

## Two Mechanisms of $K^+$ -Dependent Potentiation in Kv2.1 Potassium Channels

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**ABSTRACT** Elevation of external  $[K^+]$  potentiates outward  $K^+$  current through several voltage-gated  $K^+$  channels. This increase in current magnitude is paradoxical in that it occurs despite a significant decrease in driving force. We have investigated the mechanisms involved in  $K^+$ -dependent current potentiation in the Kv2.1  $K^+$  channel. With holding potentials of  $-120$  to  $-150$  mV, which completely removed channels from the voltage-sensitive inactivated state, elevation of external  $[K^+]$  up to 10 mM produced a concentration-dependent increase in outward current magnitude. In the absence of inactivation, currents were maximally potentiated by 38%. At more positive holding potentials, which produced steady-state inactivation,  $K^+$ -dependent potentiation was enhanced. The additional  $K^+$ -dependent potentiation (above 38%) at more positive holding potentials was precisely equal to a  $K^+$ -dependent reduction in steady-state inactivation. Mutation of two lysine residues in the outer vestibule of Kv2.1 (K356 and K382), to smaller, uncharged residues (glycine and valine, respectively), completely abolished  $K^+$ -dependent potentiation that was not associated with inactivation. These mutations did not influence steady-state inactivation or the  $K^+$ -dependent potentiation due to reduction in steady-state inactivation. These results demonstrate that  $K^+$ -dependent potentiation can be completely accounted for by two independent mechanisms: one that involved the outer vestibule lysines and one that involved  $K^+$ -dependent removal of channels from the inactivated state. Previous studies demonstrated that the outer vestibule of Kv2.1 can be in at least two conformations, depending on the occupancy of the selectivity filter by  $K^+$  (Immke, D., M. Wood, L. Kiss, and S. J. Korn. 1999. *J. Gen. Physiol.* 113:819–836; Immke, D., and S. J. Korn. 2000. *J. Gen. Physiol.* 115:509–518). This change in conformation was functionally defined by a change in TEA sensitivity. Similar to the  $K^+$ -dependent change in TEA sensitivity, the lysine-dependent potentiation depended primarily (>90%) on Lys-356 and was enhanced by lowering initial  $K^+$  occupancy of the pore. Furthermore, the  $K^+$ -dependent changes in current magnitude and TEA sensitivity were highly correlated. These results suggest that the previously described  $K^+$ -dependent change in outer vestibule conformation underlies the lysine-sensitive,  $K^+$ -dependent potentiation mechanism.

### INTRODUCTION

In many voltage-gated  $K^+$  channels, outward currents carried by  $K^+$  increase in magnitude upon elevation of external  $[K^+]$  (Pardo et al., 1992; Lopez-Barneo et al., 1993; Sanguinetti et al., 1995; Abbott et al., 1999; Grigoriev et al., 1999; Yang and Sigworth, 1998). This effect is paradoxical in that it occurs despite significant changes in driving force that would act to reduce outward current magnitude. The molecular mechanism that underlies  $K^+$ -dependent potentiation, or whether more than one mechanism contributes to the effect, is not known.

Current magnitude in wild-type *Shaker* is essentially insensitive to external  $[K^+]$  at concentrations between 0.3 and 30 mM (Lopez-Barneo et al., 1993). In channels derived from *Shaker* that have mutations at position 449, external  $K^+$  appeared to potentiate currents by making more channels available for activation (Lopez-Barneo et al., 1993). Similarly, in RCK4 channels, elevation of external  $[K^+]$  did not increase single channel conductance or mean open time, but appeared to increase the number of available channels (Pardo et al., 1992). In these channels, channel functionality

appears to require external  $K^+$  (Pardo et al., 1992; Lopez-Barneo et al., 1993; Melishchuk et al., 1998). This type of mechanism cannot account for  $K^+$ -dependent potentiation in all  $K^+$  channels, however, since many  $K^+$  channels that display  $K^+$ -dependent potentiation can operate in the absence of external  $K^+$ .

The voltage-gated  $K^+$  channel, Kv2.1, conducts well in the absence of  $K^+$  (Korn and Ikeda, 1995), and displays marked  $K^+$ -dependent current potentiation at physiological  $[K^+]$  (see Fig. 1 A). Kv2.1 contains two lysines in the outer vestibule, at positions 356 and 382, that have side chains exposed to the conduction pathway (Gross et al., 1994; Kurz et al., 1995). Following mutation of these lysines to smaller uncharged amino acids (glycine and valine, respectively),  $K^+$  interacts with site(s) associated with cation selectivity (presumably at the selectivity filter) at a lower concentration (see Immke et al., 1998, 1999). When present, these lysines also reduce the apparent affinity of the cationic pore blockers, TEA and agitoxin, for their binding sites in the outer vestibule (Gross et al., 1994; Bretschneider et al., 1999; Immke et al., 1999). Thus, these lysines appear to influence, either electrostatically or sterically, the interaction of cations with the channel pore.

The outer vestibule of Kv2.1 undergoes a conformational change as a function of  $K^+$  occupancy of the selectivity filter (Immke et al., 1999). Two outer vestibule conformations can be functionally defined (Immke and Korn, 2000).

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In one conformation, which occurs when occupancy of a  $K^+$  binding site in the pore is high, channels are TEA-sensitive. Conversely, when occupancy of this site is low, channels become TEA-insensitive. The K356G, K382V mutations eliminate the  $K^+$ -dependent change in TEA efficacy (Immke and Korn, 2000). However, these mutations do not prevent the  $K^+$ -dependent change in outer vestibule conformation that influences TEA sensitivity (Immke et al., 1999). Furthermore, these two mutations do not alter the kinetics of slow inactivation (Immke et al., 1999), which is thought to involve a different conformational change in the outer vestibule (Yellen et al., 1994; Liu et al., 1996). Taken together, these data were consistent with the hypothesis that the  $K^+$ -dependent change in outer vestibule conformation reoriented the outer vestibule lysines such that they interfered with the ability of TEA to bind to its binding site (Immke et al., 1999; Immke and Korn, 2000), which is located just external to the selectivity filter (Heginbotham and MacKinnon, 1992; Doyle et al., 1998). The observation that these lysines also influence the apparent affinity of  $K^+$  for the selectivity filter suggests that their influence on  $K^+$  in the pore could change if they reorient at different  $[K^+]$ . Thus, the  $K^+$ -dependent conformational change could influence the magnitude of  $K^+$  current through the pore. According to the model proposed by Immke et al. (1999), reorientation of the outer vestibule lysines at reduced  $[K^+]$  would decrease current magnitude. Conversely, lysine reorientation upon elevation of  $[K^+]$  would increase current magnitude.

An additional mechanism by which changes in  $[K^+]$  could potentiate current magnitude would be via an effect on slow inactivation. Elevation of external  $[K^+]$  can slow the rate of C-type inactivation (Lopez-Barneo et al., 1993; Baukowitz and Yellen, 1995; Grigoriev et al., 1999) and speed the exit of channels from the C-type inactivated state (Levy and Deutsch, 1996). A  $K^+$ -dependent change in rate constants into and/or out of the inactivated state, consistent with these observations, could account for an increase in macroscopic current magnitude if the holding potentials used placed some channels in the inactivated state. Although this mechanism was previously rejected for *Shaker* (Lopez-Barneo et al., 1993), this possibility has not been revisited in other  $K^+$  channels, some of which display mechanistic differences in the slow inactivation process (Klemic et al., 1998; Fedida et al., 1999; Immke et al., 1999).

We tested these hypotheses, and further examined the mechanism of  $K^+$ -dependent potentiation in Kv2.1. Our results indicate that potentiation in Kv2.1 can be completely accounted for by two independent mechanisms. One mechanism, which is voltage-independent, appears to involve the  $K^+$ -dependent change in the outer vestibule conformation that involves lysines 356 and 382. In addition, at membrane potentials that place some channels in the inactivated state, elevation of external  $[K^+]$  potentiates macroscopic current by removing channels from the inactivated state.

## METHODS

### Molecular biology and channel expression

Experiments were done on wild-type Kv2.1 and three mutant channels derived from Kv2.1 (Kv2.1 K356G, Kv2.1 K382V and Kv2.1 K356G, K382V). Mutagenesis details for the Kv2.1 mutants are described in Immke et al. (1999). For all channels,  $K^+$  channel cDNA was subcloned into the pcDNA3 expression vector and channels expressed in the human embryonic kidney cell line, HEK293 (American Type Culture Collection, Rockville, MD). Cells were maintained in DMEM plus 10% fetal bovine serum (Gibco BRL, Grand Island, NY) with 1% penicillin/streptomycin (maintenance media). Cells ( $2 \times 10^6$  cells/ml) were co-transfected by electroporation (Invitrogen Electroporator II; 71  $\mu$ F, 375 V) with  $K^+$  channel expression plasmid (0.5–1.5  $\mu$ g/0.2 ml) and CD8 antigen (1  $\mu$ g/0.2 ml). After electroporation, cells were plated on protamine (1 mg/ml; Sigma Chemical Co., St. Louis, MO)-coated glass coverslips submerged in maintenance media. Electrophysiological recordings were made 18–48 h later. On the day of recording, cells were washed with fresh media and incubated with Dynabeads M450 conjugated with antibody to CD8 (1  $\mu$ l/ml; Dynal, Lake Success, NY). Cells that expressed CD8 became coated with beads, which allowed visualization of transfected cells (Jurman et al., 1994).

### Electrophysiology

Currents were recorded at room temperature in the whole-cell patch-clamp configuration. Patch pipettes were fabricated from N51A glass (Garner Glass Co, Claremont, CA), coated with Sylgard, and firepolished. Data were collected with an Axopatch 200A amplifier, pClamp 6 software, and a Digidata 1200 A/D board (Axon Instruments, Foster City, CA). Currents were filtered at 2 KHz and sampled at 200–10,000  $\mu$ s/pt. Series resistance ranged from 0.5 to 2.5 M $\Omega$  and was compensated 80–90%. Holding potentials ranged from –150 mV to –70 mV and are specified in the figure legends. Depolarizing stimuli were presented once every 5–60 s, depending on the experiment.  $H_\infty$  curves were generated by a three-pulse protocol (see Fig. 4 A) and fit to the equation,

$$I/I_{\max} = ni + [(1 - ni)/(1 + \exp((V - V_h)/r))] \quad (1)$$

where  $I$  is the test current magnitude,  $I_{\max}$  is the maximum current obtained in the absence of inactivation,  $ni$  is the fraction of non-inactivating current,  $V$  is the test voltage,  $V_h$  is the voltage at which channels are half-inactivated, and  $r$  is the slope factor.

Data were analyzed with Clampfit (Axon Instruments). Curve-fitting and significance testing (unpaired Student's  $t$ -test) were done with SigmaPlot 2.0. Current magnitude was measured at the peak. All plotted data are represented as mean  $\pm$  SEM, with the number of data points denoted by  $n$ . Error values for the difference between means ( $x$  axes in Figs. 5 C and 6 C) were calculated as the quadratic sum of the errors. When a range of values is given for  $n$ , this range represents the number of cells used for each data point in a complete curve from which a single value was calculated.

### Electrophysiological solutions

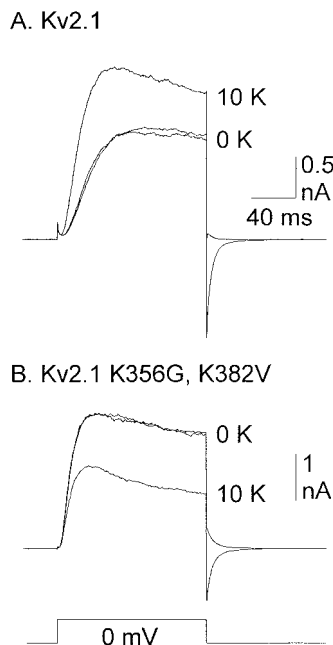
Currents were recorded in a constantly flowing, gravity-fed bath. Solutions were placed in one of six reservoirs, each of which fed via plastic tubing into a single quartz tip ( $\sim 100$   $\mu$ m diameter; ALA Scientific Instruments, Westbury, NY). The tip was placed within 20  $\mu$ m of the cell being recorded before the start of the experiment. One solution was always flowing, and solutions were changed by manual switching (solution exchange was complete within 5–10 s). Control internal solutions contained (in mM): 140 XA (X = a combination of  $K^+$  and NMG $^+$ , A = a combination of  $Cl^-$  and  $F^-$ ), 20 HEPES, 10 EGTA, 1  $CaCl_2$ , 4  $MgCl_2$ ; pH 7.3, osmolality 285.  $F^-$  was added to internal solutions to stabilize cells with holding potentials more negative than –100 mV. At –80 mV,

measurements with 0, 50, and 100 mM F<sup>−</sup> produced quantitatively identical results. Control external solutions contained (in mM): 165 XCl, 20 HEPES, 10 glucose, 2 CaCl<sub>2</sub>, and 1 MgCl<sub>2</sub>; pH 7.3, osmolality 325. In all experiments, internal and external [K<sup>+</sup>] and [F<sup>−</sup>] are reported in the figure legends. Osmotic balance was maintained with NMG<sup>+</sup>. Other additions and substitutions are listed in the figure legends.

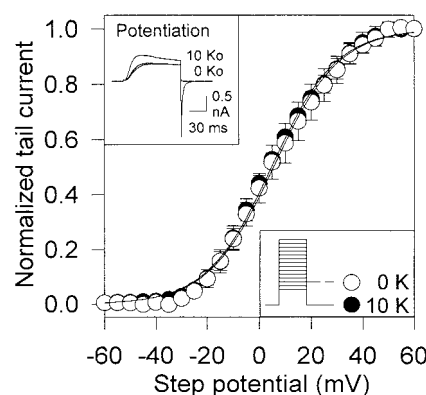
## RESULTS

Elevation of external potassium from 0 mM to 10 mM potentiated outward K<sup>+</sup> currents through Kv2.1 (Fig. 1 *A*), despite the large decrease in driving force (note the difference in tail currents with 0 and 10 mM external K<sup>+</sup>). Potentiation was accompanied by an increase in activation rate and an increase in inactivation rate. The conductance-voltage curve was identical in all respects at different external [K<sup>+</sup>] (Fig. 2), which indicates that the change in current magnitude and activation rate did not result from a K<sup>+</sup>-dependent shift in the voltage-dependence of channel activation. The magnitude of potentiation produced by elevation of external [K<sup>+</sup>] to 10 mM was identical when currents were recorded at 0 mV ( $57.5 \pm 3.9\%$ ,  $n = 6$ ), +20 mV ( $57.3 \pm 2.9\%$ ,  $n = 4$ ), and +40 mV ( $55.7 \pm 4.3\%$ ,  $n = 5$ ).

The increase in Kv2.1 inactivation rate with 10 mM external K<sup>+</sup> is associated with the increased K<sup>+</sup> occupancy of the selectivity filter (Immke et al., 1999). In Kv2.1, the



**FIGURE 1** Potentiation of wild-type Kv2.1 but not Kv2.1 K356G, K382V by 10 mM external K<sup>+</sup>. Currents were evoked by a 100-ms depolarization to 0 mV from a holding potential of −80 mV, in the presence of 0 and 10 mM external K<sup>+</sup>. The two traces recorded in 0 mM external K<sup>+</sup> were recorded before and after switching to 10 mM K<sup>+</sup>. (*A*) Currents through Kv2.1. (*B*) Currents through Kv2.1 K356G, K382V. Internal [K<sup>+</sup>] was 100 mM, internal [F<sup>−</sup>] was 0 mM.



**FIGURE 2** Voltage-dependence of activation at two different external [K<sup>+</sup>]. Internal [K<sup>+</sup>] was 30 mM (internal [K<sup>+</sup>] was reduced in these experiments to reduce current magnitude at very positive potentials). The protocol used is shown in the lower right corner. Curves were generated from normalized peak tail current magnitude, measured at −40 mV (0 K<sup>+</sup>) and −80 mV (10 mM K<sup>+</sup>). Holding potential was −80 mV. The potentiation of outward currents by 10 mM external K<sup>+</sup>, with 30 mM internal K<sup>+</sup>, is illustrated in the inset (*upper left*). Internal [F<sup>−</sup>] = 30 mM. Data points represent mean  $\pm$  SEM of 6 cells for which complete curves were obtained. Solid lines illustrate the best fit of a Boltzmann equation.

outer vestibule can take on two functionally distinguishable conformations, depending on the occupancy of a selectivity filter site by K<sup>+</sup> (Immke et al., 1999; Immke and Korn, 2000). In the high [K<sup>+</sup>] conformation channels are sensitive to TEA, whereas in the low [K<sup>+</sup>] conformation channels are TEA-insensitive. Mutation of two lysines in the outer vestibule (K356 and K382) to the smaller, neutral amino acids, glycine and valine, eliminates the influence of the K<sup>+</sup>-dependent conformational change on TEA efficacy (Immke and Korn, 2000). In Kv2.1 channels with these same mutations, elevation of external [K<sup>+</sup>] to 10 mM did not potentiate currents as in wild-type Kv2.1 (Fig. 1 *B*). Rather, current magnitude was decreased, consistent with the change in driving force produced by elevation of external [K<sup>+</sup>]. This suggested that these two lysines were also involved in the K<sup>+</sup>-dependent potentiation process. The experiments below were designed to test the hypothesis that the lysine-dependent mechanism responsible for the K<sup>+</sup>-dependent change in TEA efficacy contributed to the K<sup>+</sup>-dependent change of current magnitude.

## The [K<sup>+</sup>]-dependence of potentiation

Two possible explanations could account for the qualitatively different effects of K<sup>+</sup> on current magnitude in wild-type and mutant Kv2.1. First, K<sup>+</sup>-dependent current potentiation could have been eliminated, or reduced in magnitude, by the double lysine mutation. Alternatively, the [K<sup>+</sup>]-dependence of potentiation may have been shifted, such that potentiation occurred at lower [K<sup>+</sup>]. This possibility was suggested by previous observations that K<sup>+</sup> in-

teracted with the selectivity filter at 3–10-fold lower concentrations in the double lysine mutant than in wild-type Kv2.1 (cf. Immke et al., 1998, 1999).

Fig. 3 illustrates the  $[K^+]$ -dependence of potentiation in Kv2.1 and Kv2.1 K356G, K382V. In wild-type Kv2.1, current potentiation was initially observed at a  $[K^+]$  of 0.3 to 1 mM, and was maximal at  $[K^+]$  of 10 mM (Fig. 3 C). At  $[K^+]$  higher than 10 mM, current magnitude began to decrease due to the change in driving force. In the Kv2.1 double lysine mutant, potentiation occurred at lower  $[K^+]$ . Statistically significant potentiation occurred at 100  $\mu$ M external  $K^+$  and peaked at 300  $\mu$ M  $K^+$  (Fig. 3, B and C). At  $[K^+]$  higher than 0.3 mM, the effect of driving force change on current magnitude overwhelmed the effect of potentiation (Fig. 3 C). Two results are readily apparent from Fig. 3. First, the  $[K^+]$ -dependence of potentiation was shifted to the left in the double mutant Kv2.1, consistent with the increase in apparent affinity for  $K^+$  observed in other functional measurements. More dramatic, however, was the decrease in the magnitude of potentiation in the double mutant channel. This indicated that the differences observed in Fig. 1 between the two channels did not result simply from a change in  $K^+$  potency. Finally, in the double mutant channel,  $K^+$ -dependent current potentiation was not associated with a change in activation rate (see Fig. 3 B).

### $K^+$ -dependent removal of channels from the inactivated state

When  $K^+$ -dependent current potentiation was first described in *Shaker*-like channels, experiments suggested that it did not result from a  $K^+$ -dependent decrease in inactivation (Lopez-Barneo et al., 1993). However, elevation of external  $K^+$  can speed the recovery from inactivation (Levy and Deutsch, 1996), and therefore could also reduce the number of channels in the inactivated state. We reexamined the possibility that removal of channels from the inactivated

state contributed to the  $K^+$ -dependent current potentiation in Kv2.1.

We examined steady-state inactivation with the protocol illustrated in Fig. 4 A. Cells were held at a potential of  $-120$  mV. Following an 80-ms test pulse, cells were held at potentials between  $-140$  mV and  $0$  mV for 20 s, followed by another 80-ms test pulse. Stimuli were delivered once every 30 s. As can be observed in the initial test currents, this stimulus interval was sufficient for complete recovery from inactivation. Fig. 4 B illustrates the  $H_\infty$  curves for Kv2.1 in the presence of 0 and 10 mM external  $K^+$ . At the holding potential of  $-80$  mV with 0 mM external  $K^+$ , current was inactivated by  $\sim 12\%$ . Elevation of external  $K^+$  to 10 mM, which produced peak potentiation in wild-type Kv2.1, shifted the  $H_\infty$  curve to the right by 9.5 mV. Consequently, in the presence of 10 mM  $K^+$ , no steady-state inactivation occurred at  $-80$  mV. At  $-120$  mV, channels were completely removed from any voltage-dependent inactivated state regardless of external  $[K^+]$ .

External  $K^+$  influenced steady-state inactivation similarly in the double mutant Kv2.1 channel (Fig. 4 C). In 0 external  $K^+$ , half-maximal inactivation in the double mutant occurred at the same potential as in wild-type Kv2.1. At  $-120$  mV, channels were completely removed from the inactivated state. Elevation of external  $[K^+]$  to 0.3 mM, which produced peak potentiation in the double mutant channel, shifted the steady-state inactivation curve to the right by 11.4 mV. This removed all channels from the inactivated state at  $-80$  mV, and had no effect on channel inactivation at  $-120$  mV.

### Two mechanisms of potentiation in wild-type Kv2.1

Fig. 5 A illustrates the  $K^+$ -dependent potentiation in wild-type Kv2.1 channels held at  $-70$  mV (top),  $-80$  mV (middle), and  $-120$  mV (bottom). With holding potentials

FIGURE 3  $[K^+]$ -dependence of potentiation. (A, B) Currents recorded from Kv2.1 (A) and Kv2.1 K356G, K382V (B) in the presence of 0 and 0.3 mM external  $K^+$ . The internal solution contained 100 mM  $K^+$  and 0 mM  $F^-$ . Currents were evoked by depolarization to 0 mV from a holding potential of  $-80$  mV. (C) Magnitude of potentiation as a function of external  $[K^+]$ . Current magnitude was normalized to that in the presence of 0 mM  $K^+$  (dotted line). Data represent mean  $\pm$  SEM from 3 to 6 cells at each data point.

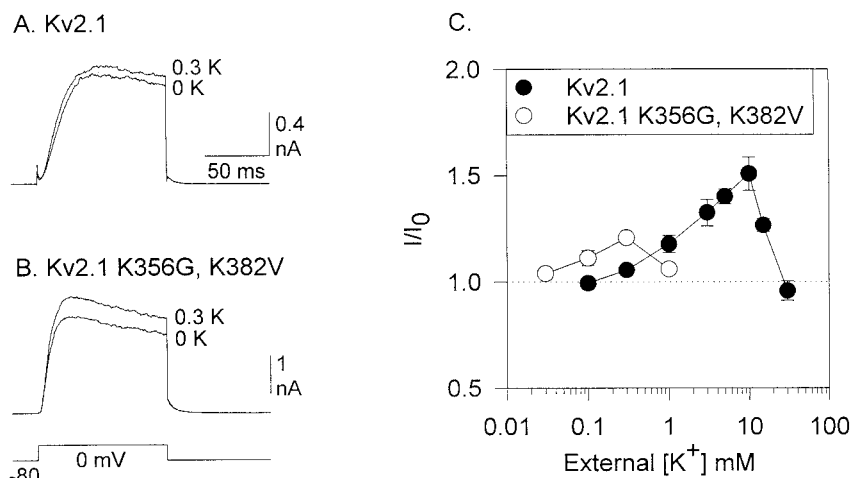
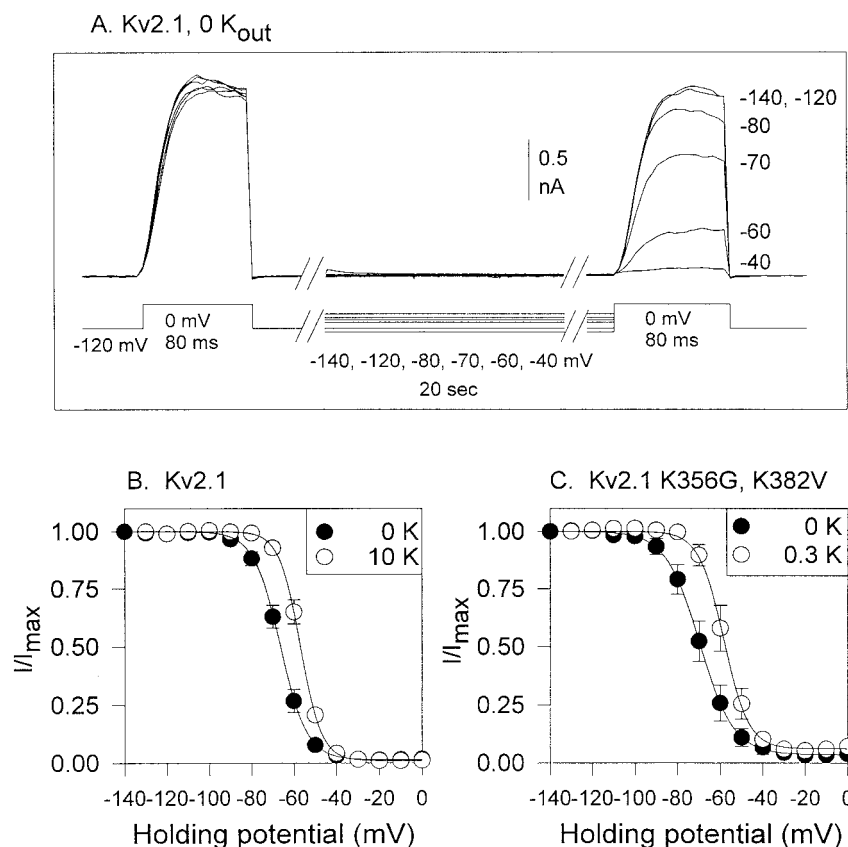




FIGURE 4 K<sup>+</sup>-dependent shift in steady-state inactivation. (A) Protocol used to measure steady-state inactivation, and currents recorded from a cell transfected with Kv2.1. Details of the protocol are described in the text. Currents were recorded with 100 mM internal K<sup>+</sup> and both 50 and 100 mM internal F<sup>-</sup>. Data were sampled at 250 Hz. (B)  $H_{\infty}$  curves for Kv2.1 in the presence of 0 and 10 mM external K<sup>+</sup>. The solid lines represent the best fit of the data to Eq. 1. In 0 and 10 mM K<sup>+</sup>,  $V_h = -66.5 \pm 0.2$  mV and  $-57.0 \pm 0.1$  mV,  $r = 6.4$  and  $5.0$ , and  $ni = 0.01$  and  $0.01$ , respectively. (C)  $H_{\infty}$  curves for Kv2.1 K356G, K382V in the presence of 0 and 0.3 mM external K<sup>+</sup>. The solid lines represent the best fit of the data to Eq. 1. In 0 and 0.3 mM K<sup>+</sup>,  $V_h = -69.8 \pm 0.1$  mV and  $-58.4 \pm 0.3$  mV,  $r = 8.0$  and  $5.8$ , and  $ni = 0.04$  and  $0.06$ , respectively.



of  $-150$  and  $-120$  mV, where no channels were in the inactivated state, 10 mM K<sup>+</sup> potentiated currents identically (percent potentiation =  $35.3 \pm 1.1$ ,  $n = 5$ ; and  $38.1 \pm 3.2\%$ ,  $n = 8$ ; respectively; Fig. 5 B). As the holding potential was made more positive into the range where voltage-dependent inactivation was observed, the magnitude of K<sup>+</sup>-dependent potentiation increased (Fig. 5, A and B). Fig. 5 C illustrates the percent potentiation produced by 10 mM external K<sup>+</sup>, plotted as a function of the K<sup>+</sup>-dependent reduction in steady-state inactivation, for three different holding potentials (shown in parentheses). The dotted line represents the magnitude of potentiation observed with a holding potential of  $-120$  mV. The additional potentiation above this level precisely corresponded to the reduction in percent inactivation produced by 10 mM K<sup>+</sup>. These data strongly suggest that two independent mechanisms were responsible for K<sup>+</sup>-dependent current potentiation in Kv2.1. One mechanism was voltage-independent at holding potentials between  $-150$  and  $-70$  mV, and was independent of inactivation. An additional component of K<sup>+</sup>-dependent potentiation could be completely accounted for by the removal of channels from the voltage-sensitive inactivated state.

#### Potentiation did not involve relief of Mg<sup>2+</sup> block

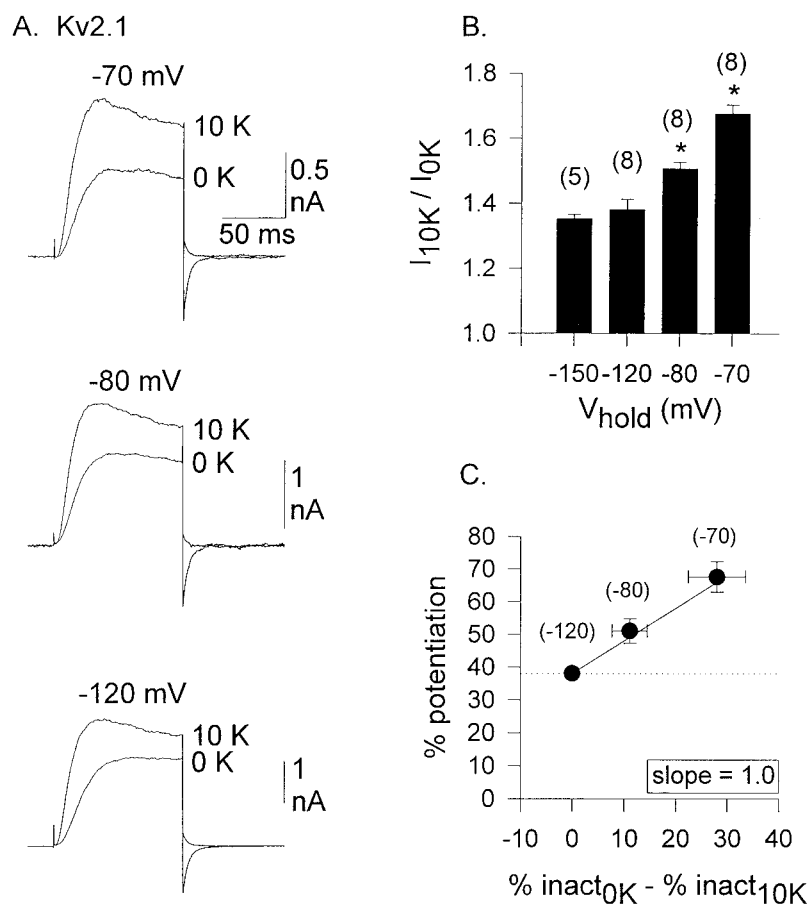
K<sup>+</sup> channels, including Kv2.1, can be blocked by intracellular Mg<sup>2+</sup> (Lopatin and Nichols, 1994; Harris and Isacoff,

1996). In Kv2.1, Mg<sup>2+</sup> block appears to be minimal at potentials lower than  $+80$  mV (Lopatin and Nichols, 1994; our unpublished data). Nonetheless, we tested the possibility that K<sup>+</sup>-dependent potentiation depended, in part, on relief of block by intracellular Mg<sup>2+</sup>. When intracellular Mg<sup>2+</sup> and Ca<sup>2+</sup> were omitted from the internal solution, and the holding potential was  $-120$  mV, 10 mM K<sup>+</sup> potentiated currents through Kv2.1 by  $37.2 \pm 2.0\%$  ( $n = 3$ ), which is statistically identical to the potentiation observed in experiments that included 4 mM Mg<sup>2+</sup> and 1 mM Ca<sup>2+</sup> in the internal solution (e.g., Fig. 5). Thus, K<sup>+</sup>-dependent potentiation did not involve relief of Mg<sup>2+</sup> block of the channel.

#### Only one mechanism of potentiation in Kv2.1 K356G, K382V

In contrast to results from wild-type Kv2.1, K<sup>+</sup>-dependent potentiation of currents through double mutant channels appeared to depend exclusively on the inactivation state of the channels (Fig. 6). When cells were held at  $-120$  mV, which completely removed channels from the inactivated state, K<sup>+</sup>-dependent potentiation in the double mutant channel was completely eliminated (Fig. 6 A, bottom; Fig. 6 B, filled circles). When cells were held at  $-80$  mV in 0 external K<sup>+</sup>, steady-state inactivation reduced current mag-

**FIGURE 5** Inactivation-dependent and inactivation-independent potentiation in Kv2.1. (A) Currents recorded in the presence of 0 and 10 mM external  $K^+$ . Currents were evoked by depolarization to 0 mV from holding potentials of  $-70$  mV (top),  $-80$  mV (middle), and  $-120$  mV (bottom). Intracellular solution contained 100 mM  $K^+$  and both 50 and 100 mM  $F^-$ . (B) Current potentiation by 10 mM  $K^+$  as a function of holding potential. Values at  $-150$  mV and  $-120$  mV were statistically identical. Asterisks denote statistical significance ( $p < 0.005$ ) compared with the adjacent bars, numbers in parentheses represent the number of cells tested. (C) Potentiation by 10 mM  $K^+$  as a function of the  $K^+$ -dependent change in percent of channels inactivated. The dashed line at 38% is the level of potentiation observed at  $-120$  mV. Potentiation data (y axis;  $n = 8$  cells) were collected as in A, inactivation differences (x axis;  $n = 5-7$  cells) were calculated from the data in Fig. 4 B. Errors for these difference measurements were calculated as the quadratic sum of the SEM for each value. The straight line has a slope = 1. Numbers in parentheses represent the holding potentials from which measurements were made.



nitude by  $21.0 \pm 6.7\%$  ( $n = 7$ ; Fig. 4 C). Elevation of external  $[K^+]$  to 0.3 mM completely removed channels from the inactivated state (Fig. 4 C), and in independent experiments, potentiated  $K^+$  currents by  $20.8 \pm 1.1\%$  ( $n = 4$ ; Fig. 3). With a holding potential of  $-70$  mV, where more channels were in the inactivated state (see Fig. 4 C), the magnitude of  $K^+$ -dependent potentiation was greater (Fig. 6, A and C). Fig. 6 C illustrates that at three different membrane potentials ( $-120$ ,  $-80$ ,  $-70$  mV), at which steady-state inactivation ranged from 0 to 48% (see Fig. 4 C), the  $K^+$ -dependent potentiation of  $K^+$  currents through the double mutant channel corresponded precisely to the  $K^+$ -dependent reduction in inactivation.

Our working hypothesis is that the inactivation-independent potentiation mechanism in wild-type Kv2.1 involved the  $K^+$ -dependent conformational change in the outer vestibule. An alternative explanation for the lack of  $K^+$ -dependent potentiation in Kv2.1 K356G, K382V was that, in these channels, the  $K^+$  binding site associated with potentiation was completely saturated in the absence of external  $K^+$ . To test this possibility, we determined the influence of external  $K^+$  on current magnitude in channels exposed to lower internal  $[K^+]$ . Reduction of internal  $[K^+]$  to 10 mM decreases  $K^+$  occupancy of the  $K^+$  binding site associated

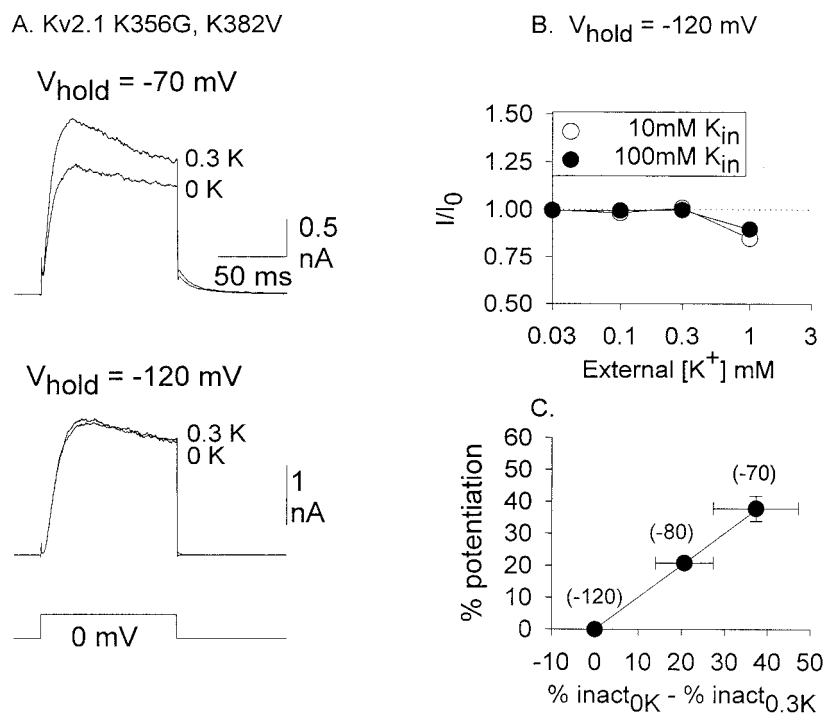
with the outer vestibule conformational change in both the double mutant and wild-type Kv2.1 (Immke and Korn, 2000). Despite this reduction in  $K^+$  occupancy, inactivation-independent potentiation did not occur in the double mutant channel (Fig. 6 B, open circles). In wild-type Kv2.1 and two other Kv2.1 mutant channels that displayed  $K^+$ -dependent potentiation in the absence of inactivation, reduction of initial  $K^+$  occupancy of the pore significantly enhanced the magnitude of potentiation (see Fig. 7 D). These results indicate that saturation of the pore by internal  $K^+$  was not the reason for the absence of potentiation in Kv2.1 K356G, K382V.

In summary, these data strongly suggest that the  $K^+$ -dependent potentiation observed in the double mutant channel was completely accounted for by the shift in steady-state inactivation, and that the K356G, K382V mutation completely abolished the Kv2.1 potentiation mechanism that was not related to voltage-dependent inactivation.

### Effects of single lysine mutations on the potentiation mechanism

Of the two outer vestibule lysines, K356 was almost entirely responsible for loss of TEA sensitivity at low  $[K^+]$ ; K382

**FIGURE 6** Lack of inactivation-independent potentiation in Kv2.1 K356G, K382V. (A) Currents recorded in the presence of 0 and 0.3 mM external K<sup>+</sup>, at holding potentials of -70 mV (top) and -120 mV (bottom). Intracellular solution contained 100 mM K<sup>+</sup>. (B) Normalized current magnitude as a function of external [K<sup>+</sup>] in cells held at -120 mV. Each data point represents the mean  $\pm$  SEM for 4–6 cells. Identical experiments were done with 100 mM internal K<sup>+</sup> (filled circles) and 10 mM internal K<sup>+</sup> (open circles), and 50 and 100 mM internal F<sup>-</sup> (both data sets). (C) Potentiation by 0.3 mM K<sup>+</sup> as a function of the K<sup>+</sup>-dependent change in percent of channels inactivated. Data were obtained from experiments and measurements similar to those used for Fig. 5 C. Potentiation values represent the mean  $\pm$  SEM from 6 cells. Inactivation differences were calculated from the data in Fig. 4 C, and errors calculated as the quadratic sum of the SEM from the two percent inactivation values. Numbers in parentheses represent the holding potentials from which measurements were made.

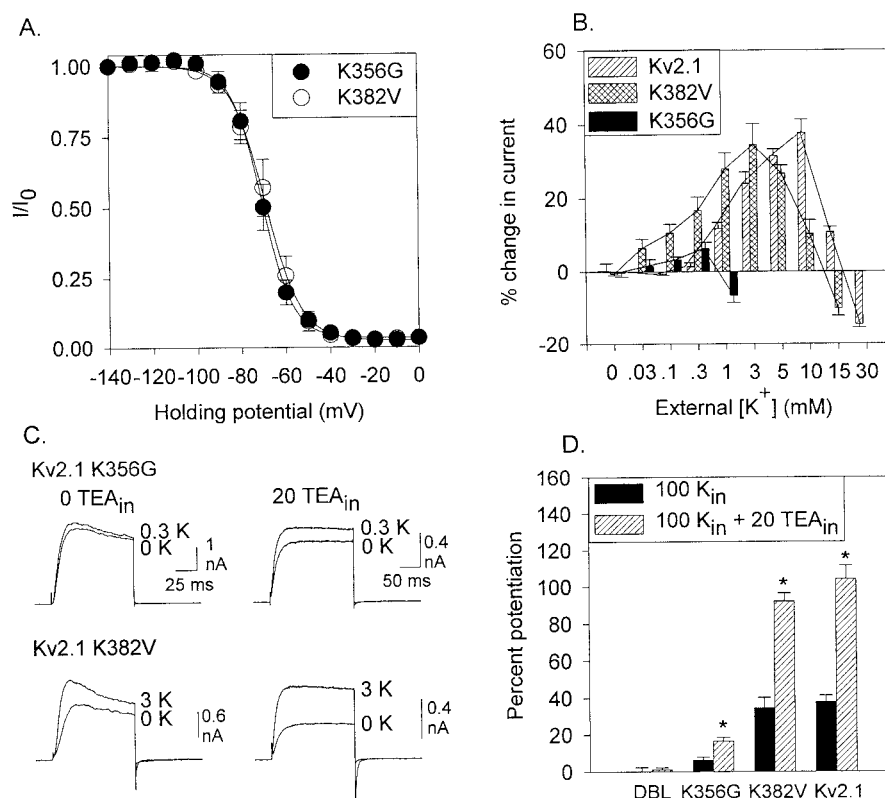


made a minor but measurable contribution (Immke et al., 1999). We next examined which of these two lysines was involved in the K<sup>+</sup>-dependent potentiation mechanism that did not involve inactivation. Two mutant channels were used, Kv2.1 K356G, which retained the lysine at position 382, and Kv2.1 K382V, which retained the lysine at position 356. In the presence of 0 mM external K<sup>+</sup>,  $H_{\infty}$  curves for the single mutant channels were essentially identical to each other and to those of wild-type Kv2.1 and the double mutant channel (Fig. 7 A). Fig. 7 B compares the [K<sup>+</sup>]-dependent potentiation of currents through the two single lysine mutants and wild-type Kv2.1. In the complete absence of voltage-dependent inactivation (holding potential of -120 mV), the magnitude of K<sup>+</sup>-dependent potentiation of Kv2.1 K382V was statistically identical to that of wild-type Kv2.1 (Fig. 7 B). However, the [K<sup>+</sup>]-dependence of potentiation was shifted  $\sim 1/2$  log unit to the left. In contrast, K<sup>+</sup>-dependent potentiation was almost completely abolished by the K356G mutation; in Kv2.1 K356G, currents were maximally potentiated by just  $6.3 \pm 1.7\%$  ( $n = 5$ ; Fig. 7 B, black bars). This value was statistically significant ( $p < 0.02$ ) when compared with the change in current magnitude upon switching between NMG solutions ( $-0.25 \pm 1.1\%$ ,  $n = 4$ ). Nonetheless, the magnitude of this difference left doubt about the validity of this apparent potentiation. For example, Fig. 7 C (top left) illustrates currents that differed in magnitude by 7.6%. Experiments described below suggest that this potentiation, albeit minimal, was real.

### Role of K<sup>+</sup> occupancy in the potentiation mechanism

Our working hypothesis postulates that when a selectivity filter cation binding site is unoccupied, one or both lysines in the outer vestibule change orientation relative to the conduction pathway and reduce the flow of cations through the pore. As initial K<sup>+</sup> occupancy of the pore is reduced, more channels will spend more time in the conformation associated with reduced K<sup>+</sup> current. Consequently, the hypothesis predicts that the magnitude of the potentiation would be increased with lower initial K<sup>+</sup> occupancy.

We lowered occupancy by two different methods. In the K356G mutant, which required just 0.3 mM external K<sup>+</sup> for maximal potentiation, we lowered the intracellular [K<sup>+</sup>] to 10 mM. Under these conditions, external application of 0.3 mM K<sup>+</sup> potentiated currents in the K356G mutant by  $12.9 \pm 0.9\%$  ( $n = 4$ ), which was significantly greater ( $p < 0.02$ ) than the potentiation when currents were carried by 100 mM internal K<sup>+</sup> ( $6.3 \pm 1.7\%$ ,  $n = 5$ ). This method of reducing K<sup>+</sup> occupancy could not be used in either Kv2.1 or the K382V mutant because of the higher external [K<sup>+</sup>] needed to produce potentiation. With just 10 mM internal K<sup>+</sup>, the driving force change produced by application of 3–10 mM K<sup>+</sup> masked potential changes in potentiation. Consequently, we lowered K<sup>+</sup> occupancy of the pore with an internal channel blocker in the presence of 100 mM K<sup>+</sup> (Baukowitz and Yellen, 1995; Khodakhah et al., 1997; Immke et al., 1999; Immke and Korn, 2000). In the Kv2.1 and Kv2.1 mutants studied here, addition of 20 mM TEA in



**FIGURE 7** Involvement of K356 and K382 residues in K<sup>+</sup>-dependent potentiation. (A)  $H_\infty$  curves for the single mutant channels, Kv2.1 K356G (filled circles), and Kv2.1 K382V (open circles).  $V_h = -70.4 \pm 0.3$  mV (K356G) and  $-68.8 \pm 0.4$  mV (K382V), slope = 6.9 (K356G) and 7.9 (K382V),  $n_i = 0.03$  (K356G) and 0.03 (K382V). Curves were generated as in Fig. 4; data points represent mean  $\pm$  SEM of 4–6 cells for each curve. (B) [K<sup>+</sup>]-dependent potentiation in the two single mutants compared with Kv2.1. Holding potential =  $-120$  mV, internal [K<sup>+</sup>] = 100 mM, internal [F<sup>-</sup>] = both 50 and 100 mM. Each bar represents the mean  $\pm$  SEM of 3–7 cells. (C) Currents recorded from Kv2.1 K356G (top) and Kv2.1 K382V (bottom), without (left) and with (right) 20 mM TEA in the pipette solution. All internal solutions contained 100 mM K<sup>+</sup> and 100 mM F<sup>-</sup>. Each pair of traces illustrates one current recorded in 0 external K<sup>+</sup> and one current recorded with 0.3 mM K<sup>+</sup> (K356G) or 3 mM K<sup>+</sup> (K382V). Illustrated currents were potentiated by 7.6% (top left), 20.0% (top right), 43.0% (bottom left), and 104.0% (bottom right). (D) Mean potentiation in cells recorded without (black bars) and with (hatched bars) internal TEA. Potentiation was produced by application of 0.3 mM external K<sup>+</sup> to K356G and DBL, 3 mM K<sup>+</sup> to K382V, and 10 mM K<sup>+</sup> to wild-type Kv2.1. Currents were recorded as in C. Each bar represents the mean  $\pm$  SEM of 4–7 cells. Asterisks denote statistical significance ( $p < 0.01$ ) between treatment groups within each channel.

the intracellular solution inhibits inward K<sup>+</sup> currents carried by 30 mM external K<sup>+</sup> by 85–90%. When channels carry outward current, 20 mM internal TEA inhibits current and significantly lowers K<sup>+</sup> occupancy of the pore, but apparently does not directly affect the outer vestibule conformational change (Immke et al., 1999).

Potentiation in wild-type Kv2.1 and both single lysine mutants was significantly enhanced when occupancy of the pore was reduced with internal TEA (Fig. 7 C right, 7 D). In each of the two single mutants and wild-type Kv2.1, currents were essentially doubled when external [K<sup>+</sup>] was elevated to the concentration that produced peak potentiation in the absence of internal TEA. Currents through the double mutant channel were not potentiated by external K<sup>+</sup> in the presence of internal TEA (Fig. 7 D), which suggests that the enhanced potentiation did not result from an unrelated mechanism produced by internal TEA. These data support two conclusions. First, these data support the hy-

pothesis that potentiation is associated with K<sup>+</sup> occupancy of the pore. Second, since potentiation in the K356G mutant was enhanced under conditions that lowered K<sup>+</sup> occupancy, but potentiation in the double mutant remained nonexistent, these data support the conclusion that K<sup>+</sup>-dependent potentiation occurred in the K356G mutant.

An alternative possibility, that the additional K<sup>+</sup>-dependent potentiation in the presence of internal TEA reflected knock off of internal TEA by added external K<sup>+</sup>, is argued against by two lines of evidence. First, in both Kv2.1 and the double mutant channel, the percent block by internal TEA was nearly identical when currents were carried by 30 mM external K<sup>+</sup> (Immke et al., 1999). This suggests that the mutations did not influence the ability of high external [K<sup>+</sup>] to knock off internal TEA. Second, in each channel that was potentiated (the two single mutants and wild-type Kv2.1), currents recorded in the presence of 20 mM internal TEA were essentially doubled by elevation of external



[K<sup>+</sup>], even though the external [K<sup>+</sup>] ranged from 0.3 to 10 mM on these different channels.

The data in Fig. 7 *D* also indicate another important point. Inclusion of internal TEA produced a nearly identical enhancement of K<sup>+</sup>-dependent potentiation in Kv2.1 and the two single mutants. These results suggest that each lysine made a quantitative contribution to the K<sup>+</sup>-dependent change in current magnitude, and that the relative contribution remained fixed despite changes in initial K<sup>+</sup> occupancy.

### Correlation of K<sup>+</sup>-dependent potentiation and TEA block

The hypothesis regarding the K<sup>+</sup>-dependent change in outer vestibule conformation was derived from experiments that examined the lysine-sensitive change in TEA efficacy. TEA efficacy was reduced at low [K<sup>+</sup>] and increased upon elevation of [K<sup>+</sup>] (Immke and Korn, 2000). Our hypothesis that the lysine-sensitive, K<sup>+</sup>-dependent current potentiation involved the same K<sup>+</sup>-dependent change in outer vestibule conformation required that the potentiation be associated with a change in TEA sensitivity. The hypothesis specifically predicted that as external [K<sup>+</sup>] is elevated, the percent block by TEA must increase if there is an increase in current magnitude. Consistent with the hypothesis, the percent block by 10 mM external TEA was highly correlated with the percent potentiation over a range of external [K<sup>+</sup>] that produced minimum to maximum potentiation (Fig. 8). The correlation coefficient, calculated for the 12 individual data points used to create Fig. 8 *C*, was 0.92.

## DISCUSSION

The primary conclusion from these experiments is that, in Kv2.1, potentiation of outward K<sup>+</sup> current by elevation of external [K<sup>+</sup>] can be completely accounted for by two independent mechanisms. The first mechanism is sensitive to mutation of Lys 356 and, to a small extent, Lys 382, in the outer vestibule. The magnitude of this lysine-sensitive, K<sup>+</sup>-dependent potentiation is independent of steady-state inactivation and insensitive to changes in either holding voltage or activation voltage. In the absence of voltage-dependent inactivation, this mechanism can completely account for the K<sup>+</sup>-dependent potentiation of current in Kv2.1. As discussed below, our results suggest that this mechanism involves a K<sup>+</sup>-dependent conformational change in the outer vestibule that was previously shown to influence TEA efficacy. The second mechanism is associated with K<sup>+</sup>-dependent removal of channels from the inactivated state. At holding potentials that result in steady-state inactivation, the magnitude of K<sup>+</sup>-dependent potentiation is precisely the sum of the current increase that is attributable to the lysine-sensitive mechanism plus the current increase attributable to K<sup>+</sup>-dependent removal of channels from the inactivated state.

### The outer vestibule of Kv2.1 changes conformation as a function of [K<sup>+</sup>]

Two different types of functionally relevant conformational changes have been proposed for the outer vestibule in voltage-gated K<sup>+</sup> channels. One conformational change, which occurs during the slow inactivation process, appears

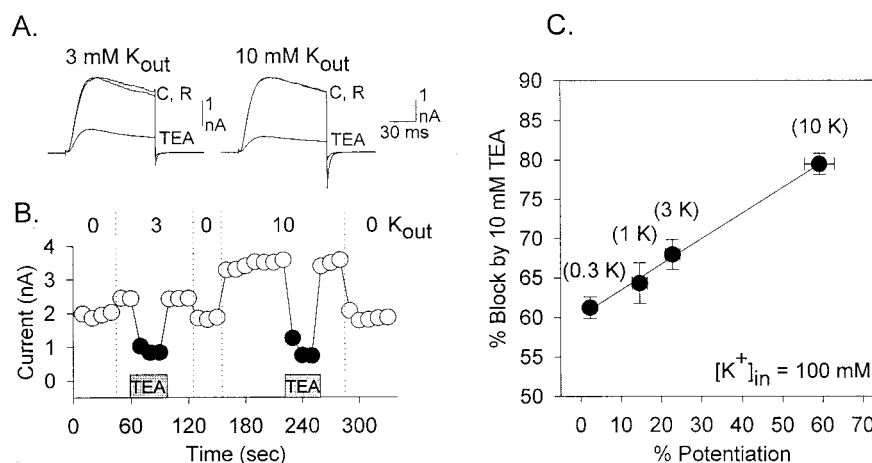


FIGURE 8 Correlation between K<sup>+</sup>-dependent potentiation and TEA block. *A* (top). Currents recorded before (C), during (TEA), and after (R) application of 10 mM external TEA. Internal [K<sup>+</sup>] was 100 mM. Currents were recorded with 3 mM external K<sup>+</sup> (left) and 10 mM external K<sup>+</sup> (right). *B* Plot from a single cell, illustrating the potentiation by two different [K<sup>+</sup>] and block by TEA at two different [K<sup>+</sup>]. *C* Correlation between percent potentiation and percent block by 10 mM TEA. Data points represent mean  $\pm$  SEM for 3 cells at each [K<sup>+</sup>]. Potentiation and TEA block data were collected on each cell, as illustrated in *B*. Numbers in parentheses denote external [K<sup>+</sup>]. The solid line illustrates a first-order linear regression (the correlation coefficient, calculated for the 12 individual data points in the plot, was 0.92).

to involve a local change in exposure of residues near the selectivity filter (Yellen et al., 1994), and perhaps a constriction at or near the level of the selectivity filter (Liu et al., 1996; Kiss et al., 1999). In several  $K^+$  channels, the rate of this conformational change is sensitive to  $K^+$  occupancy of the selectivity filter (Baukrowitz and Yellen, 1995; Kiss and Korn, 1998). The experiments in this paper describe a functional consequence of a different type of conformational change, which is also sensitive to  $K^+$  occupancy of the selectivity filter (Immke et al., 1999). This latter  $K^+$ -dependent conformational change appears to involve a re-orientation of residues both at the outer edge of the external vestibule (the turret) and internal to the selectivity filter. Although both of these postulated conformational changes are associated with properties of the slow inactivation process (Immke et al., 1999), whether there is any relationship between them remains unknown. Consequently, the following discussion pertains *only* to conformational changes associated with the  $K^+$ -dependent reorientation of the turret, which was described by Immke and co-workers (Immke et al., 1999; Immke and Korn, 2000).

Previous experiments suggested that the outer vestibule of the Kv2.1 channel can be in two functionally distinguishable conformations (Immke et al., 1999; Immke and Korn, 2000). These two conformations, which can both conduct  $K^+$ , are functionally described by differences in TEA efficacy. Kv2.1 channels can be in either a TEA-sensitive or a TEA-insensitive conformation, and which conformation they are in appears to depend on the  $K^+$  occupancy of a specific site associated with the selectivity filter (Immke and Korn, 2000). At low  $[K^+]$ , a fraction of channels are TEA-insensitive. As  $[K^+]$  is elevated, the proportion of TEA-sensitive channels increases until, at  $[K^+]$  that saturates the relevant site in the pore, all channels are TEA-sensitive. Mutation of Lys-356 and Lys-382 to the smaller, uncharged glycine and valine residues, respectively, did not prevent the  $K^+$ -dependent change in pore conformation, but did restore full TEA efficacy at all  $[K^+]$  (Immke et al., 1999; Immke and Korn, 2000). These results suggested that, as a consequence of the  $K^+$ -dependent change in outer vestibule conformation, Lys-356, and perhaps Lys-382 to a small extent, reoriented relative to the permeation pathway in such a way as to interfere with TEA block. We could not, however, conclusively determine whether the loss of TEA sensitivity was due to a direct effect of lysines on TEA block, or whether another residue in the outer vestibule was directly responsible and mutation of the lysines altered the  $K^+$ -dependent reorientation of this other residue. Nonetheless, the data demonstrated that the outer vestibule changed conformation as a function of  $[K^+]$ , and that the functional consequence of this conformational change, loss of TEA sensitivity, was abolished by the K356G K382V mutations.

## The lysine-sensitive potentiation mechanism

Several lines of evidence suggest that the lysine-sensitive component of  $K^+$ -dependent potentiation involved the  $K^+$ -dependent change in outer vestibule conformation.

### *The dependence of potentiation on the conformational change*

The involvement of the  $K^+$ -dependent change in outer vestibule conformation in the potentiation mechanism is supported by four observations. First, the external  $[K^+]$ -dependence of potentiation in three channels (K356G, K382V, and wild-type Kv2.1) closely matched the  $[K^+]$ -dependence of the change in TEA sensitivity (Immke et al., 1999; Immke and Korn, 2000). Moreover, the  $K^+$ -dependent changes in TEA sensitivity and current magnitude were highly correlated (Fig. 8). Second, the quantitative contribution of the two lysines to potentiation and TEA sensitivity was essentially identical. Both potentiation and TEA sensitivity were primarily dependent on K356, but both functional measurements were also slightly but significantly influenced by K382 (Fig. 7; Immke et al., 1999). Third, reduction of internal  $K^+$  access to the pore by intracellular TEA enhanced the magnitude of  $K^+$ -dependent potentiation in the two single mutants and wild-type Kv2.1. Similarly, internal TEA produced a marked reduction in external TEA efficacy, which was reversed by elevation of external  $[K^+]$  over the same range as that which potentiated current magnitude (Immke et al., 1999; Immke and Korn, 2000). In the Kv2.1 channel with the double lysine mutation, internal TEA neither reduced external TEA efficacy nor allowed for  $K^+$ -dependent potentiation. Taken together, these results strongly suggest that both current magnitude and external TEA sensitivity were influenced by a common  $K^+$ -dependent mechanism. Fourth, the observation that the specific contributions of K356 and K382 to potentiation remained quantitatively similar, regardless of  $K^+$  occupancy, is consistent with the hypothesis that a single event (e.g., a conformational change in the outer vestibule) was responsible for the influence of both lysines. An alternative possibility, that  $K^+$ -dependent potentiation resulted merely from increasing occupancy of the pore by  $K^+$ , is inconsistent with the observation that currents in the double lysine mutant were not potentiated under conditions in which the channel was initially less than fully occupied by  $K^+$ .

### *The specific involvement of outer vestibule lysines in potentiation*

The dependence on specific amino acids is demonstrated by two observations. First, potentiation was completely abolished by the double lysine mutation, was dramatically but incompletely reduced (>90% reduction) with the K356G mutation alone, and was reduced slightly, but significantly,

by the K382V mutation. The relative contribution of each residue to potentiation remained quantitatively similar when initial K<sup>+</sup> occupancy of the pore was reduced (Fig. 7 *D*). This observation suggests that each of these two lysines had a quantitatively specific influence on current magnitude, and is inconsistent with the possibility that either of the two mutations fundamentally altered channel function. This latter conclusion is further supported by experiments that demonstrated that the mutations did not affect the voltage-dependence of activation (data not shown), the voltage-dependence of inactivation (Figs. 4, 7 *A*), or the kinetics of slow inactivation (Immke et al., 1999). Second, a conformational change in the outer vestibule still occurs even following the K356G, K382V mutations (Immke et al., 1999). Together with the results that potentiation is enhanced almost identically in both single mutants by reduction in initial K<sup>+</sup> occupancy, these data suggest that the mutations did not alter the magnitude of K<sup>+</sup>-dependent potentiation due to prevention of, or qualitative alteration of, the outer vestibule conformational change.

### Reorientation of Lys-382

In previous studies (Immke et al., 1999), the K382V mutation in Kv2.1 slightly enhanced block by external TEA in the absence of K<sup>+</sup>. However, the effect of this mutation on TEA block in the absence of K<sup>+</sup> could not be quantified, and this mutation also slightly enhanced TEA block in the presence of K<sup>+</sup>. Consequently, these previous results were equally consistent with a K<sup>+</sup>-dependent change in the orientation of K382 with respect to the conduction pathway or with a fixed electrostatic effect of K382 on TEA block (Immke et al., 1999). The results of Fig. 7 indicate that K382 made a slight, but significant, contribution to K<sup>+</sup>-dependent potentiation. Furthermore, the relative influence of K382 and K356 appeared to remain constant under conditions of different K<sup>+</sup> occupancy (Fig. 7). These results are consistent with the hypothesis that K382 also undergoes a slight reorientation as a function of K<sup>+</sup> occupancy of the pore.

### The inactivation-dependent potentiation mechanism

In Kv2.1, elevation of external [K<sup>+</sup>] reduced steady-state inactivation (Fig. 4). Steady-state inactivation had essentially identical properties in wild-type Kv2.1 and mutant Kv2.1 channels with either or both of the lysines at positions 356 and 382 replaced. Furthermore, external [K<sup>+</sup>] that produced maximal current potentiation shifted H<sub>∞</sub> curves to the right by identical amounts in channels with and without these lysines. Several conclusions can be drawn from these observations. First, in Kv2.1, and presumably channels that undergo inactivation by a similar slow mechanism, poten-

tiation of K<sup>+</sup> currents by external K<sup>+</sup> results at least partly from K<sup>+</sup>-dependent removal of channels from the inactivated state. Second, the nature of the residues at positions 356 and 382 do not influence the probability that Kv2.1 channels will enter the voltage-dependent inactivated state. Third, reorientation of specifically these positively charged residues within the permeation pathway is not responsible for K<sup>+</sup>-dependent changes in the probability that channels will enter the inactivated state.

### Physiological relevance

In wild-type Kv2.1 carrying K<sup>+</sup> current, external K<sup>+</sup> regulates outer vestibule conformation with an apparent *K<sub>d</sub>* of ~10 mM, even in the presence of 100 mM internal K<sup>+</sup> (Immke and Korn, 2000). Consequently, at physiological [K<sup>+</sup>], this binding site is not saturated. Furthermore, due to the proximity of the apparent *K<sub>d</sub>* to the physiological concentration of external K<sup>+</sup>, small changes in external [K<sup>+</sup>] will produce significant changes in occupancy. Indeed, significant changes in current amplitude occurred at external [K<sup>+</sup>] between 3 and 10 mM (Fig. 3; potentiation of K<sup>+</sup> currents also occurred in the presence of physiological [Na<sup>+</sup>], data not shown). Because Kv2.1 is located, among other places, in both brain and heart (see Deal et al., 1996), these studies suggest that changes in K<sup>+</sup> current magnitude may occur in vivo as a result of small changes in external [K<sup>+</sup>] associated with pathological (and perhaps physiological) conditions. In brain, Kv2.1 may be particularly important for neuron repolarization during high-frequency stimulation (Du et al., 2000), which can produce elevated external [K<sup>+</sup>].

Potentiation was enhanced dramatically in the presence of the internal channel blocker, TEA. Consequently, K<sup>+</sup>-dependent changes in current magnitude may be particularly acute following clinical intervention with intracellular channel blockers (e.g., class III antiarrhythmics and some antidepressants; Valenzuela et al., 1995; Choi et al., 1999), which reduce K<sup>+</sup> occupancy of the pore. With the wide variety of biochemical regulators of Kv2.1 being discovered, it will be of interest to determine whether any of these regulators influence K<sup>+</sup> channel function via modulation of K<sup>+</sup>-sensitive conformational changes in the outer vestibule.

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