding to the modulatory site should not affect TEA block of the channel.

We measured the affinity of $TEA_o$ for the channel at pH 7.35 in the presence of 5 mM and 150 mM $K_o^+$. At +50 mV, 9 mM TEA caused 49–53% block in both cases. On the other hand, we found that $TEA_o$ could potentiate $K^+$-enhanced recovery. Fig. 5 shows that a half-blocking concentration of TEA does not speed the recovery if the bath $[K^+]$ is 5 mM (only 3 ± 3% increase in fractional recovery; $n = 5$, $p = 0.41$), but potentiates the enhancement of recovery by 31 ± 6% ($p < .01$) if the bath $[K^+]$ is 150 mM.

This potentiation of the $K^+$-enhanced recovery is mediated by TEA binding to the blocking site, because at pH 6.85 (pH at which protonation of the H399 reduces the TEA affinity by nearly one-fourth; *inset* of Fig. 5 and Kavanaugh et al., 1991) a half-blocking concentration of TEA (30 mM) was necessary to potentiate the effect of elevated $K^+$ (by 27 ± 3%) to a level similar to the potentiation by 9 mM TEA at pH 7.35 ($p < .01$). These results suggest that TEA allosterically potentiates $K^+$-enhanced recovery by interacting with the same binding site that is involved in channel block.

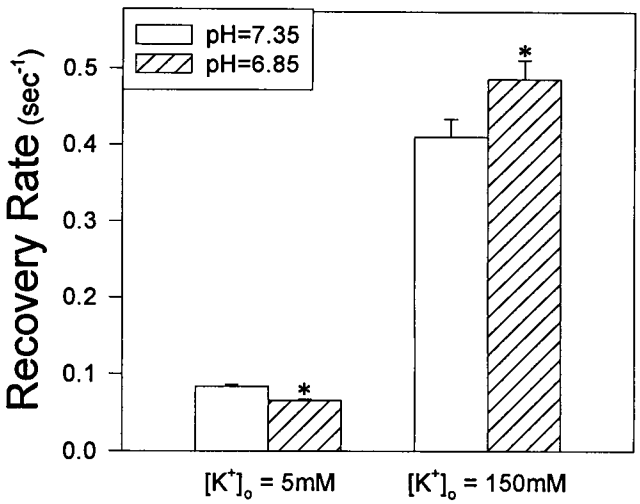



FIGURE 4 The effect of extracellular pH on recovery from inactivation. Recovery was measured as cells were continuously perfused with a bath solution of pH 7.35 (*open bars*) or 6.85 (*hatched bars*). In a 5 mM $K^+$/145 mM $Na^+$ solution, the lower pH reduced the fractional recovery by 15 ± 2%, ($p < .01$, $n = 10$) using a 10-s IPI. In a 150 mM $K^+$ bath, the lower pH increased the fractional recovery by 12 ± 3% ($p < .01$) with a 2-s ipi. To illustrate the differences between recovery in high versus low $[K^+]_o$, we show recovery rates as weighted sums of recovery rate constants, approximated by the following function, Recovery rate = −ln(1 − fractional recovery)/ipi.

How does $TEA_o$ potentiate the $K_o^+$-enhanced recovery? TEA may act on the open channel to cause entry into an unstable inactivated state, to allosterically influence the binding of $K^+$ to the modulatory site, or to prevent the exit of a $K^+$ ion from the modulatory site. Alternatively, TEA may act on the inactivated channel to allosterically promote $K^+$ binding or to further destabilize the $K^+$-bound inactivated state. Although it has been shown that $K^+$ channels cannot enter the C-type inactivated state when blocked by TEA (Grissmer and Cahalan, 1989; Choi et al., 1991), it is still possible that the TEA-binding site is accessible after the channels are in the inactivated state (e.g., see Yellen et al., 1994, or Liu et al., 1996). To test whether the effect of TEA on recovery could be induced solely by exposure of channels to TEA after the channels have entered the inactivated state, we perfused cells with a 141 mM $K^+$/9 mM choline bath solution throughout an 800-ms depolarization that caused inactivation (80 ± 2%). About 200 ms after the depolarization, the perfusate was exchanged with a solution that replaced the choline salt with 9 mM TEA (Fig. 6). Fractional recovery was calculated after correcting for the channel block during the second pulse. The recovery after a 2-s interpulse interval was enhanced to nearly the same rate

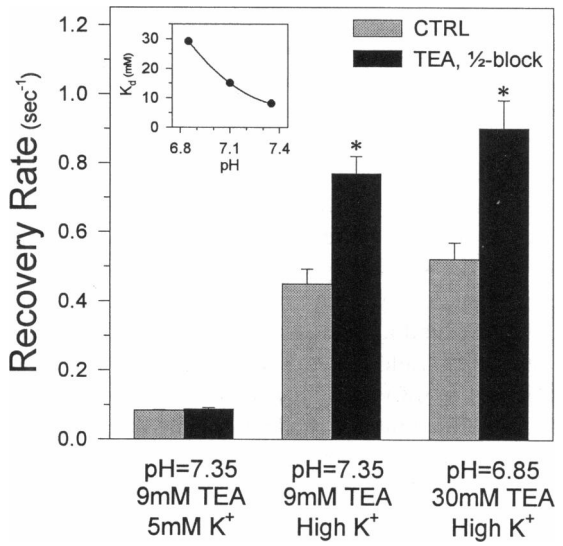



FIGURE 5 Potentiation of $K^+$-enhanced recovery by TEA. Recovery was measured in the absence (*shaded bars*) or presence (*filled bars*) of half-blocking concentrations of TEA. At pH 7.35, isotonic replacement of the primary bath cation with 9 mM TEA did not significantly change the fractional recovery in a 5 mM $K^+$/145 mM $Na^+$ bath (3 ± 3% greater, using a 6-s ipi; $p = 0.41$, $n = 5$), but caused a 31 ± 6% potentiation of enhanced recovery in a 150 mM $K^+$ bath, with an ipi of 1-s ($p < .01$). At pH 6.85, isotonic replacement of $K^+$ with 30 mM TEA potentiated the enhanced recovery by 27 ± 3%, with an ipi of 1-s ($p < .01$). In a separate experiment at pH 6.85, we measured the potentiation of $K^+$-enhanced recovery by 9 mM TEA to show that the concentrations used were not in the saturating range (11 ± 4% greater recovery versus 26 ± 4% in 30 mM; $n = 6$, not shown). The durations of interpulse intervals were chosen to give comparable fractional recovery. Recovery rates shown were estimated by the method described for Fig. 4. *Inset*: Titration of $TEA_o$ affinity by $pH_o$. The affinity for TEA was measured to confirm that block can be titrated by pH ($n = 3$; error bars fall within the symbols). The [TEA] used was 10, 20, and 40 mM in the pH 7.35, 7.10, and 6.85 solutions, respectively, and affinity was calculated by the binding isotherm: $K_d = [TEA](1 - F)/F$, where $F$ is the fraction of blocked channels.

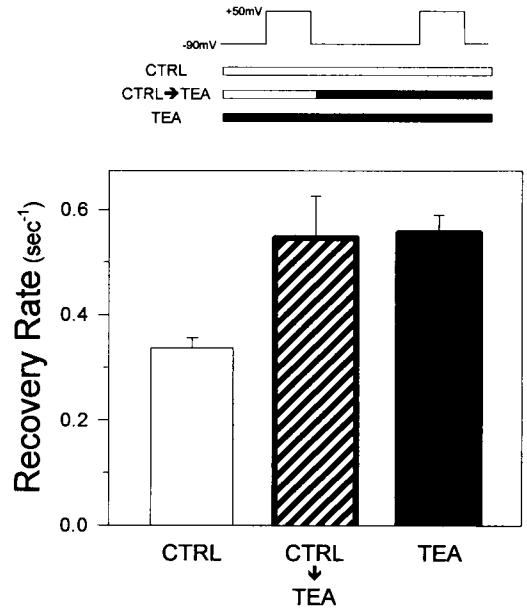



FIGURE 6 The sensitivity of $K^+$-enhanced recovery to TEA exposure after entry into the inactivated state. Cells were perfused with a 141 mM $K^+$/9 mM choline control solution for the first depolarization of a two-pulse protocol. Upon termination of the first pulse, the choline was replaced with TEA (*hatched bar*). Complete exchange of solutions occurred within the first 250 ms of the 2-s interpulse interval. Holding potential and interpulse voltage were −90 mV. Fractional recovery in the continuous presence of the control solution (CTRL) was 49 ± 2% ($n = 6$). When the bath was exchanged for the TEA solution after inactivation (CTRL→TEA), the fractional recovery rose to 65 ± 6%, which was not significantly different from the 67 ± 2% fractional recovery measured in the continuous presence of the TEA solution (TEA). Because of the presence of the TEA during only the second pulse of the experimental protocol, recovery was calculated after correcting the second peak current for the degree of block, as determined from control traces. Recovery rates shown were estimated by the method described for Fig. 4.

as when cells were perfused continuously with 9 mM TEA. This indicates that TEA potentiates the $K^+$-enhanced recovery by binding to an already inactivated channel. Furthermore, it is unlikely that TEA locks $K^+$ at the modulatory site, because the presumably rapid binding of TEA would just as likely inhibit $K^+$ association as it would dissociation.

## DISCUSSION

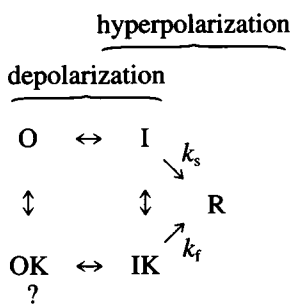
Recovery from C-type inactivation of Kv1.3 can be accelerated by the binding of extracellular potassium to the channel in a voltage-dependent fashion, such that the potency of $K_o^+$ to speed recovery is diminished at the hyperpolarized recovery voltage. Potassium-bound channels appear to have a destabilized inactivated state, with recovery approximately an order of magnitude faster than that of unbound channels. This $K^+$-enhanced recovery can also be potentiated by extracellular TEA, which interacts with the inactivated channel and allosterically either increases the affinity for $K^+$ binding or further destabilizes the inactivated state of the $K^+$ bound channel.

We measured the recovery from inactivation that was induced by successively greater depolarizing voltages and found that increased depolarization attenuates the $K^+$-enhanced recovery from inactivation. One interpretation is that this effect is a consequence of voltage-dependent binding of $K^+$ to a modulatory site located within the membrane electrical field. Another interpretation is that the stronger depolarization forces the channel into a more stable inactivated state, which recovers more slowly. If a voltage-dependent, deeply inactivated state exists independently of $K^+$ binding, we would expect recovery to be slowed after a depolarization to +70 mV in the presence of either 5 mM or 150 mM $K_o^+$. Because recovery was affected by the depolarizing voltage only when $[K^+]_o$ was elevated, then voltage-dependent recovery is most likely due to the voltage-dependent binding of a $K^+$ ion to a modulatory binding site.

In addition to a dependence of recovery on the depolarizing voltage, recovery is also made faster by the extent of hyperpolarization of the cell membrane during the inter

pulse interval (see above; Levy and Deutsch, 1996). Does voltage-dependent binding of $K^+$ entirely account for the voltage dependence of the recovery process? Recovery from N-type inactivation, for example, shows an intrinsic voltage dependence as well as voltage-dependent modulation by extracellular $K^+$ (Gomez-Lagunas and Armstrong, 1994; Demo and Yellen, 1991). Based on the sensitivity of recovery to the depolarizing voltage, we estimated an effective membrane electrical distance of ~30% for the modulatory binding site. This is much less than our previous estimation of 84%, which was determined from the dependence of recovery on the hyperpolarizing voltage during the interpulse interval (Levy and Deutsch, 1996). How can we interpret this difference? One possibility is that, in addition to the voltage dependence of $K^+$ binding, there is an intrinsic voltage dependence of the recovery process. For example, a voltage-dependent conformational change of the modulatory binding site and/or other region(s) of the channel could account for the additional voltage sensitivity observed at strongly hyperpolarized voltages. Alternatively, strong hyperpolarization could lead to a transfer of the $K^+$ ion from a relatively superficial binding site to a deeper, more destabilizing binding site.

After inactivation has occurred at either positive or negative voltages, an increase of $[K^+]_o$ will speed recovery and a decrease of $[K^+]_o$ will slow recovery. Our findings demonstrate that $K^+$ binding to the modulatory site cannot occur solely in the open state, but must also occur in the inactivated state. The state-dependent recovery can be represented by the following simplified scheme:




in which O and OK indicate the unbound and $K^+$-bound channel in the open state, respectively, and I and IK represent the unbound and $K^+$-bound channel in the inactivated state, respectively. Although we have evidence for the IK state, we have no direct evidence for the OK state (indicated in the above scheme by a question mark). At negative voltages, the inactivated channel can recover to a closed state (R) that is available for subsequent activation. The inactivated channel recovers with a slow rate ($k_s$) if $K^+$ is not bound to it (I), or a fast rate ($k_f$) if $K^+$ is bound (IK). Because the fits of Eq. 2 to the time course of recovery (as shown in Fig. 1) were excellent using two fixed time constants ($\tau_s$ and $\tau_f$) and a variable weighting factor, then the conversion of I to IK must be slow compared to the rates of recovery. Therefore, we can use these time constants to calculate not only the rate constants for recovery ($k_s = \tau_s^{-1}$, $k_f = \tau_f^{-1}$), but also to approximate the upper limits for association and dissociation kinetics of $K^+$ binding to the modulatory site at hyperpolarized voltages. The rate constants, $k_s$ and $k_f$, are 0.08 ± 0.03 and 2.0 ± 0.1 $s^{-1}$, respectively, at −130 mV. Therefore, the dissociation rate constant (IK→I) must be less than ~2.0 $s^{-1}$ and the association rate constant (I→IK) must be less than ~0.53 $M^{-1}$ $s^{-1}$. However, these kinetics must be sufficiently fast to account for the sensitivity of recovery rates to changes in $[K^+]_o$ during the hyperpolarized interpulse interval. Because the $K^+$-binding site appears to be within the membrane electrical field (see above), the binding transitions (O↔OK and I↔IK) are likely to be voltage dependent.

How does $K^+$ bind to a modulatory site in the inactivated state? One possibility is that a conformational change of the outer mouth of the pore during the inactivation process exposes a binding site. Two lines of evidence support the likelihood that such a conformational change occurs. First, external TEA block can prevent C-type inactivation (Grissmer and Cahalan, 1989; Choi et al., 1991) and second, Yellen et al. (1994) found that a *Shaker* channel T449C mutant (mutation to the external TEA-binding site) bound $Cd^{2+}$ with 45,000-fold greater affinity once the channel had inactivated. These investigators also found a similar increase of accessibility of *Shaker* residues 448–450 to thiol-specific cross-linking reagents (Liu et al., 1996). Because the affinity of agitoxin (which also interacts with the channel's outer vestibule) was not state dependent, they concluded that C-type inactivation involves a local conformational change that includes only the innermost portion of the outer vestibule. The observations of Yellen and co-workers provide evidence for the state-dependent accessibility of specific residues. Similar mechanisms may underlie a state- and voltage-dependent nature of the $K^+$-enhanced recovery from C-type inactivation.

In comparison to our previously published results (Levy and Deutsch, 1996), we have measured a greater sensitivity to $[K^+]_o$ elevated during the interpulse interval. This is likely to be due to the shorter interpulse interval used in the present experiments (2 vs. 10 s), which maximizes the ability to detect an increased weighting of the fast component of recovery. For example, the curves in Fig. 3 *B* for 150 mM $[K^+]_o$ show that at +10 mV (as opposed to +70 mV) there is a greater percentage increase in recovery for short interpulse intervals. At 2 s, there is 37% greater fractional recovery after the depolarization to +10 mV (compared to +70 mV), but at 10 s, there is only a 14% increased fractional recovery. Thus, the 2-s interpulse intervals that we used for the present studies of state-dependent $K^+$ binding have improved our ability to detect changes in recovery kinetics.

Like the modulatory $K^+$-binding site, the binding site for TEA blockade also appears to lie within the membrane electrical field (Heginbotham and MacKinnon, 1992). Because the residue critically involved in TEA binding (*Shaker* 449) also mediates the $K^+$-sensitive entry into the C-type inactivated state (Lopez-Barneo et al., 1993), we

investigated whether this location also contributes to the modulation of recovery from the inactivated state. We found no evidence, however, to show that modulation of recovery is caused by $K^+$ interacting with the equivalent residue in Kv1.3, H399. First, the TEA affinity was unaltered by raising $[K^+]_o$. Second, rather than inhibiting the $K^+$ modulation of recovery, extracellular TEA led to greater recovery. Third, acidification of bath solution, which reduces TEA binding by more than threefold, actually potentiated the $K^+$-enhanced recovery. Finally, we were unable to remove the $K^+$ sensitivity of recovery by converting this histidine residue to a tyrosine (Kv1.3 H399Y expressed in the cytotoxic lymphocyte cell line, CTLL-2; Levy, Panyi, and Deutsch, unpublished observations). Clearly, the H399 residue does not directly contribute to the modulatory binding site. On the other hand, our findings point to the possibility that additional positive charges at H399 ($H^+$ or $TEA^+$) can allosterically affect the $K^+$-enhanced recovery. We speculate that added positive charge facilitates $K^+$ destabilization of the inactivated state, or alternatively, the added charge may increase the affinity of $K^+$ binding to the nearby modulatory site, thus speeding recovery.

We believe that this is the first evidence that extracellular TEA can bind to an inactivated $K^+$ channel. Although neither Kv1.3 nor the *Shaker* $K^+$ channel can enter the C-type inactivated state when bound by extracellular TEA (Grissmer and Cahalan, 1989; Choi et al., 1991), this does not preclude the possibility that TEA can bind after the channel inactivates. In fact, Liu et al. (1996) have shown that inactivation greatly increases the affinity of the charged methanethiolsulfonate-ethylsulfonate to cysteine residues engineered at the TEA-binding site of *Shaker*. Although we have not studied the $K^+$ modulation of recovery of other *Shaker* $K^+$-channel isoforms, if they were to show a more sensitive potentiation of $K^+$-enhanced recovery by TEA, then TEA could serve as a probe to study the conformational changes involved in C-type inactivation of the channels.

The findings in this paper show that $K^+$ can regulate its own conductance by modulating recovery from C-type inactivation. This autoregulation may play an important physiological role in T-lymphocyte function. For instance, $[K^+]_o$ at sites of inflammation is significantly elevated (Silver, 1975). A rise of $[K^+]_o$ would speed recovery from inactivation, leading to an increased $K^+$-channel conductance that, in turn, would change the membrane potential. Changes in lymphocyte membrane potential influence mitogen-stimulated lymphokine production (Freedman et al., 1992; Lin et al., 1993), regulation of cell volume (Deutsch and Chen, 1993), and other lymphocyte functions that are voltage dependent. Potassium-modulated recovery may also be physiologically important for neurons expressing Kv1.3. A 25-fold increase in $[K^+]_o$ caused by ischemia (Hansen and Zeuthen, 1981), for example, could promote $K^+$ modulation that would alter the cellular membrane potential and/or attenuate cellular excitability.

We are grateful to Drs. Gyorgy Panyi and Richard Horn for many stimulating discussions. We also thank Mrs. Joan Kroc for assistance with the preparation of this manuscript. This work was supported by National Institutes of Health grant GM 52302, and a University of Pennsylvania Research Foundation grant.

## REFERENCES

Baukrowitz, T., and G. Yellen. 1995. Modulation of $K^+$ current by frequency and external $[K^+]$: a tale of two inactivation mechanisms. *Neuron.* 15:951–960.

Brett, R. S., J. P. Dilger, P. R. Adams, and B. Lancaster. 1986. A method for the rapid exchange of solutions bathing excised membrane patches. *Biophys. J.* 50:987–992.

Chandy, K. G., and G. A. Gutman. 1994. Voltage-gated potassium channel genes. *In* Ligand- and Voltage-Gated Ion Channels. R. A. North, editor. CRC Press, Boca Raton, FL. 1–71.

Choi, K. L., R. W. Aldrich, and G. Yellen. 1991. Tetraethylammonium blockade distinguishes two inactivation mechanisms in voltage-activated $K^+$ channels. *Proc. Natl. Acad. Sci. USA.* 88:5092–5095.

Demo, S. D., and G. Yellen. 1991. The inactivation gate of the *Shaker* $K^+$ channel behaves like an open-channel blocker. *Neuron.* 7:743–753.

Deutsch, C., M. Price, S. C. Lee, V. F. King, and M. L. Garcia. 1991. Characterization of high affinity binding sites for charybdotoxin in human T lymphocytes. Evidence for association with the voltage-gated K channel. *J. Biol. Chem.* 266:3668–3674.

Deutsch, C., and L. Q. Chen. 1993. Heterologous expression of specific $K^+$ channels in T lymphocytes: functional consequences for volume regulation. *Proc. Natl. Acad. Sci. USA.* 90:10036–10040.

Freedman, B. D., M. A. Price, and C. J. Deutsch. 1992. Evidence for voltage modulation of IL-2 production in mitogen-stimulated human peripheral blood lymphocytes. *J. Immunol.* 149:3784–3794.

Gomez-Lagunas, F., and C. M. Armstrong. 1994. The relation between ion permeation and recovery from inactivation of *Shaker*B $K^+$ channels. *Biophys. J.* 67:1806–1815.

Gomez-Lagunas, F., and C. M. Armstrong. 1995. Inactivation in *Shaker*B $K^+$ channels: a test for the number of inactivating particles on each channel. *Biophys. J.* 68:89–95.

Grissmer, S., and M. D. Cahalan. 1989. TEA prevents inactivation while blocking open $K^+$ channels in human T lymphocytes. *Biophys. J.* 55:203–206.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* 391:85–100.

Hansen, A. J., and T. Zeuthen. 1981. Extracellular ion concentration during spreading depression and ischemia in the rat brain cortex. *Acta Physiol. Scand.* 113:437–445.

Heginbotham, L., and R. MacKinnon. 1992. The aromatic binding site for tetraethylammonium ion on potassium channels. *Neuron.* 8:483–491.

Hille, B. 1992. Ionic Channels of Excitable Membranes. Sinauer, Sunderland, MA.

Hoshi, T., W. N. Zagotta, and R. W. Aldrich. 1990. Biophysical and molecular mechanisms of *Shaker* potassium channel inactivation. *Science.* 250:533–538.

Hoshi, T., W. N. Zagotta, and R. W. Aldrich. 1991. Two types of inactivation in *Shaker* $K^+$ channels: effects of alterations in the carboxy-terminal region. *Neuron.* 7:547–556.

Kavanaugh, M. P., M. D. Varnum, P. B. Osborne, M. J. Christie, A. E. Busch, J. P. Adelman, and R. A. North. 1991. Interaction between tetraethylammonium and amino acid residues in the pore of cloned voltage-dependent potassium channels. *J. Biol. Chem.* 266:7583–7587.

Kupper, J., M. R. Bowlby, S. Marom, and I. B. Levitan. 1995. Intracellular and extracellular amino acids that influence C-type inactivation and its modulation in a voltage-dependent potassium channel. *Pflügers Arch.* 430:1–11.

Levy, D. I., and C. Deutsch. 1996. Recovery from C-type inactivation is modulated by extracellular potassium. *Biophys. J.* 70:798–805.

Lin, C. S., R. C. Boltz, J. T. Blake, M. Nguyen, A. Talento, P. A. Fischer, M. S. Springer, N. H. Sigal, R. S. Slaughter, M. L. Garcia, G. J.

Kaczorowski, and G. C. Koo. 1993. Voltage-gated potassium channels regulate calcium-dependent pathways involved in human T lymphocyte activation. *J. Exp. Med.* 177:637–645.

Liu, Y., M. E. Jurman, and G. Yellen. 1996. Dynamic rearrangement of the outer mouth of a $K^+$ channel during gating. *Neuron.* 16:859–867.

Lopez-Barneo, J., T. Hoshi, S. H. Heinemann, and R. W. Aldrich. 1993. Effects of external cations and mutations in the pore region on C-type inactivation of *Shaker* potassium channels. *Receptors Channels.* 1:61–71.

MacKinnon, R., R. W. Aldrich, and A. W. Lee. 1993. Functional stoichiometry of *Shaker* potassium channel inactivation. *Science.* 262:757–759.

Matteson, D. R., and C. Deutsch. 1984. K channels in T lymphocytes: a patch clamp study using monoclonal antibody adhesion. *Nature.* 307:468–471.

Ogielska, E. M., W. N. Zagotta, T. Hoshi, S. H. Heinemann, J. Haab, and R. W. Aldrich. 1995. Cooperative subunit interactions in C-type inactivation of $K^+$ channels. *Biophys. J.* 69:2449–2457.

Panyi, G., Z. Sheng, L. Tu, and C. Deutsch. 1995. C-type inactivation of a voltage-gated $K^+$ channel occurs by a cooperative mechanism. *Biophys. J.* 69:896–903.

Pardo, L. A., S. H. Heinemann, H. Terlau, U. Ludewig, C. Lorra, O. Pongs, and W. Stuhmer. 1992. Extracellular $K^+$ specifically modulates a rat brain $K^+$ channel. *Proc. Natl. Acad. Sci. USA.* 89:2466–2470.

Silver, I. A. 1975. Measurement of pH and ionic composition of pericellular sites. *Phil. Trans. R. Soc. Lond. B.* 271:261–272.

Seoh, S. A., and D. M. Papazian. 1995. Mutations at D447 in the P region of *Shaker* $K^+$ channels accelerate C-type inactivation. *Biophys. J.* 68: A35 [Abstract].

Smith, P. L., T. Baukrowitz, and G. Yellen. 1996. The inward rectification mechanism of the HERG cardiac potassium channel. *Nature.* 379: 833–836.

Wolfowitz, J. 1943. On the theory of runs with some applications to quality control. *Ann. Math. Stat.* 14:280–288.

Yellen, G., D. Sodickson, T. Y. Chen, and M. E. Jurman. 1994. An engineered cysteine in the external mouth of a $K^+$ channel allows inactivation to be modulated by metal binding. *Biophys. J.* 66: 1068–1075.