Channels 5:3, 225-227; May/June 2011; © 2011 Landes Bioscience

The "structurally minimal" isoform KChIP2d modulates recovery of K_v4.3 N-terminal deletion mutant Δ2-39

Laura J. Hovind and Donald L. Campbell*

Department of Physiology and Biophysics; University at Buffalo; State University of New York; Buffalo, NY USA

Key words: K_v4.3, KChIPs, inactivation, recovery, potassium channels

Submitted: 02/28/11

Accepted: 03/01/11

DOI: 10.4161/chan.5.3.15313

*Correspondence to: Donald L. Campbell; Email: dc25@buffalo.edu

Addendum to: Hovind LJ, Skerritt MR, Campbell DL. K_v4.3 N-Terminal deletion mutant D2-39: Effects on inactivation and recovery characteristics in both the absence and presence of KChIP2b. Channels 2011; 5:43–55; PMID: 21057209; DOI: 10.4161/chan.5.1.139.

Mechanisms underlying K_v4 (Shal type) potassium channel macroscopic (open state) inactivation and recovery are unknown, as are mechanisms by which KChIP2 isoforms modulate these two processes. In a recent study (Xenopus oocytes, 2 microelectrode voltage clamp) we demonstrated that: i) Partial deletion of the K_v4.3 proximal N-terminal domain (Δ2-39; deletes N-terminal amino acids 2-39) not only slowed macroscopic inactivation, but also slowed the net rate of recovery; and ii) Co-expression of KChIP2b significantly accelerated the rate Δ2-39 recovery from inactivation. The latter effect demonstrated that an intact N-terminal domain was not obligatorily required for KChiP2b-mediated modulation of K_v4.3 recovery. To extend these prior observations, we have employed identical protocols to determine effects of KChiP2d on Δ2-39 macroscopic recovery. KChiP2d is a "structurally minimal" isoform (consisting of only the last 70 amino acids of the common C-terminal domain of larger KChIP2 isoforms) that exerts functional modulatory effects on native K_v4.3 channels. We demonstrate that KChiP2d also accelerates Δ2-39 recovery from macroscopic inactivation. Consistent with our prior Δ2-39 + KChIP2b study, these Δ2-39 + KChIP2d results: i) Further indicate that KChIP2 isoform-mediated acceleration of K_v4.3 macroscopic recovery is not obligatorily dependent upon an intact proximal N-terminal; and ii) Suggest that the last 70 amino acids of the common C-terminal of KChiP2 isoforms may contain the domain(s) responsible for modulation of recovery.

K_v4 (Shal type) potassium channels, in association with various ancillary β subunits, generate the rapidly activating and inactivating, and subsequently rapidly recovering, transient outward current phenotypes "I_A" in neurons and "$I_{to,fast}$" in working cardiac myocytes.[1-3] Unlike other much more well-studied potassium channels, such as Shaker[4-6] and KcsA,[7,8] mechanisms underlying K_v4 channel macroscopic (open state) inactivation and recovery are unknown. There is also controversy on underlying assumptions of proposed K_v4 channel gating models.[1,2,9-13] Considering the roles that K_v4 channels are recognized to play in regulating several diverse functions mediated by both the nervous and cardiovascular systems, determining mechanisms underlying K_v4 gating transitions has clear scientific and clinical implications.[1-3]

In a recent issue of Channels we presented results[14] on the K_v4.3 partial N-terminal deletion mutant Δ2-39 (which removes amino acids 2–39 of the proximal N-terminal domain). In contrast to predictions of popular K_v4 gating models,[2,9-12] our Δ2-39 analysis[14] yielded several unexpected results, among which the following two were possibly the most surprising: i) partial N-terminal deletion not only removed the prominent rapid component of macroscopic inactivation, but also slowed the net rate of recovery from inactivation; and ii) coexpression of KChiP 2b (K Channel interacting Protein 2b)[1,3,15] while having no major effect on the rate of Δ2-39 macroscopic inactivation, produced significant acceleration of the rate of Δ2-39 recovery. The first result (which is generally consistent with previous K_v4

studies[2,9-12]) indicates that deletion of the $K_v4.3$ proximal N-terminal, while mimicking the slowing effect of KChIP 2b on macroscopic inactivation,[15-17] does not mimic the acceleratory effects of KChIP 2b on recovery. The second result (which is in contrast to previous $K_v4.2$ studies[2,10,11]) indicates that an intact N-terminal proximal domain is not obligatory for KChIP2 isoforms to exert acceleratory effects upon K_v4 channel recovery. In the absence of evidence for KChiP2 isoforms activating a metabolic and/or second messenger cascade, the most straightforward interpretation of the second result[14] would be the existence of additional KChiP 2b regulatory domains located outside of the proximal N-terminus, e.g. possibly in the T1 (tetramerization) domain[18,19] and/or the C-terminus.[20-22]

Our laboratory previously identified a novel KChIP2 isoform designated KChIP 2d.[23] KChIP 2d is a "structurally minimal" isoform consisting of only the last 70 amino acids of the common C-terminal domain of larger KChIP2 isoforms.[1,3,15] It thus lacks regions reported in crystallographic studies to be required for KChIP interactions with both $K_v4.3$ N-terminal "site 1" and T1 "site 2".[18,19] KChIP 2d nonetheless modulates $K_v4.3$ gating transitions, and in particular accelerates the kinetics of recovery from macroscopic inactivation.[16,23] When our KChIP 2d results were published,[16,23] mechanisms underlying its effects were unclear. In light of our subsequent Δ2-39 + KChIP 2b results,[14] our prior KChIP 2d studies[16,23] were possibly the first demonstration of KChIP2 interaction sites existing outside of the K_v4 α subunit proximal N-terminal domain.

Based upon these prior studies,[14-17,23] we thus wondered: Is KChiP 2d also capable of accelerating Δ2-39 recovery? To address this question, we employed the same techniques used in our previous analysis of Δ2-39 + KChIP 2b (co-expression of Δ2-39 α subunits and KChiP 2d β subunits in Xenopus oocytes [cRNAs co-injected in 1:1 ratios]; two microelectrode voltage clamp analysis recordings conducted within 24 hours of cRNA injection]).[14] Employing a conventional double pulse recovery protocol (for details see **Fig. 1** caption), the mean (n = 7) macroscopic recovery waveform (HP = -100 mV) for Δ2-39 + KChiP 2d is illustrated in **Figure 1** (main panel), and overlaid with the mean Δ2-39 (basal conditions) and Δ2-39 + KChIP 2b recovery waveforms (obtained using the identical protocol) reported in our prior analysis.[14]

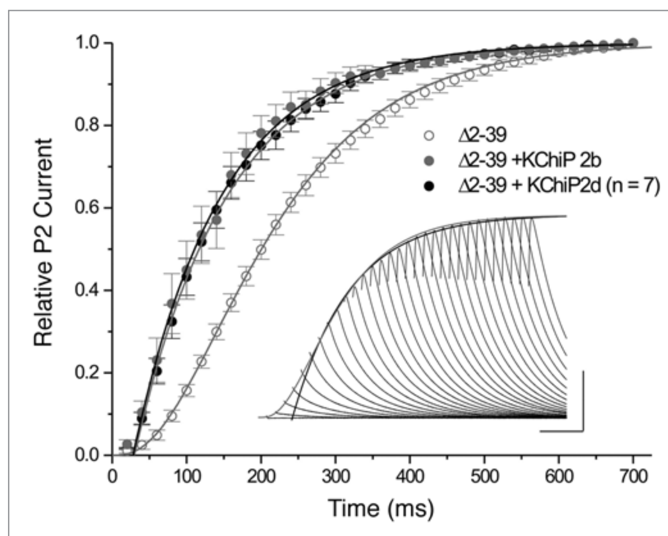

Figure 1. Effects of KChiP 2d[23] on $K_v4.3$ Δ2-39 macroscopic recovery kinetics at HP = -100 mV. Recording conditions and fitting as per our prior Δ2-39 + KChIP2 analysis[14] (Xenopus oocytes; cRNAs injected in 1:1 ratio; 2 microelectrode voltage clamp; current recordings [22°C] conducted within 24 hours of cRNA injection; external recording solution [in mM]: 2 KCl, 96 NaCl, 1 $MgSO_4$, 1.8 $CaCl_2$, 5 HEPES, pH = 7.40). Recovery (HP = -100 mV) was measured using a P1-P2 double pulse recovery protocol, with a 1 sec P1 pulse applied to +50 mV, followed by variable interpulse intervals (Δt) back to HP = -100 mV (Δt durations from 20 to 700 msec, progressively increased in 20 msec increments), and subsequent application of an identical P2 pulse. Main panel: Solid black circles, mean (n = 7) Δ2-39 + KChIP 2d recovery waveform; gray circles, mean recovery waveforms from our prior analysis[14] of Δ2-39 (basal conditions, hollow gray circles) and Δ2-39 + KChiP 2b (solid gray circles). The solid black curve is a constrained exponential fit (beginning at Δt = 60 ms) to the mean Δ2-39 + KChIP 2d macroscopic recovery data points with mean τrec = 120 msec (see text and ref. 14). The two gray lines are fits to the mean Δ2-39 (sigmoidal ["a3"] fit, τrec = 127 msec) and Δ2-39 + KChiP 2b (exponential fit constrained to begin at Δt = 60 msec, τrec = 126 msec) data points from our prior analysis.[14] Inset, representative Δ2-39 + KChIP2d P2 recovery current waveform. Solid black curve is a constrained exponential fit (begun at Δt = 80 msec for the oocyte illustrated) with τrec = 131 msec, while the gray curve is an empirical sigmoidal ("a3") fit with τrec = 105 msec (ref. 14). Calibration bars: 1 μA, 100 msec.

Compared to control basal conditions, co-expression of KChIP2d accelerated Δ2-39 macroscopic recovery, with a mean net rate very similar to that produced by the "full-length" isoform KChiP 2b.[14] As per our prior Δ2-39 + KChiP 2b analysis,[14] an initial delay (sigmoidicity) in the recovering Δ2-39 + KChiP 2d current waveforms was observed (**Fig. 1**, inset). Beginning at an interpulse interval of Δt = 60 msec (so as to "bypass" this initial complication of sigmoidicity[14]), the constrained exponential best fit to the mean Δ2-39 + KChIP 2d data points resulted in an estimated recovery time constant (τrec = 120 msec) that was very similar to that of Δ2-39 + KChiP 2b (τrec = 126 msec[14]). However, for both KChiP 2b and 2d[14] such constrained exponential fits, while well describing later recovery points, failed to adequately describe the earliest recovery amplitudes.

Our prior[14] and present results thus argue that KChiP2-mediated acceleration of K_v4 macroscopic recovery cannot be attributed solely to sequestration of proximal N-terminal domain mediated effects. We nonetheless wish to emphasize that we are not questioning results of prior crystallographic studies demonstrating the existence of two KChIP binding sites in the $K_v4.3$ N-terminal domain (proximal "site 1" and T1 "site 2").[18,19] Rather, our results functionally indicate that both KChiP2b and 2d do not exert pronounced effects on Δ2-39 macroscopic inactivation kinetics, but nonetheless still accelerate recovery. We thus propose that KChiP2-mediated modulation of native

K_v4 channel macroscopic inactivation and recovery involves at least two mechanisms: i) Sequestration of a putative rapid N-type like inactivation process due to the proximal N-terminal domain[14] (consistent with general proposals of crystallographic studies[18,19]); and ii) Acceleration of recovery by further interactions with α subunit domains located outside of the proximal N-terminus (possibilities not addressed but acknowledged in crystallographic studies[18,19]).

In summary, our prior Δ2-39 + KChiP2b results[14] indicate that KChiP2b-mediated acceleration of recovery can occur in the absence of an intact N-terminus. Our present Δ2-39 + KChIP2d results suggest that the last 70 amino acids of the common KChiP2 C-terminal[15,16,23] may contain a separate domain(s) responsible for acceleration of recovery. If this interpretation is correct, both K_v4 channel β subunits and "full length" KChiP2 isoforms would each contain at least two distinct domains for regulating the rate of development of macroscopic inactivation and its subsequent recovery. Our results thus raise several new functional questions (which will be more quantitatively addressed in future studies), and further indicate that popular K_v4 channel gating models[2,9-12] may be in need of reevaluation. Sequestration of the K_v4 channel putative N-type inactivation process by KChIP2 isoforms thus appears to be only one part of a more complex modulatory scheme.

Acknowledgements

This work was supported by grant MH08678901 from the National Institutes of Mental Health (Donald L. Campbell).

References

1. Patel SP, Campbell DL. Transient outward potassium current, "Ito", phenotypes in the mammalian left ventricle: underlying molecular, cellular and biophysical mechanisms. J Physiol 2005; 569:7-39.
2. Covarrubias M, Bhattacharji A, De Santiago-Castillo JA, Dougherty K, Kaulin YA, Na-Phuket TR, Wang G. The neuronal K_v4 channel complex. Neurochem Res 2008; 33:1558-67.
3. Niwa N, Nerbonne JM. Molecular determinants of cardiac transient outward potassium current (Ito) expression and regulation. J Molec Cell Cardiol 2010; 48:12-25.
4. Yellen G. The moving parts of voltage-gated ion channels. Quart Rev Biophys 1998; 31: 239-295.
5. Aldrich RW. Fifty years of inactivation. Nature 2001; 411:643-4.
6. Hille B. Gating: Voltage sensing and inactivation. In: Hille, B, Ed. Ion Channels of Excitable Membranes, 3rd Ed. Sunderland, MA: Sinauer Associates, Inc. 2001; 603-34. .
7. Cordelo-Morales JF, Jogini V, Lewis A, Vazquez V, Cortes DM, Roux B, Perozo E. Molecular driving forces determining potassium channel slow inactivation. Nature Struct Mol Biol 2007; 14:1062-9.
8. Cuello LG, Jogini V, Cortes DM, Perozo E. Structural mechanism of C-type inactivation in K+ channels. Nature 2010; 466:203-9.
9. Bahring R, Boland LM, Vargese A, Gebauer M, Pongs O. Kinetic analysis of open- and closed-state inactivation transitions in human $K_v4.2$ A-type potassium channels. J Physiol 2001; 535:65-81.
10. Bahring R. Dannenberg J, Peters HC, Leicher T, Pongs O, Isbrandt D. Conserved K_v4 N-terminal domain critical for effects of K_v channel – interacting protein 2.2 on channel expression and gating. J Biol Chem 2001; 276:23888-94.
11. Barghaan J, Tozakidou M, Ehmke H, Bahring R. Role of N-terminal domain and accessory subunits in controlling deactivation-inactivation coupling of $K_v4.2$ channels. Biophys J 2008; 94:1276-94.
12. Kaulin YA, De Santiago-Castillo JA, Rocha CA, Covarrubias M. Mechanism of the modulation of $K_v4.3$-KChIP-1 channels by external K^+. Biophys J 2008; 94:1241-51.
13. Wang S, Bondarenko VE, Qu Y-J. Bett GCL, Morales MJ, Rasmusson RL, Strauss HC. Time- and voltage-dependent components of $K_v4.3$ inactivation. Biophys J 2005; 89:3026-41.
14. Hovind LJ, Skerritt MR, Campbell DL. $K_v4.3$ N-Terminal deletion mutant Δ2-39: Effects on inactivation and recovery characteristics in both the absence and presence of KChIP2b. Channels 2011; 5:43-55.
15. Patel SP, Campbell DL, Morales MJ, Strauss HC. Heterogeneous expression of KChIP2 isoforms in the ferret heart. J Physiol 2002; 539:649-56.
16. Patel SP, Parai R, Parai R, Campbell DL. Regulation of $K_v4.3$ voltage-dependent gating kinetics by KChIP2 isoforms. J Physiol 2004; 557:19-41.
17. Amadi CC, Brust RD, Skerritt MR, Campbell DL. Regulation of $K_v4.3$ closed state inactivation and recovery by extracellular potassium and intracellular KChIP2b. Channels 2007; 1:305-314.
18. Pioletti M, Findeisen F, Hura GL, Minor DL. Three-dimensional structure of the KChiP1 - $K_v4.3$ T1 complex reveals a cross-shaped octamer. Nature Struc Mol Biol 2006; 13:987-95.
19. Wang HY, Yan Q, Liu Y, Huang Y, Shen L, Chen Y, Chen Q, Yang Q, Hao K, Wang K, Chai J. Structural basis for modulation of K_v4 K1 channels by auxiliary KChIP subunits. Nature Neurosci 2007; 10:32-9.
20. Kim LA, Furst J, Gutiererz D, Butler MH, Xu S, Goldstein SAN, Grigorieff N. Three dimensional dimensional structure of Ito: $K_v4.2$-KChIP2 ion channels by electronmicroscopy at 21Å resolution. Neuron 2004; 41:513-9.
21. Callsen B, Isbrandt D, Sauter K, Hartmann LS, Pongs O, Bahring R. Contribution of N– and C-terminal $K_v4.2$ channel domains to KChIP interaction. J Physiol 2005; 568:397-412.
22. Han W, Nattel S, Noguchi T, Shrier A. C-terminal domain of $K_v4.2$ and associated KChIP2 interactions regulate functional expression and gating of $K_v4.2$. J Biol Chem 2006; 281: 27134-27144.
23. Patel SP, DL Campbell, Strauss HC. Elucidating KChIP effects on $K_v4.3$ inactivation and recovery kinetics with a minimal KChIP2 isoform. J Physiol 2002; 545:5-11.