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ORIGINAL ARTICLE

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A global defect in scaling relationship between electrical activity and availability of muscle sodium channels in hyperkalemic periodic paralysis

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Abstract Hyperkalemic periodic paralysis (HyperPP) is a hereditary disorder characterized by alternate episodic attacks of muscle weakness and muscle myotonia. The most common mutation associated with HyperPP is a T704M substitution in the skeletal-muscle sodium channel. This mutation increases sodium persistent currents, alters voltage dependence of activation and impairs slow inactivation. The present study shows experimental evidence in support of a potentially important global defect caused by the T704M mutation. While the effective rate of recovery from slow inactivation, in both normal and mutated channels, is related to the duration of past activity by a power law function, the scaling power of the mutated channel is significantly greater. This difference between the channels offers a clue for an explanation to the wide range of time scales, history dependence, and the mixed myotonic/paralysis effect, which mark the clinical picture of HyperPP.

Key words Electrophysiology · Excitability · Hyperkalemic periodic paralysis · Inactivation · Power-law-scaling · Sodium channel

Introduction

The most common mutation associated with hyperkalemic periodic paralysis (HyperPP) [2] is a substitution of threonine for methionine in position 704 of the skeletal muscle voltage-gated sodium channel (SkM1) gene [10]. In a series of elegant works, researchers were able to show that in the SkM1 channel, the point mutation T704M (or its rat homologue rT698M) increases sodium persistent currents [3], alters the voltage dependence of activation [5,14] and impairs slow inactivation [4,7]. These changes eventually lead to a failure to activate the muscle, and hence paralysis. This biophysical picture marks one of the finest examples of moving up the scales from a well-defined molecular defect to its macroscopic clinical manifestations. However, several critical questions are left unanswered. While the time scales of the modified rates in the mutant channel are at the milliseconds [3] and several seconds [4,7] range, time scales of attacks of muscle paralysis or myotonia seem to be related in a complex way to past activity and spread over a wide range, up to hours [2, 11,12]. What is the mechanism that bridges this gap of time scales between the molecular defect and the clinical picture? What might be the mechanism that relates past activity to the time scale of a particular attack? Furthermore, what is the nature of the physiological process that sometimes ignites a long-lasting paralytic attack, whereas at other times causes the inverse phenomenon, namely a myotonic attack? A study of the inactivation of mammalian brain voltage-gated sodium channels [13], which are structurally similar to skeletal sodium channels, offers a clue for a more complete answer to these questions. Toib et al. [13] show that the multiplicity of slow inactivation states in brain sodium channels is expressed as an intrinsic "memory" function in the form of a scaling relationship between the duration of previous activity and the effective rate of recovery from slow inactivation. This scaling relationship persists over at least six decades of time scale, from tens of milliseconds to many minutes. A memory process, which is based on scaling of *effective* rates, is fundamentally different from a memory process, which is based on time-dependent redistribution of channels between *few* available and inactive states [9]. The former allows for the kind of diffusive time scale phenomenon that is characteristic of HyperPP; the latter does not. Taken together with reports about defective slow inactivation processes in T698M channels, the absence of a characteristic time scale for recovery from inactivation in the brain sodium channels prompted us to hypothesize that the T698M mutation modifies the

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entire scaling mechanism of the effective recovery rate from inactivation. The present work shows evidence that supports this hypothesis.

Materials and methods

Expression system and electrophysiological recordings

The plasmids pJB20-SkM1 and pRBG4-T698M were kindly provided by Dr. T. Cummins. The plasmid pRC/CMV (Invitrogen) was used for co-transfection with pRBG4-T698M. HEK293 cells were grown in DMEM medium supplemented with 10% fetal calf serum (FCS), 4 mM L-glutamine and penicillin plus streptomycin. Cells were stably transfected by the calcium phosphate precipitation or electroporation techniques. After 48 h, G-418 (Gibco) was added to select for cells expressing neomycin resistance. Two to three weeks later, colonies were picked and tested for channel expression by whole-cell recording techniques. Whole-cell recordings were conducted at room temperature using Axopatch 200 (Axon Instruments). Data were collected using a Quadra 800 (Apple computers) with PULSE software (HEKA Electronic), low-pass filtered at 5–10 kHz and sampled at 20 kHz. P/n leak subtraction routines of the PULSE software were used. Electrodes for voltage-clamp experiments were made from fire-polished aluminium silicate glass, with a resistance of 5–6 MΩ. The pipette solution contained (in mM): 140 KCl, 10 Na_2ATP, 10 EGTA, 5 HEPES, 1 $CaCl_2$ and 1 $MgCl_2$ (pH 7.3). The bath solution contained (in mM): 140 NaCl, 3 KCl, 10 HEPES, 10 glucose, 2 $MgCl_2$ and 2 $CaCl_2$ (pH 7.3 and 300–310 mosmol/l).

Pulse protocols and analysis of slow inactivation

In order to characterize the kinetics of entry into the slow inactivation state(s), a conditioning pulse from –100 mV to –10 mV was applied to the membrane for various durations (0.3, 1, 3, 10, 30, 100 and 300 s). Each conditioning pulse was followed by a recovery interval at –100 mV for 150 ms, to allow a complete recovery of the channels from rapid inactivation. A test pulse to –10 mV was applied immediately after the recovery interval. The fraction of channels in the slow-inactivated state was calculated from the ratio between the peak current response to the test pulse, and the peak current response to a test pulse that was applied a few seconds prior to the conditioning pulse. In order to monitor the process of recovery from slow inactivation, the conditioning pulse and the test pulse that are described above were followed by a series of short test pulses delivered every 1–2 s. The peak current amplitude of each nth test pulse (I_n) was observed on-line, and the pulsing was stopped when I_n approached a maximum value (I_{max}). The fraction of inactivation F at the time of the nth test pulse was normalized to: $F=1-(I_n-I_1)/(I_{max}-I_1)$, where I_1 is the peak amplitude of the current evoked by the first test pulse. The time constant τ was extracted by fitting an exponential function to F. For estimation of the scaling relationship, the values of the time constant (τ) for recovery from slow inactivation as a function of the duration (t) of the conditioning pulse were plotted on a log-log plot. These data were fitted to a power-law function, $\tau(t)=pt^D$, where p is a constant kinetic set-point, and D is a positive scaling power. Non-linear fitting was carried out using KaleidaGraph (Synergy Software).

Results

In addition to the rapid (millisecond) inactivation, most sodium ion channels exhibit a slow inactivation process that acts at the seconds time scale [9]. SkM1 channels show a similar phenomenon. Figure 1A demonstrates the slow inactivation in SkM1 channels that can be revealed

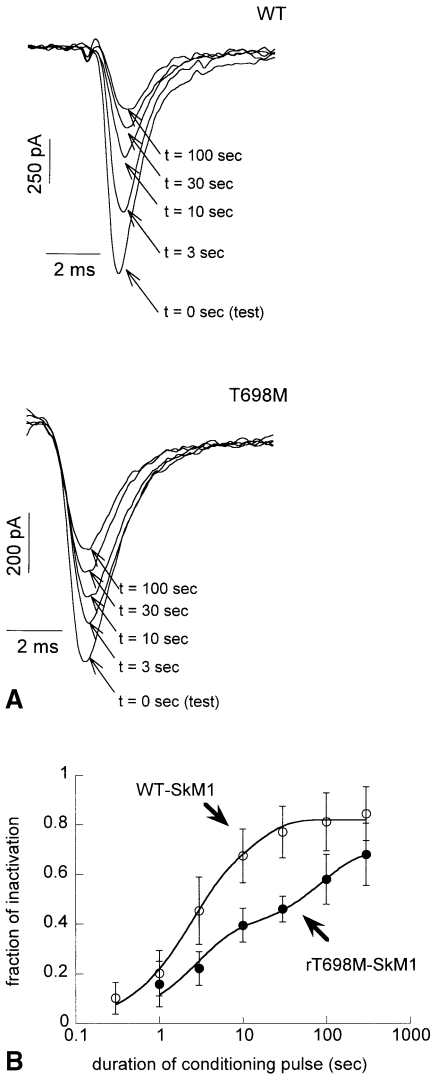

Fig. 1A, B Slow inactivation of voltage-gated skeletal muscle sodium channels. **A** Availability of WT-μI (*top*) and T698M-μI (*bottom*) channels, following conditioning depolarizing pulses at the seconds time scale. A conditioning pulse is given from a holding potential of –100 mV to –10 mV for a duration t. Following the conditioning pulse, a hyperpolarizing pulse to –100 mV for 150 ms is applied in order to allow full recovery from the rapid inactivation process. The availability of the channels for activation is then tested by applying a short depolarizing pulse to –10 mV. The resulting current traces are shown. The duration t of the conditioning pulses is depicted accordingly. The current trace depicted as t=0 is a representative test pulse applied immediately before the conditioning pulses. **B** Kinetics of entry into the slow inactivation state. The fraction of the channels in the slow inactivated state is calculated from the ratio between the peak current response to the test pulse applied after the conditioning pulse, and the peak current response to the test pulse applied before the conditioning pulse (Fig. 1A). It is shown against the duration of the conditioning pulse on a semi-log plot. (*Open circle* WT-μI channel, *filled circle* T698M-μI channel.) Data are means ±SD for each channel; n=9. *Solid lines* are double exponential fit to the data. WT-μI: τ_1=2.08 s, fraction =0.51, τ_2=12.0 s, fraction =0.31, R>0.99; T698M-μI: τ_1=2.75 s, fraction =0.36, τ_2=90.3 s, fraction =0.33, R>0.99

when the channels are activated for long periods, typically at the seconds time scale. Inactivation is seen as a reduction in the peak of the sodium current that is elicited by a depolarizing pulse from –100 mV to –10 mV, delivered 150 ms after a *t*-seconds-long depolarizing pulse. Cummins and Sigworth [4] reported that the slow inactivation of the mutant rSkM1(T698M) channel is impaired compared to the normal channel. In agreement with their results, as well as with Hayward and colleagues [7] the results of Fig. 1B show that slow inactivation is significantly slower to occur in the mutant channels compared to the normal channels. The slower time course for entry into inactivation in the mutant channel, compared with the normal channel, can be the result of a retarded rate of entry to inactivation, an expedited rate of recovery from inactivation, or both. Cummins and Sigworth [4] and Hayward et al. [7] show evidence for uniquely defined expedited rates of recovery in the mutant channel at a time scale of seconds. The main novel observation of the present study is in the experiments described below. We show that when tested over a wider range of time scales (sub-seconds to minutes), a comparison between the normal and the mutant channel uncovers a *paradoxical* behaviour: while at the several seconds (typically <10 s) range the mutant channel recovers from inactivation *faster* than the normal channel, at longer time scales the mutant channel recovers *slower* than the normal channel.

The recovery time courses after activation pulses of various lengths for both normal and mutant channels are plotted in Fig. 2. Note that the slopes of the straight lines (on linear-log plot) are equivalent to the time constants for recovery from inactivation, and that these *slopes are changing* in accordance with the duration of previous activation. This change in recovery time constant as a function of the duration of previous activation is a manifestation of the *scaling of effective recovery rate*, a property that is similar to what was observed in the brain sodium channels by Toib et al. [13]. Importantly, the relationship between past activity and the time constant for recovery from slow inactivation of the normal and mutant channels differ markedly. The mutant channels seem exceedingly more sensitive to previous activity because their recovery time constant changes more rapidly as a function of previous activity. This difference is clearly seen when the average time constants for both types of channels are plotted against the duration of previous activity in Fig. 3. This figure shows that the dependence of the time constant for recovery from slow inactivation on the duration of previous activation is steeper for the mutant channel compared to the normal channel. Thus, the mutant channel is more sensitive to previous activity over at least four decades of time scales.

Note that back extrapolation of the power-law scaling of Fig. 3 predicts a significant difference in recovery time constants at the milliseconds time scale.

A comparison between the rates of recovery from inactivation of the normal rat homologue rSkM1(698T) and the HyperPP rSkM1(T698M), at the milliseconds time scale, is presented in Fig. 4A,B. The result shows

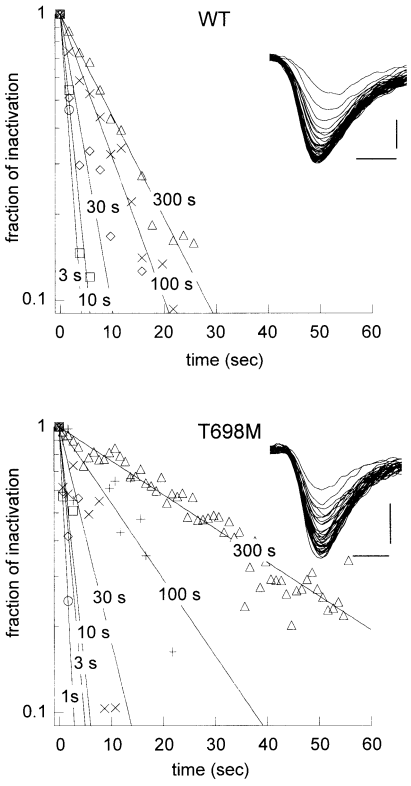

Fig. 2 Kinetics of recovery from the slow inactivation state. An example of the time course of recovery from slow inactivation induced by 3-, 10-, 30-, 100-, and 300-s-long conditioning pulses for the WT channel (*top*) and 1-, 3-, 10-, 30-, 100-, and 300-s-long conditioning pulses for the T698M channel (*bottom*). The fraction of the inactivated channels in each pulse is normalized as described in Materials and methods. *Solid lines* are single exponential fit to the data. *Insets*: representative families of current traces showing the recovery from a 300-s-long conditioning pulse, seen as a gradual increase in peak current responses to test pulses from –100 mV to –10 mV, delivered at a frequency of 1 Hz for both channels. *Inset bars*: 1 ms, 200 pA

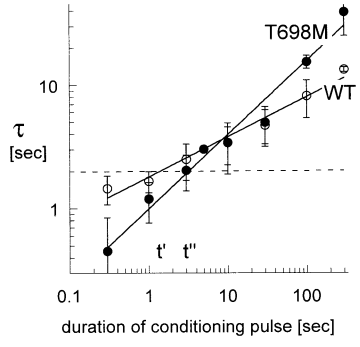

Fig. 3 Scaling relationship between the duration of the conditioning pulse and the recovery rate from slow inactivation in the sodium channels. Mean values of time constants for recovery $-\tau \pm$SD, are shown as a function of the conditioning pulse duration $-t$, on a log-log plot. Time constants were extracted from the time course of recovery as described in Material and methods. *Solid lines* are power-law functions of the form $\tau(t)=pt^D$, that were fitted to the mean values from nine cells for each channel. WT: p=1.8, D=0.32, R=0.99; T698M: p=1.0, D=0.60, R=0.99. *Broken line* is an imaginary fixed rate of recovery from inactivation of potassium channels. The intersection points between sodium and potassium curves are depicted as t' and t'' for normal and mutant channels respectively

that the difference, at this time scale, is very small, reflecting the fact that rapid and slow inactivation are two mechanistically independent processes [6].

Discussion

This study shows that rSkM1(T698M), the rat sodium channel mutant homologue that underlies HyperPP, has a modified scaling relationship between activity and recovery from inactivation over a wide range of experimentally available time scales. To illustrate the implication of this difference in sensitivity to previous activity, we have plotted an *imaginary* fixed rate of recovery from inactivation of potassium channels in the plot of Fig. 3 (broken line; see [8] for an example of a few seconds time-scale cumulative inactivation in skeletal muscle voltage-gated potassium channels). The intersection points between sodium and potassium curves are depicted as t' and t'' for normal and mutant channels respectively. During ongoing activity that lasts up to the point of intersection, each activation is followed by a net increase in excitability (recovery of the "restoring" potassium conductance from inactivation is slower than recovery of the "exciting" sodium conductance). Beyond this point, each activation results in suppression of activity (recovery of the "exciting" sodium conductance from inactivation is slower than recovery of "restoring" potassium conductance). Note the effect of the sodium recovery slope on the time of the intersection point. This picture demonstrates the wealth of phenomena that can be caused by a change in the scaling power of muscles that contain the mutated channels. Note that a train of action potentials with a comparable effect to a particular (continuous) conditioning pulse would be much longer in duration [13], and would shift t' and t'' to longer times than few seconds.

Much has been said and written about the origin of scaling relationship in the gating of ion channels (e.g. [1, 9, 13]). Most researchers and theoreticians believe that the scaling phenomenon results from a multiplicity of hidden states of the channel protein, which translates to an observed *effective* rate that changes as a function of past activity. From a physiological point of view, the scaling of effective rate of recovery from inactivation acts in the skeletal muscle sodium channels as an intrinsic memory function. This memory function enables the channels to "remember" the duration of previous activity in the form of modulated reaction rates, and to influence the excitability level of the muscle accordingly. In the T698M mutation, this memory is modified and thus the entire relationship between past activity and present status of excitability becomes biased. Furthermore, the modified scaling re-

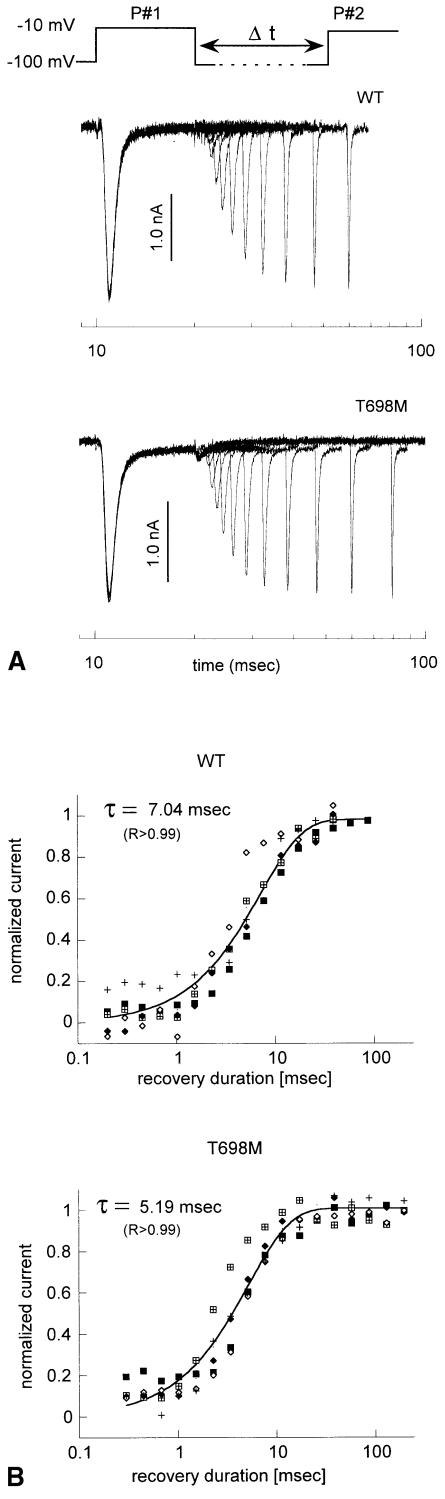

Fig. 4A, B Rapid inactivation of voltage-gated skeletal muscle sodium channels. **A** Representative families of current traces (on a semi-log plot), from HEK293 cells transfected with wild-type μI (depicted *WT, top*), and T698M-μI (depicted *T698M, bottom*) channels. The currents are elicited using a double-pulse protocol. After an initial pulse to –10 mV, from a holding potential of –100 mV for 10 ms (*P#1*), the membrane is held at a recovery voltage of –100 mV for variable time durations (0.2–100 ms). A second pulse to –10 mV is applied (*P#2*) to measure the fraction of the channels that recovered. **B** Kinetics of recovery from the rapid inactivation state. The maximum sodium current elicited in the second pulse (from the double-pulse experiment) is normalized by dividing it by the current elicited in the first pulse, and it is shown against the recovery duration on a semi-log plot. The time constant τ, for recovery from inactivation is extracted by fitting an exponential function to the average values from five cells (*continuous line*) for each channel. (WT-μI, τ=7.04 ms; T698M-μI, τ=5.19 ms; *R*>0.99)

lationship of the mutated channels changes the delicate balance between "exciting" and "restoring" forces in the muscle cell membrane, in a manner that provides a possible explanation for the paradoxical mixed paralysis-myotonia clinical symptoms of HyperPP disorder. At shorter conditioning stimulation, the mutated channels contribute to hyperactivity since they tend to be more available for further activation. At longer conditioning stimulation, the mutant channels contribute to underactivity, because they tend to be unavailable for further activation.

In summary, we have identified a global defect in the scaling relationship between the electrical activity and availability of muscle sodium channels in HyperPP. The mutant channel is more sensitive to previous activity, compared to the normal muscle sodium channel, over at least four decades of time scales. This observation explains the gap in time scales between the uniquely defined microscopically defective inactivation reactions, and the clinical picture during attacks. Furthermore, this modified scaling between activity and recovery rate offers an explanation to what seems to be a paradoxical effect of the T698M mutation – namely, the mixed paralysis-myotonia clinical picture that characterizes HyperPP patients.

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References

1. Bassingthwaighte JB, Liebovitch LS, West BJ (1994) Fractal physiology. Oxford University Press, New York
2. Cannon SC (1996) Sodium channel defects in myotonia and periodic paralysis. [Review]. Annu Rev Neurosci 19:141–164
3. Cannon SC, Strittmatter SM (1993) Functional expression of sodium channel mutations identified in families with periodic paralysis. Neuron 10:317–326
4. Cummins TR, Sigworth FJ (1996) Impaired slow inactivation in mutant sodium channels [see comments]. Biophys J 71:227–236
5. Cummins TR, Zhou J, Sigworth FJ, Ukomadu C, Stephan M, Ptacek LJ, Agnew WS (1993) Functional consequences of a Na^+ channel mutation causing hyperkalemic periodic paralysis. Neuron 10:667–678
6. Featherstone DE, Richmond JE, Ruben PC (1996) Interaction between fast and slow inactivation in Skm1 sodium channels. Biophys J 71:3098–3109
7. Hayward LJ, Brown RHJ, Cannon SC (1997) Slow inactivation differs among mutant Na channels associated with myotonia and periodic paralysis. Biophys J 72:1204–1219
8. Kalman K, Nguyen A, Tseng-Crank J, Dukes ID, Chandy G, Hustad CM, Copeland NG, Jenkins NA, Mohrenweiser H, Brandriff B, Cahalan M, Gutman GA, Chandy KG (1998) Genomic organization, chromosomal localization, tissue distribution, and biophysical characterization of a novel mammalian Shaker-related voltage-gated potassium channel, Kv1.7. J Biol Chem 273:5851–5857
9. Marom S (1998) Slow changes in the availability of voltage-gated ion channels: effects on the dynamics of excitable membranes. [Review]. J Membr Biol 161:105–113
10. Ptacek LJ, George ALJ, Griggs RC, Tawil R, Kallen RG, Barchi RL, Robertson M, Leppert MF (1991) Identification of a mutation in the gene causing hyperkalemic periodic paralysis. Cell 67:1021–1027
11. Rudel R, Lehmann-Horn F (1985) Membrane changes in cells from myotonia patients. [Review]. Physiol Rev 65:310–356
12. Ruff RL (1994) Slow Na^+ channel inactivation must be disrupted to evoke prolonged depolarization-induced paralysis. Biophys J 66:542–545
13. Toib A, Lyakhov V, Marom S (1998) Interaction between duration of activity and time course of recovery from slow inactivation in mammalian brain Na^+ channels. J Neurosci 18:1893–1903
14. Yang N, Ji S, Zhou M, Ptacek LJ, Barchi RL, Horn R, George-AL J (1994) Sodium channel mutations in paramyotonia congenita exhibit similar biophysical phenotypes in vitro. Proc Natl Acad Sci USA 91:12785–12789