Regulation of N- and C-type inactivation of Kv1.4 by pHo and K+: evidence for transmembrane communication


Kv1.4 encodes a slowly recovering transient outward current (Ito), which inactivates by a fast N-type (intracellular ball and chain) mechanism but has slow recovery due to C-type inactivation. C-type inactivation of the NH2-terminal deletion mutant (fKv1.4ΔN) was inhibited by 98 mM extracellular K+ concentration ([K+]o), whereas N-type was unaffected. In 98 mM [K+]o, removal of intracellular K+ concentration ([K+]i) speeded C-type inactivation but had no effect on N-type inactivation, suggesting that C-type inactivation is sensitive to K+ binding to intracellular sites. C-type inactivation is thought to involve closure of the extracellular pore mouth. However, a valine to alanine mutation on the intracellular side of S6 (V561A) of fKv1.4 encodes a slowly recovering transient outward current (Ito), which inactivates by a fast N-type (intracellular ball and chain) mechanism but has slow recovery due to C-type inactivation. C-type inactivation of the NH2-terminal deletion mutant (fKv1.4ΔN) was inhibited by 98 mM extracellular K+ concentration ([K+]o), whereas N-type was unaffected. In 98 mM [K+]o, removal of intracellular K+ concentration ([K+]i) speeded C-type inactivation but had no effect on N-type inactivation, suggesting that C-type inactivation is sensitive to K+ binding to intracellular sites. C-type inactivation is thought to involve closure of the extracellular pore mouth. However, a valine to alanine mutation on the intracellular side of S6 (V561A) of fKv1.4ΔN alters recovery and results in anomalous speeding of C-type inactivation with increasing [K+]o. Extracellular pH (pHo) modulated both N- and C-type inactivation through an S5-H5 linker histidine (H508) with acidosis speeding both N- and C-type inactivation. Mutation of an extracellular lysine to a tyrosine (H508K) slowed C-type inactivation and inhibited the pH dependence of both N- and C-type inactivation. These results suggest that mutations, [K+]o, and pH modulate inactivation through membrane-spanning mechanisms involving S6.

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and extracellular events, respectively, they are both strongly influenced by events occurring at pore sites physically distant from their major site of action. These global conformational changes are responsible for the pH and [K⁺]o dependency of N- and C-type inactivation. They suggest an important physiological role for allosteric coupling between the intracellular and extracellular domains of the Kv1.4 channel.

METHODS

Mature female Xenopus laevis (Xenopus Express) were cared for by standards approved by the Institutional Animal Care and Use Committee of the University at Buffalo State University of New York. Frogs were anesthetized by immersion in tricaine solution (1 g/l; Sigma). Oocytes were digested by placing them in a collagenase-containing, Ca²⁺-free OR2 solution (in mM: 82.5 NaCl, 2 KCl, 1 MgCl₂, and 5 HEPES; pH 7.4, 1 mg/ml collagenase, type I; Sigma). The oocytes were gently shaken for 1.5–2 h, with the enzyme solution refreshed at 1 h. Defolliculated oocytes (stages V-VI) were injected with up to 50 ng mRNA for a Kv1.4 channel clone originally isolated from ferret heart (9) by using the Nanoject (ALA Scientific Instruments). Microelectrodes (0.5–1.5 MΩ) were fabricated from 1.5-mm outer diameter borosilicate glass tubing (TW150–4, WPI) with the use of a two-stage puller and filled with 3 M KCl. The control extracellular solution (ND-96) contained (in mM) 96 NaCl, 2 KCl, 1 MgCl₂, 2.5 CaCl₂, and 10 HEPES; pH adjusted to 7.4. The 98 mM K⁺ solution contained (in mM) 98 KCl, 1 MgCl₂, 2.5 CaCl₂, and 10 HEPES; pH 7.4.

Giant torn-off patches (17) were taken from some oocytes and used as inside-out membrane patches in which intracellular solutions were controlled by a rapid solution switching device (ALA Scientific Instruments). Microelectrodes (0.5 MΩ) were coated with a mixture containing 5% paraffin and light mineral oil. The giant patch electrode solution contained (in mM) 98 NaCl, 2 KCl, 1 MgCl₂, 1.8 CaCl₂, and 10 HEPES; pH adjusted to 7.4. The control extracellular solution (ND-96) contained (in mM) 96 NaCl, 2 KCl, 1 MgCl₂, and 5 HEPES; pH 7.4. For giant-patch experiments, the initial bath solution was (in mM) 98 KCl, 1.8 MgCl₂, 1 EGTA, and 10 HEPES; pH adjusted to 7.4. Giant patch currents sizes were measured at +50 mV were 7.6 ± 7.1 nA in normal intracellular solution and −1.02 ± 0.78 nA in K⁺-free intracellular solution for fKv1.4 (means ± SD, n = 5) and 1.49 ± 1.29 nA in normal intracellular solution and −0.16 ± 0.14 nA in K⁺-free intracellular solution for fKv1.4ΔN (means ± SD, n = 6).

RESULTS

High [K⁺]o reduces the C-type inactivation rate (31), an observation that led to the “Foot-in-the-Door” Hypothesis (2), and subsequently the Permeation Hypothesis of coupling between N- and C-type inactivation (4). A prediction of the Permeation Hypothesis is that in the presence of saturating concentrations of [K⁺]o, C-type inactivation should be insensitive to the intracellular K⁺ concentration ([K⁺]i).

This prediction can be tested directly in torn-off giant patches (17) from Xenopus oocytes expressing ferret Kv1.4ΔN [amino acids 2–146 are deleted (9)] channels, which lack N-type inactivation but show robust C-type inactivation. Contrary to the prediction of the Permeation Hypothesis, in the presence of saturating (98 mM) levels of [K⁺]o removing [K⁺]i and replacing it with 98 mM N-methyl-D-glutamic acid increased the rate of inactivation (Fig. 1). This demonstrates that [K⁺]o is not the only coupling pathway between N- and C-type inactivation. Furthermore, this suggests that intracellular interactions have an effect on the extracellular conformation of the channel, which is consistent with the Allosteric but not the Permeation Hypothesis.

The Allosteric Hypothesis proposes that the channel can adopt different conformations with different likelihoods of entering the C-type inactivated state. It therefore predicts that certain mutations on the intra-
cellular side of the channel will alter the properties of C-type inactivation (37). Mutations near the extracellular mouth of the pore that affect C-type inactivation are well known; however, there have been few reports on intracellular mutations with similar effects on C-type inactivation. One intracellular change that does alter C-type inactivation is a spontaneous mutation in human Kv1.1 channels, which causes episodic ataxia (1). This mutation occurs at the extreme intracellular mouth of the pore on S6 and involves a valine to alanine substitution. We examined this mutation at an equivalent position in our Kv1.4 NH2-terminal deletion mutant: fKv1.4[V561A]N (Fig. 2).

Previous studies in Kv1.1 channels showed that the steady-state properties of C-type inactivation were unaffected by this mutation but inactivation was significantly faster (1). Under control conditions with 2 mM [K+]o, (to mimic amphibian extracellular fluid composition), we observed no difference in the rate of C-type inactivation between fKv1.4ΔN and fKv1.4[V561A]ΔN (Fig. 2, B and C). The apparent lack of a fKv1.4[V561A]ΔN phenotype was unexpected, so we examined this mutant construct more closely. In Kv1.1 the most striking phenotypic change associated with this mutation was a dramatic increase in the rate of recovery from C-type inactivation (37). Mutations near the extracellular side of the channel will alter the properties of C-type inactivation (37). One intracellular change that does alter C-type inactivation is a spontaneous mutation in human Kv1.1 channels, which causes episodic ataxia (1). This mutation occurs at the extreme intracellular mouth of the pore on S6 and involves a valine to alanine substitution. We examined this mutation at an equivalent position in our Kv1.4 NH2-terminal deletion mutant: fKv1.4[V561A]N (Fig. 2).

Recovery from C-type inactivation, just like development of C-type inactivation, is strongly sensitive to [K+]o (31, 37, 38). We examined the effect of [K+]o on recovery of fKv1.4ΔN and fKv1.4[V561A]ΔN. The valine-to-alanine mutation does not shift the voltage dependence of inactivation. This is in contrast to observations in the related, but not identical, Shaker channel, in which the analogous valine to alanine mutation causes a shift in voltage dependence of steady-state activation and inactivation (5).

Recovery from C-type inactivation is coupled to activation (19, 37, 38), the difference could be produced by a shift in the voltage dependence of recovery. Once the channel is sufficiently activated, inactivation proceeds with little or no voltage dependence at positive potentials. For many channels, recovery from inactivation is voltage dependent (18). Recovery is thought to be energetically linked to backward movement of the voltage sensor so that the inactivation recovery rate can be altered if the voltage dependence of inactivation is shifted. Figure 3B shows steady-state inactivation as a function of holding potential for both fKv1.4ΔN and fKv1.4[V561A]ΔN. The valine-to-alanine mutation does not shift the voltage dependence of inactivation. This is in contrast to observations in the related, but not identical, Shaker channel, in which the analogous valine to alanine mutation causes a shift in voltage dependence of steady-state activation and inactivation (5).

Recovery from C-type inactivation is strongly sensitive to [K+]o (31, 37, 38). We examined the effect of [K+]o on recovery of fKv1.4ΔN and fKv1.4[V561A]ΔN. Switching from 2 to 98 mM [K+]o greatly increased the recovery of fKv1.4ΔN but had a much smaller effect on fKv1.4[V561A]ΔN recovery (Fig. 4, A and B).

Fig. 2. A: representation of mutant fKv1.4 constructs employed in this study. Schematic representation of the topology of a single fKv1.4 a-subunit is shown. In many of the experiments in this study the NH2-terminal domain (amino acids 2–146) was deleted to allow us to study the slower C-type inactivation mechanism. The amino acid sequences of the H5 and S6 region are shown. Constructs used in this study involve mutation of the histidine at position 508, the lysine at 532, and the valine at 561. See Fig. 7 for a three-dimensional representation of the channel and mutations. B: inactivation of fKv1.4ΔN expressed in Xenopus oocytes recorded by using a two-electrode voltage clamp. Oocytes were held at −90 mV and then were clamped first for 5 s to a potential (P1) between −90 to +50 mV in 10-mV steps then stepped to a potential (P2) of +40 mV for 1 s. C: currents recorded from fKv1.4[V561A]ΔN. Under these experimental conditions (2 mM [K+]o) no significant differences were seen in the time course of C-type inactivation.
This alteration in recovery rate [K\(^+\)]\(_o\) sensitivity suggests that the intracellular V561A mutation may have changed K\(^+\) sensitivity at the extracellular site (31, 48). This prompted a reexamination of C-type inactivation at positive potentials in both fKv1.4ΔN and fKv1.4[V561A]ΔN. C-type inactivation of fKv1.4ΔN was slowed by switching from 2 to 98 mM [K\(^+\)]\(_o\), consistent with previous observations (Fig. 4C). However, switching from 2 to 98 mM [K\(^+\)]\(_o\) resulted in an increase in C-type inactivation of fKv1.4[V561A]ΔN (Fig. 4D), which is opposite to the effect observed in Kv1.4 and other K\(^+\) channels (31, 37, 38). The change in [K\(^+\)]\(_o\) affinity suggests a link between the intracellular portion of S6 and K\(^+\) binding at the opposite side of the channel and is not predicted by the Permeation Model.

The manipulations presented in Fig. 4 are relatively extreme changes in [K\(^+\)]\(_o\) and represent changes measured at only one voltage. To assess the range of [K\(^+\)]\(_o\) over which the effect occurs and whether these changes might be significant within the physiological and pathophysiological range of [K\(^+\)]\(_o\), we repeated these experiments on the fKv1.4ΔN and fKv1.4[V561A]ΔN channels at 0.5, 2, 10, 25, and 98 mM [K\(^+\)]\(_o\). The reversal in relationship between [K\(^+\)]\(_o\) and C-type inactivation is seen over this entire range (Fig. 5), with inactivation of fKv1.4[V561A]ΔN becoming faster with increasing [K\(^+\)]\(_o\). Both the fKv1.4ΔN and fKv1.4[V561A]ΔN channels retained the voltage-insensitivity characteristic of C-type inactivation rates at positive potentials (37) (Fig. 5B). Although [K\(^+\)]\(_o\) has completely opposite effects on the two channels, there is a strong relationship between [K\(^+\)]\(_o\) and inactivation time constant for the two constructs. Furthermore, the [K\(^+\)]\(_o\) dependence of inactivation rate “crosses over” at 2.0 mM. This suggests that C-type inactivation was directly modified by the mutation. For example, at 0.5 mM fKv1.4[V561A]ΔN inactivation was slower than in fKv1.4ΔN. This is important because it is possible to explain an increase in inactivation rate by mutagenesis inducing an additional inactivation “gate,” but a slowing of inactivation requires the point mutation at V561A to have slowed the intrinsic C-type inactivation.

Other extracellular factors affect both the N- and C-type inactivation characteristics of fKv1.4 channels. Changes in pH\(_o\) alter the N-type inactivation in fKv1.4 channels (Fig. 6). Extracellular acidosis increases the rate of N-type inactivation, whereas alkalosis slows the development of N-type inactivation. This pH\(_o\) dependence suggests that there is some form of transmembrane transduction, for changes in pH\(_o\) alter the characteristics of NH\(_2\)-terminal binding at the intracellular face of the channel. A parallel shift in the rate of C-type inactivation is observed in fKv1.4ΔN. This pH\(_o\) dependence of C-type inactivation occurs roughly in proportion to the changes in N-type inactivation, despite occurring on vastly different time scales.

An important aspect of the relationship between N- and C-type inactivation is that they are coupled. The increase in C-type inactivation of channel with pH might reflect a new conformational transition with altered coupling. Therefore, we examined the effect of pH\(_o\) on the rate of recovery from N- and C-type inactivation. As was the case for development of N- and C-type inactivation, the rates of recovery from inactivation for both fKv1.4 and fKv1.4ΔN were nearly identical and showed parallel shifts with changes in pH\(_o\) (Fig. 6). This suggests that pH\(_o\)-modified C-type inactivation retains the characteristic coupling between N- and C-type inactivation (for a review see Ref. 38) in which N-type inactivation promotes rapid development of C-type inactivation and recovery from both is governed by C-type inactivation.

The pH\(_o\) dependence of C-type inactivation is modulated by an extracellular histidine at position 508 (8). Mutation of this histidine to a glutamine removes the pH\(_o\) dependence of N-type inactivation of fKv1.4[H508Q]...
and C-type inactivation [normalized to pH 7.4, \( \tau_{\text{inactivation}} \) at pH 6.8 is 1.07 ± 0.04 and pH 8.0 is 0.99 ± 0.02 (\( n = 8, P < 0.01 \))]

and C-type inactivation of fKv1.4[H508Q]ΔN [normalized to pH 7.4, \( \tau_{\text{inactivation}} \) at pH 6.8 is 1.05 ± 0.07 and pH 8.0 is 1.03 ± 0.06 (\( n = 4, P < 0.01 \)), where \( \tau_{\text{inactivation}} \) is the time constant of inactivation]. The C-type nature of this coupling was confirmed using a lysine to tyrosine mutant, fKv1.4[K532Y]ΔN, which inhibits C-type inactivation (8) and abolishes the pH \( \text{o} \) dependence of both N- and C-type inactivation [normalized to pH 7.4, \( \tau_{\text{inactivation}} \) at pH 6.8 is 0.94 ± 0.02 and pH 8.0 is 0.91 ± 0.03 (\( n = 5, P < 0.01 \))]. These results provide further evidence for transmembrane coupling of a mechanism that fulfills the criteria for C-type inactivation.

Finally, we examined the combined effects of pH \( \text{o} \) and [K\(^+\)]\(_{\text{o}}\) on C-type inactivation of fKv1.4[V561A]ΔN. Changing pH \( \text{o} \) from 7.4 to 6.8 in 2 mM [K\(^+\)]\(_{\text{o}}\) results in an increase in the rate of inactivation of fKv1.4[V561A]ΔN [from 2.98 ± 0.08 to 2.51 ± 0.02 s (\( n = 3, P < 0.05 \))], which is similar to the effect pH \( \text{o} \) has on fKv1.4 and fKv1.4N channels (see Fig. 6). The rate of fKv1.4[V561A]ΔN inactivation at pH 6.8 can be further increased by switching [K\(^+\)]\(_{\text{o}}\) from 2 to 98 mM [from 2.51 ± 0.02 s to 1.87 ± 0.08 s (\( n = 4, P < 0.01 \))]. This
demonstrates that the intracellular S6 mutation at V561 results in an alteration in the [K+]o sensitivity independent of the actual kinetic rates of C-type inactivation.

**DISCUSSION**

Kv1.4 channels mediate a slowly recovering outward current with upregulated expression during ischemia. Therefore, understanding the molecular basis of the kinetic behavior of Kv1.4 channels in response to changes in pH and [K+]o may be of considerable physiological importance. Our data demonstrate that a mutation at an intracellular site on S6 can alter qualitative properties normally associated with the extracellular pore mouth in Kv1.4 channels. Conversely, pH modulation of N-type inactivation, which is a primarily intracellular event. Both [K+]o and pHo determine the recovery from inactivation of this channel in an additive manner through their interactions with C-type inactivation.

Protonation of an extracellular histidine (H508) affected N-type inactivation, an intracellular process. It is unlikely that mobility of the NH2-terminus is altered by manipulating the charge of an extracellular amino acid. Therefore, protons binding to H508 must alter either the availability of the NH2-terminal binding site or the rate of inactivation following NH2-terminal binding. Either way, our data establish a link between the extracellular H508 and conformational changes on intracellular domains associated with inactivation. This is consistent with the relationship between activation and inactivation in voltage-gated channels (19, 38). Cocrystallization of KcsA and a bound NH2-terminal domain (50) suggest that the structure of the bound NH2-terminal domain is significantly different from the NH2-terminal peptide in solution (47), which indicates that binding involves reciprocal rearrangements of the α-subunit and the NH2-terminal domain.

The effect of [H+]o on N-type inactivation can be disrupted by mutating the lysine at position 532. Given that the intracellular locus of action of N-type inactivation binding with S6 is well established, it is likely that protonation of H508 communicates with both N- and C-type inactivation through S6.

The molecular basis underlying C-type inactivation is unclear, so it is defined by a mixture of functional attributes. The slow inactivation of Kv1.4 channels fits most of the classical definitions of C-type inactivation. However, a simple point mutation on the intracellular side of S6 changes the phenotype with respect to [K+]o sensitivity. This finding might cause us to conclude that the channel no longer inactivates via a C-type mechanism.

The question of whether fKv1.4[V561A]ΔN exhibits modified C-type inactivation or has introduced or enhanced a second inactivation process is an interesting one. If we consider that fKv1.4[V561A]ΔN introduces a second independent inactivation process, then there will be two inactivation gates with opposite [K+]o dependence. In 2 mM [K+]o, fKv1.4[V561A]ΔN inactivates at the same rate as fKv1.4ΔN. At higher [K+]o, fKv1.4[V561A]ΔN inactivation becomes faster, indicating that the second V561A gate is dominant. However, when [K+]o is below 2 mM [K+]o, the two-gate hypothesis suggests that the inactivation rate would again become faster because the C-type mechanism would be dominant. However, fKv1.4[V561A]ΔN inactivation becomes slower when [K+]o is <2 mM, suggesting that the S6 mutation has modified C-type inactivation rather than introduced a new inactivation mechanism.

This complete reversal of [K+]o dependence is clearly not the result of a simple change in the K+ affinity for an external binding site. No matter how drastically the affinity was altered, there would still be the same qualitative result: an increase in [K+]o would result in a slowing of inactivation. The V561A mutation completely reversed the direction of this relationship, with an increase in [K+]o increasing inactivation. This can-
not be explained in terms of the Permeation Model. The anomalous $[K^-]_o$ dependence of C-type inactivation is strong evidence for allosteric interactions between the intracellular and extracellular faces of the channel.

The intracellular V561 mutation leading to an alteration of phenotype in response to $[K^-]_o$ manipulations is similar to response of mutated Shaker channels to heavy metal binding. A threonine-to-cysteine mutation (T449C) in the extracellular mouth of the Shaker channel reverses the relationship between extracellular zinc and the rate of C-type inactivation (48).

The pH dependence of inactivation of fKv1.4-[V561A]$\Delta$N provides further evidence that the properties of C-type inactivation have been modified. The ability of the K532Y mutation to disrupt pH dependence of Kv1.4 suggests that the pH modulation of inactivation occurs through the C-type inactivation mechanism. fKv1.4[V561A]$\Delta$N shows an increase in the rate of inactivation with acidosis similar to the wild-type channel. Given the similarity in response to acidosis of inactivation of the wild-type and V561A channels, it seems simplest to conclude that fKv1.4[V561A]$\Delta$N alters the properties of C-type inactivation rather than inducing a second inactivation mechanism.

The data in this paper demonstrate transmembrane energetic interactions on gating, which require some degree of change in protein conformation in response to the manipulations. This is particularly apparent in the ability of pH, to modulate both N- and C-type inactivation. It clearly establishes a physiologically relevant role for allosteric interactions associated with C-type inactivation, particularly in determining the role of pH and $[K^-]_o$ sensitivity, as well as recovery characteristics for coupled N- and C-type inactivation.

The relationship between N-type inactivation and C-type inactivation may have broader implications for members of the Kv family. The role of the NH$_2$-terminal domain of the $\alpha$-subunit in mediating N-type inactivation in the fKv1.4 channel (37) is virtually identical to that established by the landmark studies of Hoshi et al. (21) in Shaker K$^+$ channels. The NH$_2$-terminal domain from the Kv$\beta$1.2 subunit also accelerates C-type inactivation in fKv1.4$\Delta$N (33). The NH$_2$ terminus of the Kv$\beta$1.2 subunit has a lower affinity for the binding site and more rapid binding kinetics to the inner vestibule so the NH$_2$-terminal domain from Kv1.2 subunit functions more like a rapid open channel blocker such as quinidine (see later in text) (33, 39). This has been studied in Kv1.5 channels by Uebele et al. (41) for the Kv$\beta$1.3 subunit and Kv1.5 combination. Kv$\beta$1.3 also confers a voltage-dependent, partial inactivation (48) for the Kv1.3 subunit and Kv1.5 combination.

This has been studied in Kv1.5 channels by Uebele et al. (41) for the Kv$\beta$1.3 subunit and Kv1.5 combination. Kv$\beta$1.3 also confers a voltage-dependent, partial inactivation ($\tau = 5.76 \pm 0.14$ ms at $+50$ mV) and an enhanced slow (possibly C-type) inactivation. In this channel combination, mutation of V512A (which is homologous to V561A in Kv1.4) did not affect the rapid component of Kv$\beta$1.3-mediated inactivation, whereas a mutation at the external mouth of the pore (R485Y, equivalent to our K532Y mutation) increased the ex-
tent of fast inactivation while preventing the enhancement of slow inactivation. These studies suggest that NH$_2$-terminal binding is allosterically linked to the external pore for a variety of Kv1 channel complexes and may be a critical determinant of many key properties of channel gating.

The demonstration of physical coupling between intracellular and extracellular domains associated with C-type inactivation supports the idea that the Allosteric Hypothesis is physiologically significant. Unquestionably, permeant ion concentrations also play an important role (31). Our experiment with [K$^+$]/[H$^+$]$_i$ substitution used high [K$^+$]$_o$ to saturate an external site. This helps eliminate the role of this external site in mediating the increase in the inactivation rate observed in these experiments. However, several previous studies examining the effects of changes in permeant ionic species on different sides of the membrane suggested that there may be additional permeant ion binding sites within the channel (12, 13, 16, 45, 46). Removal of [K$^+$]$_i$ may be mediating its effects through changes in the occupancy of such sites. The nature and exact location of such sites remain unknown.

Permeant ions can exert a strong effect on gating transitions such as activation, open probability, channel dwell time, and inactivation (19). In the KcsA channel, intracellular ion binding can change the conformation of the channel at physically distant locations (30, 35). The KcsA channel pore is quite narrow, even in domains outside the selectivity filter (11). Water in such narrow pores is likely to have considerable structure, and the presence of hydrated ions in this small space may affect local protein-water interactions (23). Thus, even if a specific binding site does not exist, this is a potential mechanism by which the concentration or species of flowing ions can alter the stability of different channel conformations.

Figure 7 represents a projection of the fKv1.4 sequence onto the KcsA crystal structure (11) and shows the regions that have been altered or explored in this paper (V561, K532, and H508). Clearly, these sites are physically remote from one another and from the permeation pathway. The insensitivity of the K532 and H508 mutant channels to pH$_o$ indicates there must be some interaction between these two sites, but because of the high energy of solvation, it is unlikely that this interaction is electrostatic. It is also unlikely that there is an electrostatic interaction between the charge on H508 at the extracellular side of the membrane and the activity of the positively charged NH$_2$-terminal domain binding to the intracellular side of the channel: given the high dielectric constant of water, it is not energetically feasible. Furthermore, acidosis speeds N-type inactivation, whereas positive charge on H508 would repel the NH$_2$ terminal, thereby slowing inacti-
vation. In addition, electrostatic interactions do not seem likely with a distant and uncharged valine or alanine at position 561. The mutation of a valine to an alanine does not add any additional charge that would contribute an electrostatic component to binding of K\(^+\) at extracellular sites. The most likely mechanism is that transmembrane effects are transduced through S6 and possibly other domains, in a process somewhat analogous to activation of the KcsA channel by intracellular protons (10).

In summary, our results demonstrate a strong coupling of intracellular domains and extracellular domains associated with S6 and the flanking regions surrounding the extracellular mouth of the pore. The most striking change seen in the V561A mutation was that the [K\(^+]_o\) sensitivity of C-type inactivation was reversed, with inactivation becoming faster with increasing [K\(^+]_o\]. This qualitative behavior is similar to that observed for the Kv4 family of channels (25) and suggests that the mechanism(s) of inactivation between these two channels may be closely related, despite the disparities in biophysical properties. C-type inactivation-like mechanisms are also present in more distantly related channels, such as human ether-à-go-go-related gene (42, 43). In all cases, involvement of mutations on S6 have been noted to have a strong modulatory effect on function, despite being relatively remote from the mouth of the putative extracellular pore domain. Furthermore, it seems that C-type inactivation may have both an extracellular and intracellular “gate” similar to that proposed for activation of the KcsA channel. Understanding the coupling between these regions will enhance our understanding of the time and use dependence of many antiarrhythmic drugs.

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