Domain III S4 in Closed-State Fast Inactivation: Insights from a Periodic Paralysis Mutation

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Abstract

Heterologous expression of sodium channel mutations in hypokalemic periodic paralysis reveals two variants on channel dysfunction. Charge-reducing mutations of voltage sensing S4 arginine residues alter channel gating as typically studied with expression in mammalian cells. These mutations also produce leak currents through the voltage sensor module, as typically studied with expression in *Xenopus* oocytes. DIIIS4 mutations at R3 in the skeletal muscle sodium channel produce gating defects and omega current consistent with the phenotype of reduced excitability. Here, we confirm DIIIS4 R3C gating defects in the oocyte expression system for fast inactivation and its recovery. We provide novel data for the effects of the cysteine mutation on voltage sensor movement, to further our understanding of sodium channel defects in hypokalemic periodic paralysis. Gating charge movement and its remobilization are selectively altered by the mutation at hyperpolarized membrane potential, as expected with reduced serum potassium.

Introduction

Hypokalemic periodic paralysis is a skeletal muscle disorder characterized by flaccid weakness of limb muscles triggered by low serum potassium, and typically caused by mutation at arginine residues of transmembrane segment four (S4) in voltage-gated sodium or calcium channels (for a review see refs).[1,2] In sodium channels, charge-reducing mutations of the outer S4 arginines R1 or R2 in domains I to III produce an inwardly rectifying proton or cation current observed with hyperpolarization of membrane potential as experienced with a drop in serum potassium. [3-6] Present models of hypokalemic periodic paralysis incorporate this leak or "omega" current as a contributing factor in the pathogenesis of the disorder (for a review see refs). [6,7]

Voltage sensor mutations at R3 that produce a depolarization-activated omega current were first identified for normokalemic periodic paralysis mutations in domain II of the sodium channel $Na_V1.4$ [8] and domain IV of the calcium channel $Ca_V1.1$. [9] Recently, an R3 histidine mutation in domain IIIS4 of $Na_V1.4$ in hypokalemic periodic

paralysis was described. [10] We characterized this (R1135H) and a novel cysteine mutation (R1135C) using heterologous expression in mammalian cells to study gating properties, and in *Xenopus* oocytes to study omega currents. [11] Here, we extend our analysis of the DIIIS4 R3C mutation to include investigation of charge movement and immobilization of the gating charge. We compared the effects of the R3C mutation on gating properties and gating currents with a focus on closed-state fast inactivation. Cysteine substitution at R3 in the domain III voltage sensor enhances closed-state fast inactivation over a voltage range for which this mutation increases gating current. Interestingly, effects of mutation at R3 on recovery do not parallel those on remobilization of the gating charge across the voltage range tested. These findings suggest an important role for this voltage sensor in fast inactivation complementing that of the domain IV voltage sensor. [12]

Results

The DIIIS4 R3 hypokalemic periodic mutations R1135C/H decrease muscle fiber excitability with enhanced entry of channels into fast inactivation, and impaired exit from that state. [11] We compared the effect of the rR1128C mutation (R3C; rat $Na_V1.4$ homologue to human R1135C) on fast inactivation and charge movement to explore the biophysical mechanisms underlying effects of that mutation on excitability. First, we confirmed that R3C, constructed in the rat $Na_V1.4$ isoform and expressed in oocytes, produces similar effects on fast inactivation as reported for R1135C in mammalian cells. Then, we examined the effect of the mutation on charge movement and its immobilization by blocking ionic current with tetrodotoxin (TTX) to isolate gating current. Our primary focus was to provide a direct examination of the effect of the cysteine mutation on voltage sensor movement during entry into and exit from fast inactivation.

The DIIIS4 R3C mutation produced a significant hyperpolarizing shift of the steady-state availability curve and accelerated the entry of channels into fast inactivation from the closed state (Fig. 1A, B), whereas inactivation from the open state was unaffected (Table 1). After block of the ionic current with TTX, we compared the extent and kinetics of loss of gating charge during closed-state fast inactivation for wild type and mutant channels. Charge decrement was increased, and its kinetics accelerated, by the hypokalemic periodic paralysis R3C mutation compared to wild type channels, consistent with its effect on closed-state fast inactivation (Fig. 1C, D).

We compared the charge/voltage (Q/V) relationship in wild type and mutant channels by measuring the integrals of gating currents in response to step commands from -110 mV to 60 mV (Fig. 2). In comparison to wild type $rNa_V1.4$, R3C significantly increased gating charge movement over a voltage range of -85 mV to -45 mV (Fig. 2A). At voltages promoting activation (threshold ~ -45 mV in oocytes, data not shown), gating charge integrals were similar for wild type and R3C channels. We suspect that this effect of the cysteine mutation at R3 in DIIIS4 to selectively increase gating charge at sub-threshold voltages is causal to its effect to enhance closed-state fast inactivation. Consistent with this hypothesis are findings from molecular dynamics simulations for $rNa_V1.4$ and rR1128C channels *in silico*, predicting that a limited translocation of the voltage sensor brings the third arginine in DIIIS4 through the gating pore constriction. [11] While our results show that increased charge movement for R3C at sub-threshold voltages is correlated with enhanced closed-state inactivation, it remains to be discerned what mechanisms couple that movement to the inactivation process.

During recovery of skeletal muscle sodium channels, some portion of the activated gating charge is slow to return to a hyperpolarized favored position, a phenomenon termed charge immobilization. [13] We previously showed that defective deactivation in rR1128H/C inhibits the initial phase of recovery from fast inactivation. [11] We wished to determine if the hypokalemic periodic paralysis R3C mutation also limits remobilization of the gating charge to prolong recovery from channels inactivated from closed, or from open states. To do this we compared recovery of channels to the kinetics of charge remobilization.

As observed in mammalian cells for R1135C, recovery of channels from open-state fast inactivation (0 mV) was impaired in rR1128C mutant channels compared to wild type $rNa_V1.4$ (Table 1). In a separate set of experiments, channels were inactivated by depolarization to threshold (-40 mV) for which most channels inactivate through closed state transitions. R3C prolonged recovery after closed-state inactivation to an extent

similar to that for channels that open prior to fast inactivating (Fig. 3A, B). Thus, recovery from fast inactivation *per se* is impaired by the hypokalemic periodic paralysis R3C mutation.

Then, we blocked channels with TTX and ran identical recovery protocols to isolate the fast and slow components of charge remobilization. Following either route to fast inactivation, R3C accelerated the slow component of charge remobilization at voltages of -90 mV or more depolarized, compared to wild type rNa$_V$1.4 (Table 1, Fig. 3C, 3D). However, the voltage dependence of charge remobilization was reduced in rR1128C, such that charge remobilization was slower in mutant channels compared to wild type rNa$_V$1.4 at more hyperpolarized voltages (Fig. 3D). Thus, in addition to mechanisms such as omega current and defective deactivation that prolong recovery of hypokalemic periodic paralysis channels from fast inactivation, a prolonged return of the gating charge in these channels may contribute to a reduced excitability at more negative membrane potential.

Discussion

Our results extend the findings from our study of the DIIIS4 hypokalemic periodic paralysis mutations in muscle fibers and in heterologous expression. [11] In that study, we found that action potentials in R1135H patient muscle fibers were slow to develop and reduced in amplitude. Expression of that mutation and a novel R1135C mutation in mammalian cells and in oocytes revealed gating defects that include enhancement of fast and slow inactivation, prolonged recovery, depolarization-induced outward omega current and impaired deactivation. The present findings extend the known defects associated with mutation of Na$_V$1.4 at DIIIS4 R3 and further explain the hypoexcitability of muscle fibers promoted by hyperpolarization of membrane potential as experienced during bouts of hypokalemia.

Common gating defects caused by sodium channel hypokalemic periodic paralysis mutations include an enhanced entry into both fast and slow inactivation, and prolonged recovery from either state (for review see ref). [2] However, these and other gating defects do not completely explain the phenotype of reduced membrane excitability in response to a drop in serum potassium. The majority of hypokalemic periodic paralysis mutations discovered to date in sodium or calcium channels involve reduction of charge in the voltage sensing S4 segments. These mutations promote an aberrant leak current through the narrow constriction of the gating pore of the voltage sensor module. Sodium channel mutations in hypokalemic periodic paralysis at R1 or R2 produce a robust inward cationic leak current over the hyperpolarized voltage range. This "omega" current is thought to promote the transition between two stable membrane potentials P1 and P2 during hypokalemia, and thus elicits paralysis of muscle fibers (for review see refs). [6,7]

In contrast, sodium channel normo- and hypokalemic mutations at R3 promote omega current over the depolarized voltage range (domain II, R675Q/G/W; [8] domain III, R1135H/C). [11] For R3 mutations at either of these loci, the outwardly directed omega current may inhibit action potential initiation. Also, deactivation is impaired by R3 mutations in domain II (from slow inactivation) or in domain III (from fast inactivation). For mutations of R3 in DIIIS4, defective deactivation contributes to a prolonged recovery. We hypothesized that since this voltage sensor is immobilized during sodium channel fast inactivation, [13] mutations such as R3C/H that prolong recovery might limit the remobilization of the gating charge. Our finding that the R3C mutation causes this effect at hyperpolarized voltages is consistent with the association of the mutation with hypokalemic attack and membrane hyperpolarization. However, since the mutation prolongs recovery over a broader voltage range, we speculate that impaired deactivation prior to recovery is the more general effect of the mutation.

We also observed that charge movement in R3C is selectively enhanced at sub-threshold voltages compared to wild type sodium channels. This effect of R3C may enhance entry into fast inactivation by promoting closed-state transitions over the voltage range for which R3C decreases the steady state availability of channels. The artificial mutation R1135E may produce a similar effect to decrease the slope of the Q/V curve, increase charge movement at hyperpolarized voltages, and prolong recovery (Groome, unpublished). Studies on skeletal muscle sodium channels indicate that DIIIS4 is the first voltage sensor to move to promote activation of the channel.

[14,15] The present findings show that R3 in DIIIS4 is an important determinant of closed-state transitions, and may limit the movement of the voltage sensor during the earliest steps of the activation process. Gating defects caused by mutation at this locus in DIIIS4 are comparable to those found in certain cardiac sodium channel DIVS4 mutations; namely that reduction of charge in the voltage sensor is associated with an enhanced entry of closed channels into a fast-inactivated state (for review see ref). [16] Closed-state transitions may thus be an important determinant of the effect of mutations of S4 arginine residues of domains III and IV of the sodium channel.

Methods

Methods for molecular biology and the cut-open oocyte technique used for these experiments are detailed elsewhere. [11] Briefly, the hypokalemic periodic paralysis mutation rR1128C was constructed in rNa$_V$1.4 / pGEMHE, generously provided by Dr. Steven Cannon, University of Texas SW Medical Center, Dallas, TX. Messenger RNA for wild type and mutant channels were co-injected with β$_1$ subunit mRNA into *Xenopus* oocytes, and cultured for 3-7 days prior to recordings. Two modifications of the cut-open recording technique were employed. First, the external bath solution contained 20 mM Na$^+$ to reduce current amplitude and permit accurate voltage control during recordings of ionic current. Second, we used leak subtraction to measure gating currents after addition of 2 uM TTX to the top chamber. Gating charge Q was measured from the integrals of anodic charge as Ig$_{ON}$. Charge decrement during closed-state fast inactivation was determined from a single exponential fit to the plot of Ig$_{ON}$ at time t normalized to that at time zero. The slow component of charge remobilization was determined from a double exponential fit to the normalized remobilization curve of Ig$_{ON}$ test / Ig$_{ON}$ control, where Ig$_{ON}$ test is anodic charge at time t and Ig$_{ON}$ control is anodic charge during the initial peak.

Acknowledgements

References

1. Cannon SC. Pathomechanisms in channelopathies of skeletal muscle and brain. Ann Rev Neurol 2006; 29:387-415.

2. Jurkat-Rott K, Holzherr B, Fauler M, Lehmann-Horn F. Sodium channelopathies of skeletal muscle result from gain or loss of function. Pflugers Arch Eur J Physiol 2010; 460:239-248.

3. Sokolov S, Scheuer T, Catterall WA. Gating pore current in an inherited ion channelopathy. Nature 2007; 446:76-78.

4. Struyk AF, Markin VS, Francis D, Cannon SC. Gating pore currents in DIIS4 mutations of Na$_V$1.4 associated with periodic paralysis: saturation of ion flux and implications for disease pathogenesis. J Gen Physiol 2008; 132:447-464.

5. Francis DG, Yabalchenko V, Struyk A, Cannon SC. Leaky sodium channels from voltage sensor mutations in periodic paralysis, but not myotonia. Neurology 2011; 76:1-7.

6. Jurkt-Rott K, Groome J, Lehmann-Horn F. Pathophysiological role of omega pore current in channelopathies. Front Pharmacol 2012; 3:1-15.

7. Cannon SC. Voltage sensor mutations in channelopathies of skeletal muscle. J Physiol 2010; 588:1887-1895.

8. Sokolov S, Scheuer T, Catterall WA. Depolarization-activated gating pore current conducted by mutant sodium channels in potassium-sensitive normokalemic periodic paralysis. Proc Natl Acad Sci USA 2008; 105:19980-19985.

9. Fan C, Lehmann-Horn F, Weber M-A, Bednarz M, Groome JR, Jonsson MKB, Jurkat-Rott K. Transient compartment-like syndrome and normokalemic periodic paralysis due to a $Ca_V1.1$ mutation. Brain 2013; 136:3775-3786.

10. Sung CC, Cheng CJ, Lo YF, Lin MS, Yang SS, Hsu YC, Lin S-H. Genotype and phenotype analysis of patients with sporadic periodic paralysis. Amer J Med Sci 2012; 343:281-285.

11. Groome JR, Lehmann-Horn F, Fan C, Wolf M, Winston V, Merlini L, Jurkat-Rott K. $Na_V1.4$ mutations cause hypokalemic periodic paralysis by disrupting IIIS4 movement during recovery. Brain 2014; 137:998-1008.

12. Capes DL, Goldschen-Ohm MP, Arcisio-Miranda M, Bezanilla F, Chanda B. Domain IV voltage-sensor movement is both sufficient and rate limiting for fast inactivation in sodium channels. J Gen Physiol 2013; 142:101-112.

13. Cha A, Ruben PC, George AL Jr, Fujimoto E, Bezanilla F. Voltage sensors in domains III and IV, but not I and II, are immobilized by Na+ channel fast inactivation. Neuron 1999; 22:126-133.

14. Chanda B, Bezanilla F. Tracking voltage-dependent conformational changes in skeletal muscle sodium channel during activation. J Gen Physiol 2002; 120:629-645.

15. Gosselin-Badaroudine P, Delemotte L, Moreau A, Klein ML, Chahine M. Gating pore currents and the resting state of $Na_V1.4$ voltage sensor domains. Proc Natl Acad Sci USA 2012; 109:19250-19255.

16. Jones DK, Ruben PC. Biophysical defects in voltage-gated sodium channels associated with long QT and Brugada syndromes. Channels 2008; 2:70-80.

Figure Legends

Figure 1. Closed-state fast inactivation in $rNa_V1.4$ and R3C channels. A. Steady state availability: channels were conditioned for 300 ms at voltages from -120 mV to 25 mV, prior to test at -20 mV. Traces show test responses following conditioning at -120 mV (solid line) or -60 mV (dotted line), with voltage dependence below. B. Kinetics of sodium current inactivation; channels were conditioned at voltages from -85 mV to -30 mV for variable duration, prior to test at -20 mV. Traces show responses for selected sweeps up to 25 ms conditioning at -60 mV. Plots of voltage dependence are shown below, with arrows representing fast inactivation ranges of recovery, closed-state entry, and open-state entry for wild type $rNa_V1.4$. Calibration for A, B: vertical 2 μA; horizontal 10 ms. C, D. Charge movement from experiments as in (B) after block of ionic current with 2 μM TTX. Normalized charge (Q/Q_{MAX}) after 300 ms conditioning is shown in (C), with kinetics of charge decrement shown in (D). Values represent mean ± SEM from 10 to 29 ($rNa_V1.4$) or 11 to 32 (rR1128C) experiments.

Figure 2. Charge movement in wild type $rNa_V1.4$ and rR1128C channels. A. Traces comparing responses at -60 mV and at 0 mV, from a holding potential of -120 mV. Calibration: vertical 100 nA (-60 mV) or 500 nA (0 mV); horizontal 10 ms. B. Voltage dependence of charge movement (Q/V relation) in response to 20 ms depolarization, from -110 mV to 60 mV. Values represent mean ± SEM from 34 ($rNa_V1.4$) or 18 (rR1128C) experiments.

Figure 3. Recovery and charge remobilization in wild type $rNa_V1.4$ and R3C channels, following closed-state fast inactivation with depolarization to -40 mV. A. Traces of recovery of ionic current with -90 mV hyperpolarizing commands and test at -20 mV, with voltage-dependence shown at right. B. Traces and voltage dependence of the slow component of charge remobilization in experiments after TTX block, using 0 mV test. Calibration for traces: vertical 1-2 μA (A), 400 nA (B); horizontal 10 ms. Values represent mean ± SEM from 16 to 21 ($rNa_V1.4$) or 8 to 10 (rR1128C) experiments.

Table 1. Gating parameters for wild type and mutant skeletal muscle channels. SS FI; steady-state fast inactivation. OS FI; open-state fast inactivation. CS FI; closed-state fast inactivation.

Parameter	$rNa_V1.4$	n	rR1128C	n
Sodium Currents				
SS FI midpoint	-59.8 ± 0.9 mV	29	-75.0 ± 1.2 mV ***	32
SS FI slope factor	4.57 ± 0.17	29	3.96 ± 0.10 **	32
OS FI tau, 0 mV	1.24 ± 0.05 ms	13	1.23 ± 0.04 ms	9
CS FI tau, -60 mV	38.0 ± 1.8 ms	22	18.2 ± 1.9 ms ***	11
OS FI recovery				
tau, -90 mV	7.63 ± 0.49 ms	18	19.9 ± 2.18 ms ***	8
CS recovery				
tau, -90 mV	8.12 ± 0.45 ms	16	20.4 ± 0.78 ms ***	10
Gating Currents				
Q/V midpoint	-35.3 ± 0.8 mV	34	-36.2 ± 2.2 mV	18
Q/V slope factor	1.84 ± 0.05	34	1.51 ± 0.08 **	18
CS Q immobilization				
tau, -60 mV	28.5 ± 2.34 ms	10	15.1 ± 2.81 ms **	12
OS Q remobilization				
tau (slow), -90 mV	13.5 ± 0.63 ms	8	8.33 ± 0.97 ms**	7
CS Q remobilization				
tau (slow), -90 mV	13.7 ± 0.35 ms	21	10.8 ± 0.63 ms**	8

* p ≤ .05   ** p ≤ .005   *** p ≤ .0001

A

rNa$_V$1.4, 20 mM Na+

rR1128C, 20 mM Na+

- ◆ rNa$_V$1.4
- ○ rR1128C

I$_{Na}$ Recovery (ms)

Voltage (mV)

B

rNa$_V$1.4, 2 μM TTX

rR1128C, 2 μM TTX

- ◆ rNa$_V$1.4
- ○ rR1128C

Q Remobilization (ms)

Voltage (mV)