Amino-terminal Determinants of U-type Inactivation of Voltage-gated K\(^+\) Channels*

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The T1 domain is a cytosolic NH\(_2\)-terminal domain present in all Kv (voltage-dependent potassium) channels, and is highly conserved between Kv channel subfamilies. Our characterization of a truncated form of Kv1.5 (Kv1.5/N190) expressed in myocardium demonstrated that deletion of the NH\(_2\) terminus of Kv1.5 imparts a U-shaped inactivation-voltage relationship to the channel, and prompted us to investigate the NH\(_2\) terminus as a regulatory site for slow inactivation of Kv channels. We examined the macroscopic inactivation properties of several NH\(_2\)-terminal deletion mutants of Kv1.5 expressed in HEK 293 cells, demonstrating that deletion of residues up to the T1 boundary (Kv1.5/N19, Kv1.5/N91, and Kv1.5/N191) did not alter Kv1.5 inactivation, however, deletion mutants that disrupted the T1 structure consistently exhibited inactivation phenotypes resembling Kv1.5/N209. Chimeric constructs between Kv1.5 and the NH\(_2\) termini of Kv1.1 and Kv1.3 preserved the inactivation kinetics observed in full-length Kv1.5, again suggesting that the Kv1.5 T1 domain influences slow inactivation. Furthermore, disruption of intersubunit T1 contacts by mutation of residues Glu\(^{131}\) and Thr\(^{132}\) to alanines resulted in channels exhibiting a U-shaped inactivation-voltage relationship. Fusion of the NH\(_2\) terminus of Kv2.1 to the transmembrane segments of Kv1.5 imparted a U-shaped inactivation-voltage relationship to Kv1.5, whereas fusion of the NH\(_2\) terminus of Kv1.5 to the transmembrane core of Kv2.1 decelerated Kv2.1 inactivation and abolished the U-shaped voltage dependence of inactivation normally observed in Kv2.1. These data suggest that intersubunit T1 domain interactions influence U-type inactivation in Kv1 channels, and suggest a generalized influence of the T1 domain on U-type inactivation between Kv channel subfamilies.

The inactivation mechanisms exhibited by different voltage-gated potassium (Kv)\(^1\) channels provide important physiological means by which the duration of action potentials in many excitable tissues is regulated at different frequencies and potentials. Inactivation of Kv channels has historically been divided into two categories, fast (N-type) inactivation which involves occlusion of the inner pore by an NH\(_2\)-terminal ball, and slow (C-type) inactivation which involves a concerted constriction of the outer mouth of the channel pore (1–3). However, recent studies have distinguished a second slow inactivation phenotype termed U-type inactivation, which has been characterized in several voltage-gated K\(^+\) channels, including Kv2.1 (4) and most recently in Shaker and Kv3.1 (5). U-type inactivation has been named for its characteristic U-shaped inactivation-voltage relationship, showing maximal inactivation at intermediate potentials where only a fraction of channels are open, and less pronounced inactivation at more positive potentials where channel opening has saturated (5). This U-shaped voltage-dependence of inactivation is caused by preferential inactivation from channel closed states, although the conformational changes underlying U-type inactivation remain unclear. Interestingly, whereas C-type inactivation is slowed by elevation of extracellular K\(^+\), this condition generally accelerates U-type inactivation, suggesting a distinct mechanism for channel inactivation (4, 5). In addition, our laboratory has recently demonstrated that a naturally occurring NH\(_2\)-terminal truncated form of the cardiac potassium channel Kv1.5 (Kv1.5/N209) exhibits a U-type inactivation phenotype (6–8). This finding clearly suggests that Kv1.5 possesses machinery to undergo both C-type and U-type inactivation, and directed our attention toward an investigation of the NH\(_2\) terminus as a potential regulatory site of U-type inactivation in Kv1.5 and other channels.

A number of recent studies have investigated a modular architecture of potassium channel gating machinery, considering the membrane-bound segments of potassium channels as interchangeable pore modules and voltage-sensing modules (9, 10). However, structural and biochemical evidence for modularity within Kv channels is strongest with respect to the NH\(_2\)-terminal T1 domain, a roughly 120-amino acid cytosolic region that is highly conserved within Kv channel subfamilies. Crystal structures determined for T1 domains of both Shaker and Shao family channels demonstrate that the T1 domains are arranged as a rotationally symmetrical tetramer which is thought to lie in alignment with the channel pore (11). Despite significant primary sequence differences between different Kv channel subfamilies, the structural scaffold of the T1 domain is common to all Kv channels (12–14). Tetramerization of the T1 domains is an early step in channel biosynthesis, and crystallographic, biochemical, and electrophysiological evidence suggest that the T1 tetramer exists as a module distinct from the transmembrane channel core, adopting a “hanging gondola” structure away from the inner pore of intact Kv channels (13, 15–18). The T1 domain influences numerous fundamental
channel functions, including interaction of channels with Kvβ subunits (19–22), interaction with many intracellular signaling molecules (23–25), and prevention of heteromultimerization between different Kv channel subfamilies (14, 18, 26–28). Although the interactions between the T1 domains and gating elements of intact channels have not been identified, a number of recent studies have characterized the influence of the T1 domain on channel gating. Specifically, it has been demonstrated that deletion mutations and point mutations in the T1 domain can substantially alter the voltage dependence and kinetics of activation in Shaker-related channels, suggesting conformational coupling of the T1 domain and the transmembrane segments of the channel (29–31). The influence of the T1 domain on channel inactivation has yet to be established.

To investigate the molecular basis for the modulation of slow inactivation by the Kv1.5 NH2 terminus, we have characterized the gating properties of a series of NH2-terminal deletions of human Kv1.5, and chimeric constructs that we substituted the NH2 terminus of Kv1.5 with the NH2 terminus of other Kv1 channels. Our study demonstrates that the NH2-terminal region responsible for modulation of slow inactivation in Kv1.5 lies within the T1 domain. Furthermore, we demonstrate that fusion of the NH2 terminus of Kv2.1 to the transmembrane segments of Kv1.5 imparts a U-shaped inactivation-voltage relationship to Kv1.5, whereas the NH2 terminus of Kv1.5 attenuates the U-type inactivation properties of Kv2.1.

**Experimental Procedures**

**Cell Preparation and Transfection—**Unless otherwise stated, experiments were carried out on transiently transfected HEK 293 cells grown in minimal essential medium with 10% fetal bovine serum, at 37°C in an air, 5% CO2 incubator. In a few experiments, mouse ltk– cells were used when expression of a construct in HEK 293 cells proved difficult. One day before transfection, cells were plated on sterile glass coverslips in 35-mm Petri dishes with 20–30% confluence. On the day of transfection, cells were washed once with minimal essential medium with 10% fetal bovine serum. To identify the transfected cells efficiently, channel DNA was co-transfected with the vector pHook-1 (Invitrogen). This plasmid encodes the production of an antibody to the hapten phOx, which was expressed and displayed on the cell surface. Channel DNA was incubated with pHook-1 (1 μg of pHook, 1–3 μg of channel DNA) and 4 μl of LipofectAMINE 2000 (Invitrogen) in 100 μl of serum-free DMEM in the dishes containing HEK 293 cells grown in minimal essential medium with 10% fetal bovine serum. Cells were allowed to grow overnight before recording. One hour prior to experiments, cells were treated with beads coated with phOx. After 15 min, excess beads were washed off with cell culture medium, and cells that had beads stuck to them were used for electrophysiological recordings. For some experiments, stable HEK 293 cell lines expressing full-length (FL) Kv1.5, Kv1.5ΔN209, or Kv2.1 were employed. These HEK 293 cells were stably transfected with FL Kv1.5, Kv1.5ΔN209, or Kv2.1 cDNAs in pcDNA3 using LipofectACE reagent (Invitrogen).

**Solutions—**Patch pipettes contained (in mM): NaCl, 5; KCl, 135; Na3ATP, 4; GTP, 0.1; MgCl2, 1; EGTA, 5; HEPES, 10; and was adjusted to pH 7.2 with KOH. The bath solution contained (in mM): NaCl, 135; KCl, 5; HEPES, 10; sodium acetate, 2.8; MgCl2, 1; CaCl2, 1; and was adjusted to pH 7.4 with NaOH. All chemicals were from Sigma.

**Electrophysiological Procedures—**Data were sampled at 10 kHz and filtered at 5–10 kHz. Membrane potentials have not been corrected for small junctional potentials between bath and pipette solutions. Throughout the text the data are presented as mean ± S.E.

**Molecular Biology and Channel Mutations—**The mammalian expression vector pcDNA3 was used for expression of all channel constructs used in this study. All primers used were synthesized by Sigma-Genosys (Oakville, Ontario, Canada). All constructs were sequenced to check for sequence errors, and to ensure the correct reading frame. The Kv1.5ΔN19, Kv1.5ΔN91, and Kv1.5ΔN162 mutants were generated by Bal31 exonuclease digestion from the 5′ end of hKv1.5. Resulting fragments were ultimately subcloned into a pcDNA3 vector cut with Neol (which was blunted to introduce a start codon) and Xbol restriction sites. The Kv1.5ΔN141 and Kv1.5ΔN140 mutants were generated by PCR amplification of the cDNA encoding residues 120 or 141 to the COOH terminus of hKv1.5. The 5′ primers used were 5′-CCGAAGCT-TATGCAGGGCGCTCCATCACATC-3′ for Kv1.5ΔN119, and 5′-CCCAACCTTATGGCCACCCCTGCGAGTTTC-3′ for Kv1.5ΔN140 (introduced restriction sites are underlined). The resulting channels were ultimately subcloned in a pcDNA3 vector using HindIII and NotI restriction sites. The Kv1.5ΔN185 mutant was generated by removal of the Neol-HindIII fragment of Kv1.5. Kv1.5ΔN209 was generated by removal of the Neol-Neol fragment of Kv1.5. Kv2.1ΔN101 was generated by removal of sequence up to the Nav1 restriction site in rKv2.1.

For preparation of the Kv1.1N/Kv1.5 and Kv1.3N/Kv1.5 chimeric channels, DNA encoding the Kv1.5 channel domain core beginning at the Neol site encoding residue Met186 was subcloned into homologous Neol sites and Xbol sites, encoding residues 141 to the NH2 terminus of Kv1.1 and Kv1.3 using Neol and Xbol restriction sites. The resulting fusion protein was then subcloned into pcDNA3 for mammalian expression as an EcoRI/EcoRI fragment, followed by screening for correct orientation of the insert.

For preparation of Kv1.5N/Kv2.1 T19+163/Kv2.1, DNA encoding amino acids 180 to the COOH terminus of hKv1.5 were amplified by PCR, such that a Bspe restriction site at Pro180 of hKv1.5, and a Xbol site at residue 163 were introduced. The following primers were used: 5′-ATGTCGAGATGCTGGTGCCTGCGCCAGGAC-3′ for the 5′ end, and 5′-GCTCTAGACCTCTTCGTTAGGACCACAGGAC-3′ for the 3′ end of the amplicon (restriction sites are underlined). The resulting fragment was used to replace the analogous region of Kv1.5 encoding amino acids 243 to the COOH terminus of Kv1.5, in a pcDNA3 vector encoding Kv1.5 or Kv1.5T19+163 using Bspe and Xbol restriction sites.

For preparation of Kv2.1N/Kv1.5, the NH2 terminus of rKv2.1 was amplified by PCR, introducing a EcoRI restriction site preceding the start codon, and a Bspe restriction site at 180 of rKv2.1. The primers employed were 5′-GGGATTTCGCCATGCAAGCATGGC-3′ as the 5′ primer and 5′-GCGTTCGCCGATACTCCAGCAGATCCCAGAG-3′ as the 3′ primer (introduced restriction sites are underlined). The resulting fragment was used to replace the analogous Kv1.5 sequence encoding residues 11 to 243 of the Kv1.5 NH2 terminus up to residue 243, by subcloning into pcDNA3 vector encoding Kv1.5 using HindIII and Bspe restriction sites.

Site-directed mutagenesis of Kv1.5 was performed using the QuikChange method from Stratagene. For preparation of the Kv1.5ΔAQL primers, the primers were: GGCGTCCCAGCTGGCGGCAGCTGGGACCCGT and its complement.

**RESULTS**

**C- and U-type Inactivation in Kv1.5—**In intact channels, the T1 domain is thought to be structurally dissociated from the transmembrane segments of the channel, forming a hanging gondola (shown schematically in Fig. IA). Interestingly, however, disruptions of the NH2 termini of Kv channels can significantly affect the gating properties of the channel. A good example of this phenomenon is Kv1.5ΔN209, the naturally occurring short form of Kv1.5 comprising a deletion of greater than 80% of the cytosolic NH2 terminus of Kv1.5, which exhibits its activation and inactivation properties substantially different from the long-form of Kv1.5 (FL Kv1.5) (8). Throughout this study, we examined the voltage-dependent activation of Kv channels using the double pulse protocol described in Fig. 1B. Cells were stepped from a holding potential of −80 mV to potentials between −65 mV and +50 mV in 5-mV steps for 200 ms (P1, Fig. 1B), followed by a brief repolarization to −40 mV (P2, Fig. 1B). The magnitude of the tail currents observed at −40 mV was proportional to the number of channels activated during P1 (Fig. 1B). Inactivation-voltage relationships were
derived using the triple-pulse protocol described in the legend to Fig. 1C. From a holding potential of −80 mV, cells were given a 100-ms control pulse to 60 mV (P1, Fig. 1C), followed by a brief repolarizations to −40 mV (P2). Tail current amplitudes were measured isochronally (marked with an asterisk), normalized, and fit with single Boltzmann equations. C, to study inactivation, cells were given a 100-ms control pulse to 60 mV (P1), rested for 2 s at −80 mV, stepped from voltages between −70 and +60 mV in 10-mV steps for 5 s (P2), followed by a test pulse to +60 mV (P3). The interpulse interval was 35 s. Peak currents during P3 were normalized to peak currents during P1, and fit with single Boltzmann equations. D, V_{1/2} values for activation in Kv1.5, Kv1.5ΔN209, and Kv2.1 were −10.8 ± 0.8, −20.3 ± 1.7, and −2.8 ± 1.7 mV, respectively. E, V_{1/2} values for inactivation in Kv1.5, Kv1.5ΔN209, and Kv2.1 were −21.0 ± 1.2, −32.8 ± 0.9, and −26.3 ± 0.7 mV, respectively.

The activation and inactivation properties of Kv1.5ΔN209 differ substantially from those observed in FL Kv1.5. When stably expressed in HEK 293 cells, Kv1.5ΔN209 exhibited a half-activation potential of −10.8 ± 0.8 mV (Fig. 1D), and a half-inactivation potential of −21.0 ± 1.2 mV (Fig. 1E), which was consistent with previous studies from our laboratory and others (32, 34, 35). Kv1.5 exhibits a number of other features distinguished from typical C-type inactivation by a marked upturn of its inactivation-voltage relationship. These observations clearly distinguish inactivation in FL Kv1.5 from the U-type inactivation phenotype of Kv1.5ΔN209 and Kv2.1, and suggest that the NH2 terminus of Kv1.5 may influence the inactivation properties of the channel.

The shape of the inactivation-voltage relationship in Kv1.5 was consistent with a C-type inactivation mechanism, generally viewed as a voltage-independent inactivation process that is coupled to channel opening (33). This observation was also consistent with previous studies of Kv1.5 and other Kv1 channels (32, 34, 35). Kv1.5 exhibits a number of other features consistent with a C-type inactivation mechanism. First, although residue Arg467 (corresponding to Thr449 in Shaker) renders wild-type Kv1.5 insensitive to extracellular TEA, inactivation in the R487T mutant of Kv1.5 was inhibited by TEA (Fig. 2A). The application of extracellular TEA diminished the peak currents observed through Kv1.5 R487T channels, with
10 mM extracellular TEA resulting in a 51 ± 2% (n = 3) block of peak current. Normalized data demonstrating the effect of 10 mM extracellular TEA on the inactivation time course of Kv1.5R487T are shown in Fig. 2A. Currents through the R487T mutant channel inactivate by 38 ± 2% during 5-s depolarizations to 60 mV under control conditions, but inactivate by only 25 ± 3% in the presence of 10 mM extracellular TEA. To confirm a C-type mechanism of inactivation in Kv1.5, and to contrast the inactivation mechanisms in Kv1.5 and Kv1.5ΔN209, we also examined the effects of elevation of extracellular K⁺ on inactivation in both channels. Clearly, elevation of extracellular K⁺ results in deceleration of Kv1.5 inactivation (Fig. 2B), which suggests a C-type mechanism of inactivation in FL Kv1.5, and was consistent with previous studies on Kv1.5 and other Shaker homologues (32, 36). In contrast, Kv1.5ΔN209 exhibits an opposite sensitivity to extracellular K⁺, with more rapid inactivation observed in 135 mM extracellular K⁺. This paradoxical sensitivity of inactivation to extracellular K⁺ appears to be a common feature of channels that exhibit a U-shaped inactivation-voltage relationship (5).

The Kv1.5 T1 Domain Influences U-type Inactivation—To confirm and extend these findings, we attempted to define the NH₂-terminal region involved in altering the inactivation phenotype of Kv1.5. We began by constructing a series of NH₂-terminal truncated forms of Kv1.5 (Fig. 3A). These were transiently expressed in HEK 293 cells, and their activation and inactivation properties were examined. The activation and inactivation curves of FL Kv1.5 and Kv1.5ΔN209 have been included for comparison. We examined three constructs comprising progressive deletions of the Kv1.5 NH₂-terminal residues up to the T1 boundary (Kv1.5ΔN19, Kv1.5ΔN191, and Kv1.5ΔN119, see Fig. 3A). None of these deletion constructs exhibited any remarkable differences in activation and inactivation gating from FL Kv1.5, although the half-inactivation voltage for Kv1.5ΔN191 was -11.0 ± 0.6, -12.0 ± 0.5, and -11.5 ± 1.5 mV, respectively. In contrast, a longer NH₂-terminal deletion into the T1 domain (Kv1.5ΔN209) exhibited half-inactivation voltages of -23.8 ± 0.7 mV, respectively. D and E, inactivation-voltage relationships were derived as described in the legend to Fig. 1. V₁⁄₂ values of inactivation for Kv1.5ΔN19, Kv1.5ΔN191, and Kv1.5ΔN119 were: -11.0 ± 0.6, -12.0 ± 0.5, and -11.5 ± 1.5 mV, respectively. Important, all three constructs exhibited a flat voltage dependence of inactivation at positive potentials, which was consistent with the appearance of the inactivation-voltage relationship characteristic of FL Kv1.5. These data suggest that the first 120 amino acids of Kv1.5 exert little effect on the activation or inactivation properties of the channel.
main (Kv1.5ΔN188, Fig. 3A) resulted in leftward shifts of both activation and inactivation, and a U-shaped inactivation-voltage relationship closely resembling that reported for Kv1.5ΔN209 (Fig. 3, C and E). These observations were consistent with a U-type inactivation phenotype in both Kv1.5ΔN188 and Kv1.5ΔN209, and demonstrate that deletions into the T1 domain were required to produce the U-shaped inactivation-voltage relationship characteristic of Kv1.5ΔN209. Kv1.5ΔN188 exhibited half-activation and half-inactivation potentials of −24.7 ± 1.3 and −29.9 ± 4.0 mV, respectively. To further characterize the influence of the T1 domain, we generated several deletion mutants intermediate to Kv1.5ΔN119 and Kv1.5ΔN188, namely Kv1.5ΔN140 and Kv1.5ΔN162 (Fig. 3A). Interestingly, despite fairly expressible points of shorter and longer deletion constructs, repeated transfections with these intermediate deletion mutants failed to generate any detectable macroscopic currents. Nevertheless, the data collected suggested that the first 70 amino acids of the Kv1.5 T1 domain (between residues 119 and 188) exert a critical influence on U-type inactivation in Kv1.5.

We also examined the activation and inactivation properties of a construct in which we fused the first 19 amino acids of the Kv1.5 T1 domain to Kv1.5ΔN162 (see Kv1.5T19+163 in Fig. 3A). Surprisingly, this manipulation of the T1 structure both restored channel expression and resulted in a U-shaped voltage dependence of inactivation (Fig. 3, B and C). Kv1.5T19+163 exhibited a strongly leftward shifted half-activation potential of −23.8 ± 0.7 mV, and a half-inactivation potential of −32.1 ± 1.9 mV. These data suggested that disruption of the first 42 residues of the Kv1.5 T1 domain was sufficient to reproduce the U-type inactivation phenotype observed in Kv1.5ΔN209. We examined the location of homologous residues in the recently solved structures of the T1 domains of aplysia Kv1.1 (Molecular Modeling Database accession number 13897) (14) and rat Kv1.2 (Molecular Modeling Database accession number 14393) (31), and noticed that this region contains several amino acids that form intersubunit contacts between T1 domains. This observation was intriguing, given a recent demonstration that mutation of residues at the intersubunit T1 interface can strongly influence the activation gating properties of Kv1.2 (31).

**NH2 Terminals of Kv1.1 and Kv1.3 Channels Restore Wild-type Inactivation in Kv1.5**—To confirm the importance of the T1 domain, we replaced the NH2 terminus of Kv1.5 up to residue Met210 with the corresponding NH2-terminal sequence of either Kv1.1 or Kv1.3 (shown schematically in Fig. 4A), and examined activation and inactivation properties after transient expression in HEK 293 cells (Fig. 4). Sequence alignment illustrates that channels within the Kv1 family exhibit roughly 85% identity between their T1 domains, and essentially no homology in the remainder of their cytosolic NH2 termini (Fig. 4B) (37). Thus, restoration of a flat inactivation-voltage relationship in these chimeric constructs would further implicate the T1 domain in the regulation of U-type inactivation. The Kv1.1N/Kv1.5 and Kv1.3N/Kv1.5 chimeras exhibited half-activation potentials of −18.0 ± 1.1 and −13.5 ± 0.9 mV, respec-
Suggested changes to the text:

In the text, there are some inconsistencies in the use of symbols and subscripts. For example, the symbol 'K' is used without a subscript, and there are instances where 'K' is used with a subscript 'v'. The text also contains some unusual spacing and formatting issues, which may affect the readability. Additionally, there are some instances where the text appears to be incomplete or cut off, which may require further context to understand the full meaning.

The text discusses the inactivation of potassium channels, specifically focusing on the T1 domain and its role in the activation and inactivation processes. It mentions the use of chimeric constructs to study the effects of the NH2 terminus of Kv1.5 on the inactivation properties of Kv2.1. The text describes experiments where the NH2 terminus of Kv1.5 was fused with Kv2.1, and the resulting channels exhibited slow inactivation, unlike the fast inactivation of wild-type Kv1.5.

The text also discusses the use of point mutants, such as Kv1.5AAQL, to study the effects of mutations in the T1 domain. These mutants showed altered inactivation properties, indicating the importance of specific amino acids in the T1 domain for inactivation.

Overall, the text provides a comprehensive overview of the mechanisms underlying potassium channel inactivation, with a focus on the T1 domain and its role in the activation and inactivation processes.
of the kinetics of activation and essentially complete ablation of slow inactivation of the channel, evident in Fig. 6D, and consistent with a previous publication (38). This contrasts sharply with currents recorded from the Kv1.5N/Kv2.1 chimera, which exhibit markedly faster kinetics of activation, and substantial inactivation during a 5-s pulse (Fig. 6D). As a further control, we characterized a chimeric channel consisting of the NH2 terminus of Kv1.5T19/H11001163 (Fig. 3A) fused to the transmembrane domains of Kv2.1. This NH2-terminal construct results in a U-shaped voltage dependence of inactivation in Kv1.5 (Fig. 3), but results in activation and inactivation kinetics in Kv2.1 that were effectively indistinguishable from the NH2-terminal deletion of Kv2.1 (Fig. 6D). This suggests that the inactivation phenotype observed in the Kv1.5N/Kv2.1 chimera results from a specific effect of the intact Kv1.5 NH2 terminus.

DISCUSSION

The current hanging gondola model of voltage-gated K+ channel structure suggests that the T1 tetramer was structurally dissociated from membrane-bound segments of the channel (13, 15, 18). Paradoxically, it has been demonstrated that deletion mutations, and even highly conservative point mutations, can exert strong effects on channel activation (30, 31). These observations were particularly interesting in light of several studies demonstrating the expression of truncated isoforms of several Kv channels in vivo, including Kv1.5 and KvLQT1 (6, 7, 39). NH2-terminal truncated channels likely exhibit an altered sensitivity to modulation by signaling mechanisms as the T1 domain has been identified as a region of interaction with Gβγ subunits, tyrosine kinases, and Kvβ subunits (23–25, 35). However, the influence of the T1 domain on channel gating, and particularly inactivation, has not been widely studied.

Our characterization of T1 deletions of Kv1.5 together with recent studies in Shaker (5) suggest that Shaker family K+ channels are able to exhibit phenotypes characteristic of both C-type and U-type inactivation. In addition, the data presented in our study suggests that the cytosolic NH2 terminus of Shaker homologues, such as Kv1.5, strongly influences the extent of U-type inactivation, and hence determines the balance between C- and U-type inactivations. FL Kv1.5 channels exhibit no features consistent with U-type inactivation (32), and deletions up to the T1 border (residue 120) exerted no significant effects on gating (Fig. 3). However, deletions within the T1 domain consistently disrupted both activation and inactivation properties of Kv1.5, resulting in a shift to a U-type inactivation phenotype in the T1-deleted forms of the channel (Fig. 3). Using approaches of progressive channel deletion mutations (Fig. 3), chimERIC constructs of Kv1 channels (Fig. 4), and site-directed point mutations (Fig. 5), we have demonstrated that this function is localized within the Kv1 T1 domain.

The results of our studies in Kv1.5 suggest that the amino acids forming intersubunit T1 contacts may be involved in regulating the U-type inactivation phenotype, although the
U-shaped inactivation-voltage relationship of the Kv1.5AAQL construct is not as marked as that observed in Kv1.5N209 or other T1 deleted forms of Kv1.5 (Fig. 3). This may be because the Kv1.5AAQL mutation only disrupts a fraction of the inter-subunit T1 interactions, as inter-subunit contacts were made between several residues throughout the T1 domain (see Fig. 5A). It should be noted that mutations throughout the T1 domain have been demonstrated to exert varying effects on the activation gating of Kv1 channels. This has been most carefully examined among residues at the inter-subunit T1 interface, but residues lining the central T1 pore, and even residues in the T1-S1 linker region have been shown to affect channel gating (29, 31, 40, 41). In addition, point mutations within T1 have been shown to cause both hyperpolarizing and depolarizing shifts of the activation relationship, depending on both the position and properties of the substituted amino acid. In summary, we can say with certainty that disruption of the inter-subunit T1 contact residues examined in our study alter the slow inactivation properties of Kv1.5. However, without a more complete mutagenic analysis of the T1 domain, we cannot rule out the possibility that more global conformational changes in T1 are involved in the influence of this domain on channel gating.

A generalized role for the NH\textsubscript{2} terminus in regulation of U-type inactivation was also supported by our chimeric studies of Kv1.5 and Kv2.1. These experiments examined the effects of switching the NH\textsubscript{2} termini of C-type inactivating channel (Kv1.5) and a U-type inactivating channel (Kv2.1). Substitution of the Kv1.5 NH\textsubscript{2} terminus with the Kv2.1 NH\textsubscript{2} terminus resulted in channels that exhibited a U-shaped inactivation-voltage relationship, similar to the inactivation phenotype observed in Kv1.5N209 (Fig. 6). However, the inverse substitution resulted in a deceleration of inactivation compared with Kv2.1 (Fig. 6). Our interpretation of these observations was that the Kv2.1 NH\textsubscript{2} terminus adopts a conformation or interacts with channels in a manner that promotes U-type inactivation. In contrast, the Kv1.5 T1 domain may interact with channels in a manner that prevents U-type inactivation, which was consistent with the absence of U-type inactivation in Kv1.5, and the deceleration of inactivation in Kv1.5N209. Interestingly, the influence of the Kv1.5 NH\textsubscript{2} terminus on the inactivation of Kv2.1 was extremely similar to the influence of Kv2.3 (also known as Kv8.1) (42). In particular, coexpression of Kv2.1 and Kv2.3 results in deceleration of both Kv2.1 activation and inactivation, and studies of chimeric constructs of Kv2.1 and Kv2.3 have localized this effect to the NH\textsubscript{2} terminus of Kv2.3, specifically within the COOH-terminal side of T1 and the T1-S1 linker (42). Similar effects are seen upon coexpression of Kv2.1 and Kv6.1, although the regulatory domains in Kv6.1 have not yet been determined (43). In addition, as observed in our Kv1.5N209/Kv2.1 channel, chimeric channels consisting of this NH\textsubscript{2}-terminal segment of Kv2.3 and the transmembrane domains of Kv2.1 exhibit deceleration of Kv2.1 inactivation (42). These findings raise the possibility that more global conformational changes underlying U-type inactivation remain unclear, the cytosolic T1 domain of Kv channels appears to be intimately involved in the regulation of U-type inactivation properties. As a docking site for a number of intracellular regulatory molecules, the T1 domain may provide an important mechanism for transient alteration of both activation and inactivation properties of Kv channels.

Acknowledgments—We thank Dr. Rolf Johe for providing the rKv2.1 cDNA, and Sandy Wang and Linda Sui for preparation of cells.

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