The Roles of N- and C-terminal Determinants in the Activation of the Kv2.1 Potassium Channel*

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The human and rat forms of the Kv2.1 channel have identical amino acids over the membrane-spanning regions and differ only in the N- and C-terminal intracellular regions. Rat Kv2.1 activates much faster than human Kv2.1. Here we have studied the role of the N- and C-terminal residues that determine this difference in activation kinetics between the two channels. For this, we constructed mutants and chimeras between the two channels, expressed them in oocytes, and recorded currents by two-electrode voltage clamping. In the N-terminal region, mutation Q67E in the rat channel displayed a slowing of activation relative to rat wild type, whereas mutation D75E in the human channel showed faster activation than human wild type. In the C-terminal region, we found that some residues within the region of amino acids 740–833 (“CTA” domain) were also involved in determining activation kinetics. The electrophysiological data also suggested interactions between the N and C termini. Such an interaction was confirmed directly by using a glutathione S-transferase (GST) fusion protein with the N terminus of Kv2.1, which we showed to bind to the C terminus of Kv2.1. Taken together, these data suggest that exposed residues in the T1 domain of the N terminus, as well as the CTA domain in the C terminus, are important in determining channel activation kinetics and that these N- and C-terminal regions interact.

Voltage-gated potassium channels play critical roles in the function of excitable cells, such as maintenance of membrane potential, repolarization of the action potential, and regulation of firing frequency, and they are also the sites of pathologies such as inherited disorders (1). Although there are several molecular domains that underlie differences in activation kinetics between rat and human channels, this study shows that specific interactions between N and C termini affect the gating process.

EXPERIMENTAL PROCEDURES

Preparation of Mutants and Chimeras for Electrophysiology—The human Kv2.1 clone in pGEMHE was provided by O. Pongs, Hamburg, Germany, and the rat Kv2.1 clone in pBluescript SK− was provided by A. M. Brown, Cleveland, OH. The mutants Q67E,E75D in rKv2.1 and E67Q,D75E in hKv2.1 were generated by a PCR method (QuikChange, Stratagene) using PfuTurbo polymerase enzyme (Stratagene) together with appropriate primers. 18 cycles were carried out, each cycle consisting of 30 s at 95 °C, 1 min at 55 °C, and 2 min/kb at 68 °C. Products were digested with DpnI (Sigma). The hKv2.1 sequence has an ATG 12 bases before the assumed start methionine in rat and human Kv2.1. This ATG was mutated to ATA, again by the QuikChange method. Chimeric channels between rat Kv2.1 and human Kv2.1 were produced using either appropriate restriction enzyme digestion and subsequent ligation or the PCR overlap extension method. For restriction digestion and ligation, joins were made at the sites cut by the enzymes BssHII (Promega), Bsp1407I, Bsu36I, Bsm1, and NotI (Fermentas) with ligation using T4 DNA ligase (Fermentas). Silent mutations were made (QuikChange) to introduce a Bsu36I site into hKv2.1 and a Bsm1 site into hKv2.1 at corresponding positions to the sites in hKv2.1 and rKv2.1, respectively. For the overlap extension method, first round PCR fragments were generated using appropriate primers and PfuTurbo polymerase (25 cycles, 30 s at 95 °C, 30 s at 55 °C, and 2 min/kb at 68 °C). Products were then digested with Bsp1407I and NotI and ligated into the similarly restricted wild type rKv2.1 in pBlue- script, using T4 ligase. All mutants and chimeras were confirmed by DNA sequencing.

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The abbreviation used are: GST, glutathione S-transferase; CTA, C-terminal activation (domain).
For GST fusion protein studies, an N-terminal construct of the rat Kv2.1 channel (from the second amino acid to residue 181, just before S1) was subcloned in-frame into the pGEX-4T-3 vector (Amersham Biosciences). For this, a corresponding N-terminal PCR product was obtained using sense and antisense primers with EcoRI and XhoI (plus stop codon) overhangs respectively and subcloned into the vector using these enzymes. For in vitro transcription-translation under the T7 promoter, a C-terminal construct of the rat Kv2.1 channel (from just after S6 at residue 413 to the end) was obtained by deleting amino acids 1–412 from the rat Kv2.1 channel in pBluescript SK+. For this, a corresponding PCR product was obtained using a sense primer with an EcoRI overhang and a downstream primer without overhang. The PCR product was digested with EcoRI and Bsp1407I (the latter restriction site is native to the sequence), ligated back into rKv2.1 in pBluescript, and digested with the same enzymes.

In Vitro Expression of Glutathione S-Transferase Proteins and Binding Studies—The N-terminal GST fusion construct was transformed into BL21(DE3) Escherichia coli cells (Invitrogen). Large scale cultures were grown for 5 h, induced with 0.25 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG), and grown for a further 90 min at 37 °C. Cells were lysed by freeze/thaw, and insoluble material was removed by centrifugation. The 35S-labeled, C-terminal protein was synthesized using the reticulocyte lysate T7 coupled transcription/translation system (Promega) according to the manufacturer’s protocol. In vitro binding assays were carried out by adding 20 μl of glutathione-agarose beads (Amersham Biosciences) (50% slurry) to 1 ml of N-terminal GST fusion lysate. Tubes were incubated at room temperature for 45 min and washed three times with 100 μl of 1× phosphate-buffered saline. 20 μl of the C-terminal 35S-labeled protein product was then added, and the samples were incubated for a further 45 min at room temperature. The samples were again washed three times, the GST fusion protein was eluted from the beads using 10 μl of elution buffer per sample (Amersham Biosciences) and subjected to 12% SDS-PAGE and autoradiography.

Electrophysiology—Xenopus oocytes were prepared and injected with 50 nL of wild type, mutant, or chimeras RNA (5 ng for constructs in pBluescript, 0.5 ng for those in pGEMHE), and two-electrode voltage-clamp recordings were made at room temperature (22 °C) as described previously (6). Oocytes were held in a 50-μl chamber and continually perfused (2 mL/min) with Ringer’s solution (115 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, and 10 mM HEPES, pH 7.2). To obtain current-voltage (IV) curves, the membrane potential was held at −80 mV, and 500-ms duration test depolarizations at 0.1 Hz were applied in 10 mV increments from −70 mV to +70 mV. All currents were leak-subtracted by using twenty 10 mV hyperpolarizing steps (500 ms, 0.1 Hz). Activation times were taken as the time for currents to rise from 10–90% of maximum. Where possible, data were obtained for mutants/chimeras together with both wild types in the same batch to control for variations between batches of oocytes. Data were expressed as mean ± S.E., and Student’s t test was used to test statistical significance.

RESULTS

Wild Type Channels—We first compared the properties of wild type rat and human Kv2.1 channels expressed in oocytes using two-electrode voltage-clamping. As found previously (20, 21), currents were outwardly rectifying, with little inactivation during 500-ms pulses (Fig. 1, A and B). For the IV curves, there were no significant differences between currents for the rat and human wild type channels at all test potentials studied (Fig. 1D). The activation times were significantly faster for the rat channel than for the human channel at all test potentials studied (Fig. 1C and E), which was in agreement with earlier comparisons (20). The amino acid sequence differences between rat and human Kv2.1 channels are shown schematically in Fig. 1F, and the aim of this paper is to investigate the role of these amino acid differences in determining activation times.

Role of N-terminal Determinants—The human Kv2.1 sequence (but not the rat Kv2.1 sequence) has an ATG located 12 bases before the assumed start methionine. In principle, a start of translation at this ATG could also underlie the slower activation of hKv2.1. However, mutation of this ATG to ATA did not affect the activation kinetics (data not shown), suggesting that this preceding ATG has no functional role, and, indeed, it was not preceded by a Kozac sequence in the vector.

To investigate the roles of the two N-terminal amino acids that differ between rat and human channels (Fig. 1F), these two residues were mutated in the rat channel to those in the human. These mutations in rat Kv2.1 (i.e. Q67E, E75D, and Q67E/E75D) were expressed in oocytes, and currents were recorded under voltage clamp. The IV curves for the mutants were not significantly different from those for rat wild type (data not shown), as would indeed have been expected, because the wild type IV curves themselves did not differ between rat and human (see above). Mutation Q67E in the rat channel caused a slowing of activation at all test potentials as compared with rat wild type (Fig. 2A), and, in fact, the activation times were not significantly different from those for the human wild type channel. On the other hand, mutation E75D in the rat channel did not affect activation times, which remained fast (Fig. 2B). The double mutant rKv2.1 Q67E/E75D showed slow activation as in the human channel. These results suggest that, for the rat channel (i.e. in the context of the rat C terminus), residue 75 did not affect activation, whereas residue 67 of the N terminus must be Gln (rather than Ghu) for activation kinetics to be fast.

Experiments were also carried out on the human Kv2.1 channel, this time mutating the two residues at 67 and 75 to the corresponding rat residues (i.e. E67Q, D75E, and E67Q/D75E). Again, IV curves were not affected by these mutations (data not shown). Mutation E67Q in the human channel did not affect activation times as compared with human wild type (Fig. 3A). On the other hand, mutation D75E in the human channel was characterized by faster activation than in the wild type human channel and, in fact, was not significantly different from that for the rat channel (Fig. 3B). The double mutant E67Q/D75E showed similar slow activation times as for human wild type (Fig. 3C). These results imply that, for the human channel (i.e. in the context of the human C terminus), residues 67 and 75 must both be Gln for the activation to be fast. At first glance, these results appear surprising and contradictory to those reported above with the rat channel (i.e. rat C terminus); indeed, only the combinations Gln67-Glu75-rC, Gln67-Asp75-rC, and Gln67-Glu75-rC (with rC and hc denoting the rat and human C-terminal regions, respectively) lead to channels with fast activation times. The most obvious explanation is that the C terminus is also involved in determining activation times and interacts with these N-terminal residues, albeit in a complex way. Thus the nature of the C terminus also determines the functional effects of mutations in the N terminus.

Role of C-terminal Determinants—To explore further the possible roles in activation kinetics of the 49 amino acids that differ in the C terminus between rat and human Kv2.1 (Fig. 1F), chimeras were made between the two channels and expressed in oocytes. Again, consistent with the lack of difference between IV curves for rat and human wild types, the IV curves for all the chimeras described in this section were not significantly different (data not shown) from wild type IV curves (except for one chimera wherein only very small differences were seen).

Experiments were first carried out in the context of the rat channel N terminus. Replacing C-terminal amino acids 108 to 528 of the rat channel with corresponding human sequence (chimera I) did not change the activation time as compared with rat wild type (Fig. 4A). Because of the near identity of the two channels, this in fact corresponds to swapping only the four amino acids that are different between the two channels at the
C terminus (see Fig. 4D for diagrammatic representation). The data indicate that these residues are not involved in determining differences in activation time between the two channels. Replacing residues 529 to 740 in the rat channel with the human sequence (chimera II) also did not change the activation time (Fig. 4B). In this region, 30 amino acids are different between the two channels (again shown schematically in Fig. 4D), so the data suggest that these residues are also not involved in determining differences in activation times between the two channels. To confirm this, the entire region from residue 108 to 740 was swapped from human into rat channel (chimera III) and, as expected, the activation time was not significantly different from rat wild type. Therefore, none of the residues in the region 108–740 contribute to differences in
To investigate whether residues from 741 to 853 at the C terminus are involved, we constructed chimera IV with these residues in the rat replaced by their corresponding residues in human. This stretch comprises 15 amino acids that are different between the two channels (Fig. 5D). For this chimera, the activation time was slower than for rat and, in fact, not significantly different from that for human (Fig. 5A). This indicates that amino acids in this region determine differences in activation kinetics between the two channels. In confirmation of this, chimeras V and VI, which also include
this region, likewise showed slow activation times similar to human wild type (Fig. 5, B and C). Therefore, taken together, the data show that up to 15 residues that are different in the domain 741–853 determine differences in activation times between the two channels (again in the context of the rat N terminus). We refer to this region as the C-terminal activation or CTA domain.

In an attempt to further narrow down this domain, we made chimeras VII and VIII, which subdivide the swapped CTA domain into two subdomains (741–795 and 796–853, Fig. 6C). However, activation times for both these chimeras remained fast, like rat wild type (Fig. 6, A and B). In combination with the previous results for chimeras IV to VI, the data therefore suggest that there are residues in both subdomains (i.e. 741–795 and 796–853) that are involved in determining differences in activation times between the two channels. More precisely (also taking into account the numbers of residues that are different in each domain), the data show that from 1 to 5 residues are involved in 741–795, and from 1 to 10 residues are involved in 796–853.

Further experiments were also carried out in the context of the human N terminus while making chimeras in the C-terminal regions with the rat channel. In chimera IX, we replaced residues 529–853 of the human channel with the corresponding rat residues. For this chimera, the activation times were slow, as in the human wild type channel (Fig. 6D). This is consistent with our previous results for the rat N-terminal double mutant (Q67E/E75D), because the C-terminal CTA do-

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**Fig. 4. Activation times for C-terminal chimeras I-III.** A, chimera I (residues 108–528 of rat replaced by human) (n = 7); human wild type (n = 4); rat wild type (n = 10). B, chimera II (residues 529–740 of rat replaced by human) (n = 8); human wild type (n = 7); rat wild type (n = 3). C, chimera III (residues 108–740 of rat replaced by human) (n = 6); human wild type (n = 5); rat wild type (n = 5). D, schematic diagrams of the chimeras, with vertical lines representing amino acid differences between rat and human wild type channels (F, fast activation like rat; S, slow activation like human). Activation times are indicated as follows: rat wild type (○); human wild type (■); and chimera (▲). Significant differences (p < 0.05) between chimera and wild type are signified by an asterisk (*).
main of chimera IX is rat type, whereas the N-terminal residues are Glu67 and Asp75, just as in the rat double mutant Q67E/E75D (Fig. 2C), which is also slowly activating. Finally, we constructed chimera X with residues 108–740 of the human sequence replaced by rat channel residues. This was also slowly activating, as for the human wild type (Fig. 6E). This again can be understood in terms of our earlier results, because chimera X contains the human C-terminal CTA domain together with N-terminal residues Glu67 and Asp75, as for the human wild type itself, which was also slow.

It is noteworthy that none of the mutations and chimeras (whether at the N or C terminus) affected the voltage dependence of the activation times (Figs. 2–6) in the sense that the voltage dependence was either the same as that for human or rat wild type channels.

In summary, the data, taken together, indicate that residues 67 and 75 at the N terminus and some residues within 741–853 at the C terminus are involved in determining activation kinetics. The data furthermore suggest an interaction between the N and C termini in determining activation kinetics.

**Binding Studies**—The most likely model for interaction between the N and C termini is by a direct binding between these two regions in the channel. To investigate this further, we carried out binding studies between these regions of the channel. For this, we examined binding of the C terminus (from S6

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**Fig. 5. Activation times for C-terminal chimeras IV-VI**. A, chimera IV (residues 741–853 of rat replaced by human) (n = 8); human wild type (n = 6); and rat wild type (n = 7). B, chimera V (residues 594–853 of rat replaced by human) (n = 8); human wild type (n = 6); and rat wild type (n = 22). C, chimera VI (residues 529–853 of rat replaced by human) (n = 29); human wild type (n = 25); and rat wild type (n = 22). D, schematic diagrams of the chimeras, as in Fig. 4. Activation times are indicated as follows: rat wild type (○); human wild type (■); and chimera (▲). Significant differences (p < 0.05) between chimera and wild type are indicated by an asterisk (*).
We first expressed the C-terminal protein as a 35S-labeled protein using an *in vitro* reticulocyte lysate system. This radio-labeled product was readily detected by autoradiography on SDS-PAGE gels (left lane in Fig. 7A, Kv2.1-C), and the 35S-labeled C terminus product was indeed expressed at approximately the expected size. Next, the GST-N-terminal fusion protein was expressed in *E. coli* and purified with glutathione. This GST fusion protein was then exposed to the above 35S-labeled C-terminal protein, and any unbound protein was washed off while retaining the GST fusion protein (as well as any protein that had bound to it). This GST-N-terminal protein, together with any bound protein, was also run on SDS-

**FIG. 6. Activation times for C-terminal chimeras VII-X.** A, chimera VII (residues 741–795 of rat replaced by human) \( n = 6 \); human wild type \( n = 5 \); and rat wild type \( n = 4 \). B, chimera VIII (residues 796–853 of rat replaced by human) \( n = 6 \); human wild type \( n = 6 \); and rat wild type \( n = 5 \). C, schematic diagrams of the chimeras VII–VIII. D, chimera IX (residues 529–853 of human replaced by rat) \( n = 33 \); human wild type \( n = 23 \); and rat wild type \( n = 3 \). E, schematic diagrams of chimeras IX–X. Activation times are indicated as follows: rat wild type (●), human wild type (■), and chimera (◆). Significant differences \( p < 0.05 \) between chimera and wild type are indicated by an asterisk (*).
**Fig. 7. N- and C-terminal interactions and models.** A, GST-N-terminal fusion protein was assayed for interaction with in vitro translated \(^{35}\text{S}\)-labeled C-terminal protein. The autoradiograph of an SDS-PAGE gel has lanes as follows: Kv2.1-C, \(^{35}\text{S}\)-labeled rKv2.1 C terminus protein; Kv2.1-N, GST-N-terminal rKv2.1 fusion protein after incubation with \(^{35}\text{S}\)-labeled rKv2.1 C terminus protein; and GST, GST protein alone after incubation with \(^{35}\text{S}\)-labeled rKv2.1 C terminus protein. B, computer predictions of three-dimensional structures (www.expasy.org/swissmod/ SWISS-MODEL.html) for the T1 domain of Kv2.1 by threading amino acids 27–137. Single subunits are shown, each oriented with the C-terminal end that faces the membrane at the top and with subunit-subunit interface regions at the left. The figure shows predictions for the mutated residues at positions 67 and 75 of Kv2.1. Both residues are exposed at the outside face of the subunit. C, schematic model of the Kv2.1 channel, showing the membrane spanning part and the intracellular domains T1, Kv2, and CTA. The linkers comprise both N-terminal (T1-S1) and C-terminal (S6-Kv2) parts. The model was guided by the electron microscope structure of the Shaker channel (19) but allows for the much larger size of the C-terminal of Kv2.1 (we assumed a constant density and increased the size accordingly). The CTA domains are shown overlapping the T1 residues 67 and 75, and the C linker (S6-Kv2) has been drawn as directly connecting to the bottom of T1, as for Shaker (25). The Kv2 domain has been located schematically in the remaining space. D, alignment using ClustalW of rat and human Kv sequences. The CTA domain is shown as a bar, with dashed bars indicating regions of uncertainty (i.e. identical residues between human and rat Kv2.1). Arrows indicate various computer predictions (www.compbio.dundee.ac.uk/~www-jpred/submit.html) of \(\beta\)-strands, shown as guidelines only.
PAGE gels and examined by autoradiography for any $^{35}$S-labeled product (which must have come from bound labeled C terminus). This is shown in the middle lane in Fig. 7A (Kv2.1-N), and it can be seen that a $^{35}$S-labeled band was indeed detected in this lane (albeit weakly) at the same size as the C terminus product, indicating binding of the C terminus with the GST-N terminus fusion protein. The right lane in Fig. 7A (GST) was obtained like the middle lane, except that GST was used instead of the GST-N-terminal fusion protein. No band was seen in this control lane, indicating that the C terminus protein did not bind to GST itself. Taken together, the data therefore confirm that the N and C termini can indeed bind to each other.

**DISCUSSION**

In this study, we have investigated the underlying molecular regions in the N- and C-terminal regions of the Kv2.1 potassium channel that are responsible for the marked differences in activation time between rat and human channels. At the N terminus, residues 67 and 75 were important in determining activation rate, whereas at the C terminus some residues within 741–853 (CTA domain) also determined activation rate. The data suggested interaction between N and C termini, because effects on either terminus depended on the nature of the (mutated) residues in the other terminal region. Furthermore, direct evidence for an interaction between the N and C termini was obtained using binding studies with GST fusion proteins. Although effects on the activation rate at the N terminus were localized to just two amino acids, at the C terminus two or more amino acids were involved. For the latter region (741–853) there are 15 amino acids, spanning residues 749–838, that are different between the two channels. Attempts to further narrow down this region indicated that 1–5 residues are involved in the subdomain 749–795, as well as 1–10 residues in the subdomain 796–838.

The N-terminal residues 67 and 75 are located in the T1 domain of Kv2.1. Although the three-dimensional structure for the Kv2.1 T1 domain is not available, three-dimensional structures are available for the closely related channels Kv1.1, Kv1.2, and Kv3.1 (7–11), and it seems reasonable to assume that the T1 domain for Kv2.1 has a rather similar structure and, indeed, that it also hangs like a gondola below the membrane, as for Kv3.1 (17–19). To pursue this further, we have used computer-based homology modeling for the Kv2.1 T1 domain (Fig. 7B). Residues 67 and 75 are apparently located at exposed positions on the outside of the T1 tetramer, with residue 67 at the lower part of the exposed side and residue 75 underneath in the “stirrup.” Using the same homology-based model, which includes energy minimization, it can be seen that a marked local change is produced by mutation between Gln and Glu at position 67; the region moves outward, probably repelled by neighboring negative charges. Although there is no such alteration for Asp and Glu mutations at residue 75, there is obviously a change in bulk for this residue. Of course, this is only a computer model, but it does illustrate the kind of changes that might occur. Mutation of these residues cannot change activation rate by interacting directly with the S4 region or its nearby intracellular loops, because neither residue faces the membrane. On the contrary, our data suggest instead that residues 67 and 75 may interact with the CTA domain that we have identified in the C-terminal region.

The conclusion from our data of interactions between N and C termini is supported by earlier reports. For instance, deletions of the N terminus (139 residues) caused slowing of activation time in rKv2.1, but the activation times were speeded up (almost back to normal) by the further deletion of 318 residues from the C terminus (22). On the other hand, deleting these C-terminal residues in channels with an intact N terminus did not affect activation kinetics. Thus, the activation kinetics depend on interactions between N and C termini. Also, in our earlier work (24) we have shown that phosphorylation of the rKv2.1 channel by protein kinase A takes place at the C terminus, but the extent of phosphorylation is influenced by the presence or absence of the N terminus, even though phosphorylation does not occur on the N terminus. Furthermore, under oxidizing conditions, a disulphide bond can form between an N-terminal cysteine (located just below the T1 tetrameric structure) and a C-terminal cysteine (26 residues from S6) in Kv1.1, indicating that these residues are, or can be, in the vicinity of each other (25). This suggests that, on leaving S6, the polypeptide chain leads directly to the underside of T1. Further support for an interaction between N and C termini comes from the electron microscope structure (19) of the Kv1.1 channel, where it seems that the hanging gondola structure occupies a greater volume than T1 alone and, presumably, the remaining space is taken up by the C terminus, which seems to envelop it. Contact between the T1 region and the C terminus has also been hypothesized on the basis of the hydrophobicity of the outer surface of the T1 tetramer (11).

The C-terminal region in Kv2 channels can be divided into three separate domains, i.e. an S6 linker region (50 amino acids), a “K2” domain (Pfam 3521, ~250 amino acids), and the CTA domain (~110 amino acids) (Fig. 1F). The S6-Kv2 linker region shows considerable homology between Kv2 channels and has a computer-predicted helix-strand-helix structure. This linker may, together with the T1-S1 linkers, form the “ropes” that suspend the gondola and, by analogy with Kv1 (see above), may reach the underside of the gondola (Fig. 7C). The Kv2 domain is characteristic of the Kv2 channels and also shows some predicted secondary structure as well as some hydrophobic regions and may also contact the T1 region. The CTA domain that we have identified shows partial homology across the Kv2 family (Fig. 7D) and is predicted to possess several $\beta$-strands as well as some hydrophobic parts, and it may form a structure that interacts with T1 around residues 67 and 75, as has already been suggested in the present paper (Fig. 7C).

Although the role of the Kv2.1 C terminus has not previously been systematically studied (except for deletion studies), the role of the N-terminal T1 domain has been well characterized. Besides the T1 deletions that cause slowing of activation (22, 23), various mutations within the T1 domain have also been shown to cause changes in activation kinetics as well as shifting IV curves (10–12, 14, 16, 17, 26). Some of these mutations have been located at the interface regions within the T1 tetramer, apparently without disrupting its structure, and the conclusion has been made that the conformational change of gating involves movement of both the membrane-gating machinery and the T1 region as a whole. Our data suggest the C terminus might also be involved in this conformational change within the model of a hanging gondola composed of a T1 core and an interacting C terminus (Fig. 7C). Deletion of the core T1 region might leave the C terminus waggling in the wind, which may affect gating via its direct connection to S6 and the pore that it lines. Indeed, alterations of the T1 region may in fact mainly affect gating via its interaction with C-terminal protein, particularly for Kv2 channels with their very large C-terminal region. Obviously these possibilities remain the subject for further experimental testing.

It had previously been thought that the voltage dependence of activation was different between rat and human Kv2.1 channels based on studies in different laboratories under different experimental conditions (20, 21). However, our results showing
identical IV curves for the two channels under the same experimental conditions have shown that, in fact, the voltage dependence of steady-state activation is identical between the two channels. Thus, one might suggest that the extent of S4 movement and the stability of the open and closed states are probably not altered between these channels. However, the kinetics of S4 movement and/or the kinetics of intervening steps that lead to opening may be different between rat and human channels, leading to differing activation kinetics. Our experiments suggested a voltage-independent mechanism. Comparison with N-terminal deletion and cysteine binding experiments suggests that the slowing of activation that we have observed might be due to delayed first latency of opening by interfering with transitions in the activation pathway before first opening of single channels (23).

It is worth mentioning that interactions between the N and C termini are not confined to Kv channels like Kv2.1. Thus, for instance, interactions between these intracellular regions have been implicated for ether-a-go-go channels, plant KAT channels, and inward rectifier Kir channels (27–29). It may well be that such interactions generally form an intimate part of channel-gating machinery.

Regarding physiological relevance of our results, Kv2.1 channels are widely expressed in a range of tissues, including brain (cell bodies and dendrites), heart, skeletal muscle, retina, cochlea, eye, germ cells, lung, pulmonary arteries, and pancreatic β cells (30). The physiological function of the channel appears to be concerned with maintaining membrane potential and in modulating electrical excitability in neurones and muscle. In particular, in the brain where expression of the Kv2.1 channel is marked, the channel may play an important role in the control of synchrony, burst firing, and oscillations. Indeed the time scale of activation of these channels (tens of milliseconds) is appropriate to influence such dynamically occurring processes. It is tempting to speculate that the marked difference in activation time for Kv2.1 between rat and human (60 and 120 ms, respectively, at 0 mV) might contribute physiologically to differences in dynamic firing patterns between rat and human.

In conclusion, our results for Kv2.1 show that residues 67 and 75 in the T1 domain, as well as some residues within the CTA domain (741–853) at the C terminus, affect activation kinetics and that these regions interact. The T1 domain might affect channel activation kinetics via its connection with the C-terminal region and, hence, from there to S6 and its associated pore.

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