Structural Determinants of KvLQT1 Control by the KCNE Family of Proteins*

Received for publication, November 28, 2000

Yonathan F. Melman**, Anna Domènech†, Susana de la Luna‡, and Thomas V. McDonald¶

College of Medicine, Bronx, New York 10461 and the Down Syndrome Research Group Medical and Molecular Genetics Center-IRO, Hospital Duran i Reynals Gran Via Km 2.7 08907 L’Hospitalet, Barcelona, Spain

KvLQT1 is a Shaker-like voltage-gated potassium channel that when complexed with minK (KCNE1) produces the slowly activating delayed rectifier $I_{kr}$. The emerging family of KCNE1-related peptides includes KCNE1 and KCNE3, both of which complex with KvLQT1 to produce functionally distinct currents. Namely $I_{kr}$, the slowly activating delayed rectifier current, is produced by KvLQT1/KCNE1, whereas KvLQT1/KCNE3 yields a more rapidly activating current with a distinct constitutively active component. We exploited these functional differences and the general structural similarities of KCNE1 and KCNE3 to study which physical regions are critical for control of KvLQT1 by making chimerical constructs of KCNE1 and KCNE3. By using this approach, we have found that a three-amino acid stretch within the transmembrane domain is necessary and sufficient to confer specificity of control of activation kinetics by KCNE1 and KCNE3. Moreover, chimera analysis showed that different regions within the transmembrane domain control deactivation rates. Our results help to provide a basis for understanding the mechanism by which KCNE proteins control K⁺ channel activity.

KvLQT1 is a voltage-activated Shaker-type potassium channel widely expressed in heart, kidney, colon, pancreas, and inner ear (1–3). KvLQT1 (also known as KCNQ1) complexes with minK (KCNE1) to form a slowly activating delayed rectifier current, $I_{kr}$ (4, 5). KCNE1 is a member of a gene family that includes a number of one-spanning membrane proteins whose association with potassium channels controls their activity (6, 7). $I_{kr}$, when compared with KvLQT1 homotrameric channels, shows an augmentation of whole cell current amplitude, a depolarizing shift of the voltage dependence of activation, removal of inactivation (8), and a slowing down of both activation and deactivation kinetics (4, 5). Mutations in either KvLQT1 (1, 9) or KCNE1 (10, 11) genes can lead to Romano-Ward and Jervell and Lange-Nielsen syndromes, both characterized by a prolongation of QT interval due to delayed cardiac repolarization and, in the case of Jervell and Lange-Nielsen syndrome, congenital sensorineural deafness. The nature of the interaction between KCNE1 and KvLQT1 has been the subject of much investigation. An extensive mutagenesis study of KCNE1 (12) originally uncovered a region of the protein within the N-terminal portion of the cytosolic domain that appeared critical for KCNE1 function; changes within this stretch were poorly tolerated. Romey et al. (13) provided biochemical evidence in support of an interaction between the pore region of KvLQT1 and the intracellular C terminus of KCNE1. Cysteine-scanning mutagenesis (14, 15) provided evidence that residues in the transmembrane domain line the channel conduction pore. Tapper and George (16), using KCNE1 deletions and chimeras with a sodium channel β-subunit, showed that the KCNE1 C terminus alone cannot modify the KvLQT1 channel but rather that the presence of the KCNE1 transmembrane domain is also needed for this function.

Recently, a novel KCNE family member, KCNE3, was shown to interact with KvLQT1 to produce a potassium current with a constitutive component (2). This current resembles that seen in colonic epithelia, where both KCNE3 and KvLQT1 are expressed. Here we have exploited the marked differences in the regulation of KvLQT1 by these two proteins to investigate which regions of these two proteins mediate the differences in effects on KvLQT1. The approach of KCNE1/KCNE3 chimeras has the advantage of placing structural portions of KCNE1 in a homologous environment, but one that can modulate KvLQT1 function quite differently from KCNE1. By using this approach, we find that a three-amino acid segment within the transmembrane domain of KCNE1 is sufficient and necessary to determine the specificity of KCNE control of channel activation. Additionally, our results show a physical separation of determinants of activation and deactivation.

MATERIALS AND METHODS

Cloning of KCNE3—We detected human ESTs with homology to KCNE1 via TBLASTN search. By overlapping the ESTs we constructed the full-length cDNA. Forward and reverse primers from the 5′- and 3′-ends of the predicted coding region (5′ATGGAGAATCACTATGGAACGGAG and 3′TATGACCATAGACACACGTTGCTT) were used in 5′- and 3′-rapid amplification of cDNA ends PCR using the Marathon Ready cDNA kit (CLONTECH) and human stomach cDNA. Several rapid amplifications of cDNA end clones were sequenced and assembled to get the full-length cDNA. The sequence has been reported to GenBank under accession number AF302494.

Construction of KCNE1/KCNE3 Chimera—Chimeras were constructed using a PCR-based approach. Briefly, using PCR with Pfu turbo (Stratagene) and 5′-phosphorylated primers, we produced blunt-ended PCR fragments that corresponded to the regions of interest from KCNE1 and KCNE3. Blunt-ended fragments from KCNE1 were gel-purified, ligated overnight to a blunt-ended PCR product corresponding to the appropriate region of KCNE3, and re-amplified by PCR for subcloning into PCR Blunt II TOPO (Invitrogen). Chimera cDNAs were digested using HindIII and EcoRI and ligated into the mammalian expression plasmid pcDNA3 (Invitrogen). Nomenclature of the primers...
Functional Domains of KCNE1 and KCNE3

RESULTS

**KCNE1 and KCNE3 Control KvLQT1 Activity**—Fig. 2 shows whole cell current recordings from CHO cells transfected with KvLQT1 cDNA alone or in combination with either KCNE3 or KCNE1. As previously reported, coexpression with KCNE1 results in an increase in whole cell current amplitude (2.2-fold, see Table I) and the kinetics of both activation and deactivation are slowed (Fig. 2, a and b, and Table I) (4, 5). Coexpression of KCNE3/KvLQT1, as previously reported (2), resulted in a current with a constitutively active component (Fig. 2c), as viewed on faster time scales and hyperpolarizations to −150 mV (Fig. 2c, inset). We also observed a distinct time voltage-dependent component of activation and deactivation for the KvLQT1/KCNE3 current (Fig. 2, c, e, and f). The voltage-dependent component had altered activation (KvLQT1, 24.2 ± 2.3 ms, and KCNE3, 21.1 ± 1.9 ms) and deactivation kinetics (KvLQT1, 39 ± 1.1 ms, and KCNE3, 16 ± 2.1 ms) with a shift in $V_h$ (KvLQT1, −15.2 ± 0.52 mV, and KCNE3, 52.2 ± 2.15 mV), and lacked obvious voltage-dependent inactivation (Fig. 2c), indicating that it too was a consequence of the association of KvLQT1 with KCNE3. Whole cell current amplitudes were also augmented relative to KvLQT1 alone, to a similar extent as KCNE1 (KvLQT1, 2.13 ± 0.14-fold, and KCNE3, 2.22 ± 0.09-fold, relative to KvLQT1, 1.00). Expression of KCNE3 alone did not yield currents (Fig. 2d).

**KCNE Transmembrane Regions Determine Specificity of KCNE Control of KvLQT1 Kinetics**—To determine the regions of KCNE1 and KCNE3 that are responsible for the different effects on KvLQT1 function, we swapped various segments of the transmembrane domain. We observed that it too was a consequence of the association of KvLQT1 with KCNE3. Whole cell current amplitudes were also augmented relative to KvLQT1 alone, to a similar extent as KCNE1 (KvLQT1, 2.13 ± 0.14-fold, and KCNE3, 2.22 ± 0.09-fold, relative to KvLQT1, 1.00). Expression of KCNE3 alone did not yield currents (Fig. 2d).
and KCNE1, respectively (Fig. 3, b, c, and Fig. 5). Chimeras that exchanged the intracellular domains plus 4 amino acids of the transmembrane domain, M(1–63)K, with KCNE3, showed no constitutive component and slow activation (rise time 691 ± 34.9 ms). These chimeras showed that activation kinetics follow amino acids 57–59 in the KCNE1 transmembrane region in finer detail, we subdivided it by constructing chimeras KM(48–56)K that had a clear constitutive component (14). The equivalent residue of KCNE3 is a valine, a substitution that like T59C abolishes the hydrogen bonding potential of the threonine hydrogen bond.

FIG. 2. Differential control of KvLQT1 channel activity by KCNE1 and KCNE3. a, whole cell recording in response to depolarizing steps in CHO cells transfected with KvLQT1 alone. b, cotransfection with KCNE1. c, cotransfection with KCNE3; inset, same cell recorded at faster time scale. d, transfection with KCNE3 alone. e, voltage-dependent activation curves. f, normalized activation curves were fitted using the Boltzmann equation. y scale bars are a and d, 400 pA; b and c, 10,000 pA; e, 200 pA; c (lower trace), 11,000 pA. x scale bars are c (lower trace), 50 ms; all others, 500 ms.

Functional Domains of KCNE1 and KCNE3

Functional Domains of KCNE1 and KCNE3...

A constitutively active current was detected for MK(57–59)M; however, it composed a smaller fraction of the total than that seen with wild-type KCNE3 (Table I), indicating that additional residues outside this region may be involved in generating the constitutive component.

Separation of KCNE Determinants of Activation and Deactivation Rates—In contrast to currents seen with KCNE1 or other I_L-like chimeras (Table I and Figs. 5 and 6b), the kinetics of deactivation of KM(57–59)K are greatly accelerated (39 ± 1.0 ms versus 146 ± 13.5 ms for KvLQT1/KCNE1 currents). The voltage-dependent current of chimera MK(57–59)K shows activation kinetics that are somewhat slower than the KCNE3 voltage-dependent current alone (MK(57–59)M, 65 ± 6.4 ms, KCNE3, 21.1 ± 1.9 ms, and Fig. 6a) but nonetheless are 9-fold faster than the rise time of KvLQT1/KCNE1(70 ± 27.5 ms). Deactivation kinetics with this chimera (123 ± 4.8 ms) are nearly as slow as that of KCNE1 (146 ± 13.5 ms), however. Comparison of deactivation rates for chimeras KM(48–59)K and KM(57–59)K implicates a role for KCNE1 residues 48–56, lying N-terminal to this stretch, in determining the slow kinetics of deactivation seen with I_L.

In contrast, incorporation of KCNE3 residues either N-terminal (MK(48–59)M) or C-terminal (MK(57–63)M) to this stretch resulted in fast kinetics of deactivation, similar to KCNE3 (Table I). A possible explanation is that residues 48–56 of KCNE1 confer slow deactivation, whereas in KCNE3, C-terminal residues (60–63) control fast deactivation. When both segments are present in the same protein, however, the KCNE3 residues may act in a dominant fashion, but only when residues 57–59 are present as well (compare KM(48–56)K and MK(57–63)M, Table I).

KCNE3 Induces a Constitutively Active K^+ Current through Complex Physical Interaction with KvLQT1—We determined the presence of a constitutively active component to KCNE3 currents by looking for the appearance of an instantaneous current following depolarization, manifested as a sharp corner before the onset of voltage-activated current (see Figs. 2c, 3, and 4). From the height of this step we estimated the fraction of total current that was attributable to the constitutive component (Table I, last column). The presence of constitutively active current was also seen using very fast sampling rates and membrane hyperpolarizations to eliminate the possibility of a very fast-activating voltage-dependent current (Figs. 2c and 4e). All chimeras containing KCNE3 amino acids 71–73 exhibited such a current. In contrast, chimera KM(57–59)K which consists essentially of KCNE3 with amino acids 71–73 replaced with KCNE1 residues 57–59 does not exhibit any observable constitutive component to its current, demonstrating that these residues are necessary for generating the constitutive component (Fig. 4, d and e). The fraction of total current provided by this constitutive component, however (0.18 ± 0.03), was smaller for chimeras containing these and surrounding residues than for KCNE3 (constitutive component is 0.302 ± 0.03 of total current). Thus, although residues 71–73 are necessary for constitutive activation, they are not sufficient for its full expression. Our results suggest that full control of the constitutively active component may be more complex than kinetics of voltage activation. Interestingly, Cd^2+ treatment of a T59C KCNE1 mutant (T58 in human KCNE1) leads to currents with a constitutively active component (14). The equivalent residue of KCNE3 is a valine, a substitution that like T59C abolishes the hydrogen bonding potential of the threonine hydroxyl group and introduces a larger residue in its place.

DISCUSSION

Our work has determined that small regions within the transmembrane domain of KCNE family members control the activation and deactivation rates of KvLQT1. Specifically, a...
Functional Domains of KCNE1 and KCNE3

TABLE I
Quantitative analysis of currents from KCNE1/KCNE3 chimeras

| Channel       | Relative current | Rise time  | tau deact | Vh   | Slope factor | n    | Constitutive component |
|---------------|------------------|------------|-----------|------|--------------|------|------------------------|
| kvlqt alone   | 1.00             | 24.2 ± 2.3 | 39 ± 1.1  | -15.2 ± 0.52 | 13.1 ± 0.55  | 8    | ND                     |
| minK          | 2.13 ± 0.14      | 704 ± 27.5 | 146 ± 13.5| 30.5 ± 1.65 | 20.1 ± 1.1   | 20   | ND                     |
| NCNE3         | 2.22 ± 0.09      | 21.1 ± 1.9 | 16 ± 2.1  | 52.2 ± 3.97 | 36.2 ± 1.5   | 9    | 0.30 ± 0.03            |
| N termini swapped | 1.587 ± 0.12 | 442 ± 15   | 203 ± 18  | 7.16 ± 5.43 | 27 ± 1.73    | 10   | ND                     |
| C termini swapped | 0.23 ± 0.06 | 27 ± 1.6   | 47 ± 6.6  | 33.0 ± 10.2 | 45 ± 7.3     | 5    | 0.18 ± 0.08            |
| Kv1–47M       | 2.30 ± 0.16      | 143 ± 5.86 | 183 ± 3.43| 40.7 ± 4.82 | 40.5 ± 1.33  | 10   | 0.24 ± 0.07            |
| M1–63M        | 0.48 ± 0.04      | 591 ± 70   | 112 ± 5.5 | 67.8 ± 12.6 | 11.4 ± 5.9   | 7    | ND                     |

Interspersed segments

| KM(48–63)K   | 2.09 ± 0.07      | 825 ± 81.8 | 182 ± 15.5| 13.0 ± 3.65 | 24.5 ± 2.7   | 5    | ND                     |
| KM(48–59)K   | 2.83 ± 0.17      | 691 ± 34.9 | 116 ± 10.6| 49.2 ± 2.59 | 18.2 ± 1.1   | 10   | ND                     |
| KM(57–59)K   | 0.86 ± 0.13      | 632 ± 26.2 | 39 ± 1.02 | 60.8 ± 1.52 | 18.1 ± 1.3   | 9    | ND                     |
| KM(48–56)K   | 2.52 ± 0.43      | 42.5 ± 3.22| 28.5 ± 3.5| Not measured | measured    | 8    | 0.75 ± 0.17            |
| MK(48–59)M   | 1.13 ± 0.15      | 44 ± 2.5   | 24 ± 1.4  | 42.4 ± 2.56 | 30.2 ± 2.8   | 5    | 0.12 ± 0.07            |
| MK(57–59)M   | 0.30 ± 0.07      | 81 ± 3.13  | 123 ± 4.7 | 75.0 ± 9.9  | 32.4 ± 4.3   | 5    | 0.17 ± 0.03            |
| MK(57–63)M   | 0.28 ± 0.35      | 57.2 ± 1.48| 17 ± 3.2  | 10.4 ± 2.1  | 33.1 ± 2.3   | 8    | 0.18 ± 0.02            |

Three amino acids stretch is sufficient and necessary to confer specificity of activation kinetics to KvLQT1, but neighboring regions appear to control the rates of deactivation. These same three amino acids also appear sufficient and necessary for at least partial generation of the constitutive component of current seen with KCNE3/KvLQT1 complexes.

Several groups have provided evidence that the KCNE1 C terminus is a region critical for its function (12, 13, 21). Most recently, Tapper and George (16) have shown that the C-terminal domain alone is not sufficient for slow activation when placed in a chimera with a sodium channel β-subunit and that the C-terminal half of the transmembrane domain was also necessary. Our work defines that portion of the transmembrane domain that is sufficient and necessary to confer functional specificity to KCNE1 and -3. Our results do not, however, rule out a role for the C terminus in KCNE1 function. The greatest homology between KCNE1 and KCNE3 resides in the previously determined critical C-terminal amino acids (Fig. 1). This region may be needed for anchoring, binding, or positioning the transmembrane domain that we now identify as the effector. This would explain the results of earlier mutagenesis studies that showed the C-terminal region to be relatively intolerant to amino acid substitutions, as well as an analysis of KvLQT1 mutations that affect KvLQT1 function only in association with KCNE1 (22).

Our results indicate a separation of structural determinants of activation and deactivation kinetics. Thermodynamics can help elucidate the mechanism of KCNE control of channel activity by relating experimentally determined rates to energy states of the channel as it opens and closes. Changes in the voltage dependence of activation (Vh) are indicators of changes...
in the relative free energy of the closed and open states of the channel ($\Delta G$ indicates change in Gibbs' free energy). Rates of opening and closing of K channels, in contrast, reflect changes in the activation energy ($\Delta G^\ddagger$). This is the energy required to reach the transition state, the least stable intermediate conformation along the reaction coordinate of a molecule undergoing a physical change. KCNE1 and KCNE3 affect the rates of both opening and closing of the channel and the voltage dependence of activation. Our results provide a physical basis for each of these effects.

That we observed nearly identical kinetics of activation between KCNE1 and chimera KM(57–59)K suggests that this region is necessary and sufficient to modulate the slow gating of activation seen with KvLQT1-KCNE1 complexes. We observed a ~30-mV difference in the $V_h$ between the two, however. This result indicates that the substitution of the KCNE1 residues 57–59 in KCNE3 was able to confer the KCNE1-like kinetics of activation of KvLQT1 (by increasing $\Delta G^\ddagger$) independently of the $V_h$ ($\Delta G$). The implication therefore is that KCNE1 residues 57–59 interact with the transition state of the channel during the closed → open transition but do so somewhat independently of the relative free energy of the closed and open states ($\Delta G$). In addition, our results have dissected the region of KCNE1 controlling activation rate from that controlling deactivation rate. This result is not consistent with a view of KCNE1 acting simply as an “enzyme,” modulating the activation energy of a reversible reaction. If that were true, we would expect the regions controlling activation and inactivation to be identical. Our observations imply that KCNE1 modulates the kinetics of KvLQT1 by interacting with different transition states during activation (the closed → open transition) and deactivation (the open → closed transition). While such a scenario is impossible in simple chemical equilibria, in the case of voltage-gated ion channels the two transitions take place under conditions of different electric field across the membrane. Channel structure is expected to be different during activation (depolarization) and deactivation (repolarization). Thus, the transition states for each process are also expected to be different.

Our data are consistent with a model in which the conformation of KvLQT1, when altered by the change in voltage across the membrane, presents different regions of the channel for interaction with KCNE1 and KCNE3. Following a depolarization, it appears that KCNE1 residues 57–59 (KCNE3 residues 71–73) are available to interact with a transition state to modulate the activation kinetics. Upon repolarization, the different conformations of KvLQT1 and KCNE1/3 allow other neighboring transmembrane regions of the proteins to interact. This interaction then modulates the stability of the reverse transition state ($\Delta G^\ddagger_{\text{rev}}$).
Our study has thus elucidated structural components of KCNE1 and KCNE3 that are necessary and sufficient to modulate the gating kinetics of KvLQT1 by interacting with several transition states. The technique of perturbing specific structural components by moving them into a homologous environment can serve to complement results from gating charge (23), crystallographic (24), and structural data (25, 26). Further work and analysis of the interactions of KCNE family of proteins with K\(^+\) channel \(\alpha\)-subunits may help to improve our understanding of gating mechanisms in a new way.

REFERENCES

1. Neyroud, N., Tesson, F., Denjoy, I., Leibovici, M., Donger, C., Barhanin, J., Faure, S., Gary, F., Coumel, P., Petit, C., Schwartz, K., and Guicheney, P. (1997) *Nat. Genet.* 15, 186–189
2. Schroeder, B. C., Waldegger, S., Fehr, S., Bleich, M., Warth, R., Greger, R., and Jentsch, T. J. (2000) *Nature* 403, 196–199
3. Yang, W. P., Levesque, P. C., Little, W. A., Conder, M. L., Shalaby, F. Y., and Blanar, M. A. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 4017–4021
4. Barhanin, J., Lesage, F., Guillemare, E., Fink, M., Lazdunski, M., and Romey, G. (1996) *Nature* 384, 78–80
5. Sanguinetti, M. C., Curr, M. E., Zou, A., Shen, J., Spector, P. S., Atkinson, D. L., and Keating, M. T. (1996) *Nature* 384, 80–83
6. Takumi, T., Ohkubo, H., and Nakashiki, S. (1989) *FASEB J.* 5, 331–337
7. Abbott, G. W., and Goldstein, S. A. (1998) *Q. Rev. Biophys.* 31, 357–398
8. Tristani-Firouzi, M., and Sanguinetti, M. C. (1998) *J. Physiol. (Lond.)* 510, 37–45
9. Wang Q. C. M., Splawski, I., Burn, T. C., Millholland, J. M., VanRaay, T. J., Shen, J., Timothy, K. W., Vincent, G. M., de Jager, T., Schwartz, P. J., Toubin, J. A., Moss, A. J., Atkinson, D. L., Landes, G. M., Connors, T. D., and Keating, M. T. (1996) *Nat. Genet.* 12, 17–23
10. Duggal, P., Vesely, M. R., Wattanasirichaigoon, D., Villafane, J., Kaushik, V., and Beggs, A. H. (1998) *Circulation* 97, 142–146
11. Tyson J. T., L. Bellman, S., Wren, C., Taylor, J. F., Batheik, J., Aslaksen, B., Sorland, S. J., Lund, O., Pal, M., Pembrey, M., Bhattacharya, S., and Ritner-Glindzicz, M. (1997) *Hum. Mol. Genet.* 6, 2179–2185
12. Takumi, T., Mori, K., Arai, T., Ishii, T., Okada, Y., Ohkubo, H., and Nakashiki, S. (1991) *J. Biol. Chem.* 266, 22192–22198
13. Barhanin, J., Lesage, F., Guillemare, E., Ahtie, I., Guicheney, P., Barhanin, J., and Lazdunski, M. (1997) *J. Biol. Chem.* 272, 16713–16716
14. Tai, K. K., and Goldstein, S. A. (1998) *Nature* 391, 605–608
15. Wang, K. W., Tai, K. K., and Goldstein, S. A. (1998) *Neuron* 16, 571–577
16. Tapper, A. R., and George, A. J. (2000) *J. Gen. Physiol.* 116, 379–390
17. van den Hoff, M. J., Mooreman, A. F., and Lamers, W. H. (1992) *Nucleic Acids Res.* 20, 2802
18. Kagan, A., Yu, Z., Fishman, G. I., and McDonald, T. V. (2000) *J. Biol. Chem.* 275, 11241–11248
19. McDonald, T. V., Yu, Z., Ming, Z., Palma, E., Meyers, M. B., Goldstein, S. A. N., and Fishman, G. I. (1997) *Nature* 388, 289–292
20. Hammill, O. P., Marti, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981) *Pfluegers Arch.* 391, 85–100
21. Sesti, F., and Goldstein, S. A. (1998) *J. Gen. Physiol.* 112, 651–663
22. Chouabe, C. N. N., Richet, P., Denjoy, I., Hainque, B., Romney, G., Driess, M. D., Guicheney, P., and Barhanin, J. (2000) *Cardiovasc. Res.* 45, 971–980
23. Seoh, S. A., Sigg, D., Papazian, D. M., and Bezanilla, F. (1996) *Neuron* 16, 1159–1167
24. Doyle, D. A., Cabral, J. M., Pusztzser, R. A., Kue, A., Golbue, J. M., Cohen, S. L., Chait, B. T., and Matrin, R. (1998) *Science* 280, 67–77
25. Cha, A., Snyder, G. E., Selvin, P. R., and Bezanilla, F. (1999) *Nature* 402, 809–813
26. Glann, K. S., Mannuzzu, L. M., Gandhi, C. S., and Isacoff, E. Y. (1999) *Nature* 402, 813–817
