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Different effects of mexiletine on two mutant sodium channels causing paramyotonia congenita and hyperkalemic periodic paralysis

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Abstract

Effects of the antiarrhythmic and antimuscarinic drug mexiletine were studied on two sodium channel mutants causing paramyotonia congenita (R1448H) and an overlap paramyotonic and hyperkalemic paralytic syndrome (M1360V). Channels were expressed in human embryonic kidney cells and studied electrophysiologically, using the whole-cell patch-clamp technique. Compared to the wild-type, both mutants showed alterations of inactivation, i.e. slower inactivation, left shift of steady-state inactivation and faster recovery from inactivation. Mexiletine caused a significantly larger use-dependent block of the R1448H mutant when compared to M1360V and wild-type channels. This can be explained by a prolonged recovery from mexiletine block as observed for R1448H channels, since the affinity of mexiletine for the inactivated state was similar for all three clones. The use-dependent block of sodium channels by mexiletine reduces repetitive series of action potentials and therefore improves muscle stiffness in myotonic patients. The enhanced use-dependent block as seen with R1448H may explain the extraordinary therapeutic efficacy of mexiletine in most patients with paramyotonia congenita. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

To date about 20 different naturally occurring mutations have been found in the human skeletal muscle sodium channel α -subunit which may cause either of three neurological syndromes, hyperkalemic periodic paralysis (HyperPP), paramyotonia congenita (PC) and potassium-aggravated myotonia (PAM) [1]. Hallmarks of PC are paradoxical myotonia and cold-induced muscle weakness. Paradoxical myotonia is muscle stiffness increasing with continued exercise, which is in contrast to the ‘warm-up’ phenomenon observed with the chloride channel myotonias Thomsen and Becker. In contrast, temperature sensitivity is not a typical sign of HyperPP and PAM [1,2]. HyperPP is characterised by episodic weakness sometimes accompanied by myotonia. Weakness usually appears at rest after heavy

exercise or is precipitated by depolarising agents such as potassium. Potassium-aggravated myotonia is characterised by muscle stiffness induced or aggravated by a potassium load occurring typically without weakness [2].

Normal excitability of muscle fibres requires a high resting potential and short-lasting action potentials. These requirements are not fulfilled in PC and HyperPP as shown with microelectrode recordings on excised muscle fibres from patients. Voltage-clamp studies on single fibres have revealed that inactivation of sodium currents is incomplete [3]. A defective inactivation with increased sodium influx results in depolarisation of muscle fibres. The degree of depolarisation determines the clinical phenotype; a slight depolarisation causes membrane hyperexcitability and muscle stiffness, whereas a substantial and sustained depolarisation induces membrane inexcitability and muscle paralysis. Patch-clamp studies on mutant sodium channel causing PC, PAM or HyperPP expressed in human cell lines not only revealed detailed mechanisms of the pathophysiology of these diseases but also pointed to parts of the protein important for channel function [4–8].

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The standard therapy in myotonia patients is the administration of sodium channel blocking agents of the lidocaine type. Mexiletine is favoured because of less serious side-effects compared to tocainide or lidocaine [9]. Under therapy with mexiletine or tocainide, the muscle stiffness and weakness in patients with PC, as well as myotonia in other myotonic syndromes, tend to disappear completely [10,11]. The therapeutic effect of mexiletine in HyperPP patients is not satisfactory [12]. The severity and duration of symptoms can be reduced by administration of hydrochlorothiazide, acetazolamide or β -mimetic agents.

Lidocaine causes a resting, or tonic block of sodium channels and a phasic block, also called use-dependent block. The blocking mechanism can be explained by a change of the channel affinity for the drug in different kinetic states. The resting block of sodium current by lidocaine-like local anaesthetics is attributed to the affinity of the drug to the resting state of the channel, whereas the additional phasic block occurs due to an increased affinity to the inactivated state [13]. Regions in the sodium channel that can be involved in binding of local anaesthetics are the cytoplasmic end of segment S6 in the domain IV and the intracellular loop between domains III–IV containing the proposed inactivation particle [14,15].

We used the patch-clamp technique to study the effects of mexiletine on two sodium channel mutants causing paramyotonic and/or hyperkalemic paralytic syndromes. Although both mutants show similar inactivation defects, mexiletine induced a larger resting and phasic block in the PC-causing mutant. Our results indicate that an increased mexiletine block of mutant channels reduces the pathologic sodium inward current in a diseased muscle, and causes a relief of symptoms in PC.

2. Methods

The Arginine-1448-Histidine (R1448H) and Methionine-1360-Valine (M1360V) substitutions were created by site-directed mutagenesis using the altered sites system (Promega Corporation, Madison, WI) as described [6,16]. Wild-type (WT) and mutant constructs were assembled in the mammalian expression vector pRC/CMV and transfected into human embryonic kidney cells (HEK293) by the calcium phosphate precipitation method. Since the channel expression after transient transfection was low (<10%) stable cell lines were obtained by antibiotic selection as described [7].

Standard whole cell patch-clamp recordings [17] were performed at 20°C (temperature controller II, Luigs and Neumann, Ratingen, Germany) using an EPC-9 amplifier with 'Pulse' software (HEKA, Lambrecht, Germany). The voltage error due to series resistance was <5 mV. Leakage and capacitative currents were subtracted automatically by a prepulse protocol (-P/4). All data were filtered at 5 kHz and digitised at 20 kHz. Analysis was based on HEKA, Excel

(Microsoft corporation) and Sigma Plot (Jandel Scientific, San Rafael, CA) software. The solutions were as follows (in mM, pH 7.4): pipette: 135 CsCl, 5 NaCl, 2 MgCl₂, 5 EGTA and 10 HEPES; bath: 140 NaCl, 4 KCl, 2 CaCl₂, 1 MgCl₂, 4 dextrose and 5 HEPES. Mexiletine was added to the bathing solution at concentrations of 0.1, 0.5 or 1.0 mM.

The amplitude of sodium currents in non-transfected cells was always below 0.5 nA (0.25 ± 0.05 nA, $n = 8$). A maximum peak current in transfected cells was up to 20 nA. To minimise both serial resistance and contribution of endogenous sodium channels, data were only recorded from cells with currents between 2 and 5 nA (WT: 2.63 ± 0.4 nA; R1448H: 2.75 ± 0.4 nA, M1360V: 2.81 ± 0.6 nA, $n \geq 14$). The mean cell capacitance was 12.9 ± 0.18 pF with no significant difference between cells expressing mutant and wild-type channels.

Student's *t*-test for comparison of two means and the Bonferroni *t*-test for comparison of more than two means were applied for statistic analysis. All data are presented as mean \pm SEM.

3. Results

The S4 segments in all four domains contain positively charged residues, either arginines or lysines, and are proposed to be the voltage sensors of voltage-gated channels [18–20]. For the PC causing R1448H mutant, a positively charged arginine in the S4 segment of domain IV of the skeletal muscle sodium channel α -subunit is substituted by a histidine which is predominantly neutral at pH 7.4 (Fig. 1). The M1360V mutation is located in the S1 segment of domain IV (Fig. 1). Although the exact role of this segment in sodium channel function is not known, there is evidence that this part of the channel is important for inactivation [16].

3.1. Characterization of the mutant channels

3.1.1. Inactivation time course in WT and mutant channels

Fig. 2A shows typical sodium currents of WT, R1448H and M1360V elicited by a depolarising step to 0 mV from a holding potential of -100 mV after a 10 ms prepulse to -120 mV. The inactivation time course of either mutant, in particular that of R1448H, is slower than that of the WT. The current decay was best described by a sum of two exponentials yielding two time constants of inactivation, τ_{h1} and τ_{h2} , with a relative weight of the faster time constant, τ_{h1} , of more than 90 %. τ_{h1} was significantly larger for R1448H > M1360V than for WT channels (Fig. 2B). In addition, for R1448H, τ_{h1} showed an altered voltage dependence (Fig. 2B). τ_{h2} was not significantly different between WT and mutant channels (not shown).

3.1.2. Steady-state inactivation

To characterise steady-state inactivation, which determines the probability of Na⁺ channels being inactivated, a

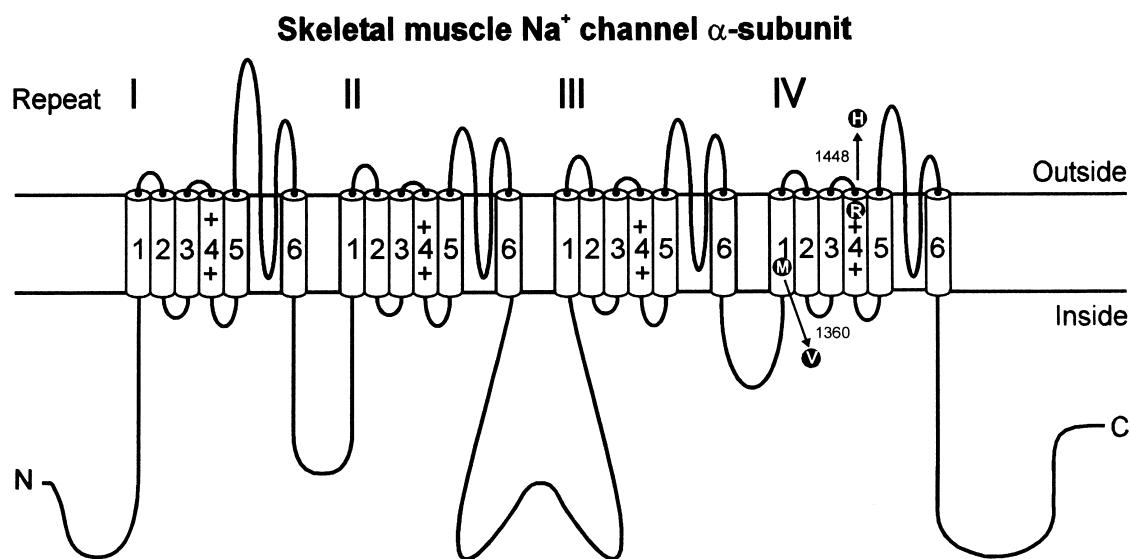


Fig. 1. Model of the human skeletal muscle sodium channel α -subunit containing four domains each having six transmembrane segments. The M1360V mutation causing a hyperkalemic paralytic and paramyotonic syndrome is located in the first segment of the fourth domain (D4/S1). The R1448H mutation causing paramyotonia congenita is located in the fourth segment of the same domain (D4/S4).

variable 20-ms prepulse ranging from -150 to -5 mV in 5-mV steps was applied prior to a test pulse to 0 mV. The steady-state inactivation curve was fit by a standard Boltzmann equation ($I/I_{max} = (1 + \exp[q(V - V_{0.5})/k])^{-1}$). The midpoint of the inactivation curve ($V_{0.5}$) was shifted for both mutants to the left (Fig. 2C, Table 1). The shift was more pronounced and the slope of the curve more markedly reduced for R1448H than for M1360V (Table 1). These changes have two important implications with regard to the weakness in PC: (i) at the resting potential, the number of Na^+ channels available for an action potential is markedly reduced (at -80 mV only 70% of mutant channels versus 95% of WT) and (ii) at less negative potentials, the increased overlap of the activation and inactivation curves results in a larger permanent ‘window’ current. The first

point directly reduces excitability, the second furthers membrane depolarisation, i.e. also reduces excitability.

3.1.3. Steady-state activation

Steady-state activation was determined by using a series of depolarizations in 5 mV steps, starting with -50 mV from a holding potential of -100 mV. Boltzmann fits of the data revealed a -6 mV shift of the steady-state activation curve for R1448H ($V_{0.5}$ in mV: WT: -15.0 ± 2.0 versus R1448H: -21.1 ± 4.3 versus M1360V: -14.8 ± 1.8 ; $n \geq 4$). This shift might represent a real alteration of the activation process, but it could also just reflect the influence of slowed inactivation on our usual procedure for determining the activation curve: all currents are normalised to the largest peak current which was delayed in R1448H.

Table 1
Midpoint ($V_{0.5}$) and slope of the steady-state inactivation curve in control solution and mexiletine (0.1, 0.5, 1.0 mM)

	WT	<i>n</i>	R1448H	<i>n</i>	M1360V	<i>n</i>
<i>Control solution</i>						
$V_{0.5}$ (mV)	-51.9 ± 1.6	6	-68.2 ± 3.4^a	4	-58.2 ± 2.4^a	4
Slope	2.8 ± 0.1		1.9 ± 0.1^a		2.8 ± 0.1	
<i>Mexiletine 0.1 mM</i>						
$V_{0.5}$ (mV)	-55.8 ± 1.6	3	-71.2 ± 2.3^a	3	-60.3 ± 0.6^a	4
Slope	2.0 ± 0.2		1.7 ± 0.1		2.4 ± 0.1	
<i>Mexiletine 0.5 mM</i>						
$V_{0.5}$ (mV)	-57.4 ± 1.3	3	-74.0 ± 2.3^a	3	-65.8 ± 1.3^a	3
Slope	1.9 ± 0.1		1.3 ± 0.1^a		2.2 ± 0.2	
<i>Mexiletine 1.0 mM</i>						
$V_{0.5}$ (mV)	-71.4 ± 1.9	4	-83.0 ± 4.1^a	4	-76.5 ± 1.8	3
Slope	1.5 ± 0.1		1.3 ± 0.1		1.7 ± 0.1	

^a Data was significantly different for mutant channels when compared to WT (mean \pm SEM, $P < 0.01$).

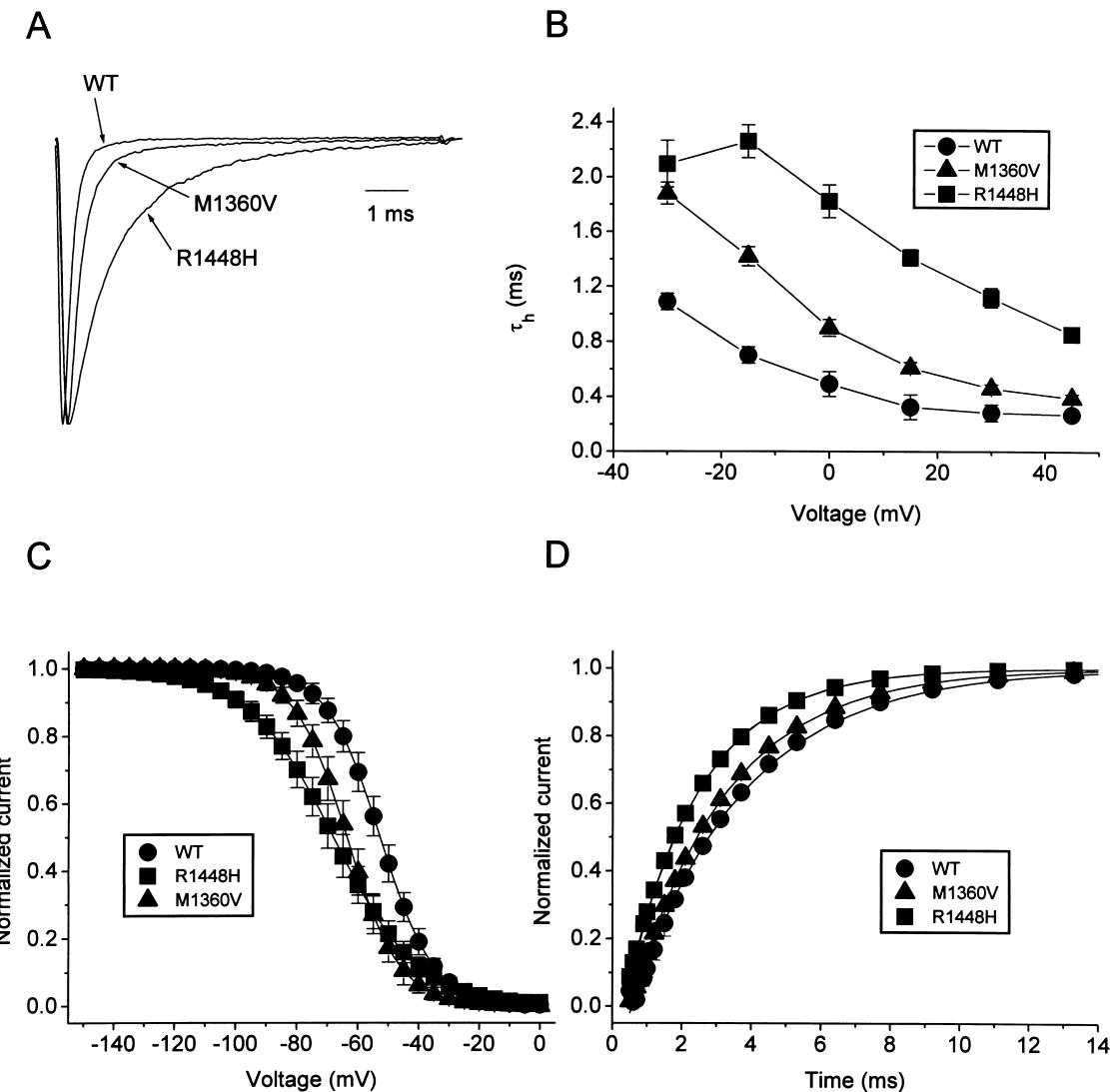


Fig. 2. Inactivation and recovery from inactivation. (A) Normalised sodium currents through WT, M1360V and R1448H channels were elicited by depolarisation from -100 to 0 mV for 10 ms. Current amplitudes were 2.33, 3.01 and 3.71 nA for WT, R1448H and M1360V channels respectively. Both mutations inactivate more slowly than the WT channel. (B) Fast time constant of inactivation τ_h plotted versus test potential for all three clones ($n = 3-6$). (C) Steady-state inactivation curves for WT, R1448H and M1360V channels. Lines represent fits of standard Boltzmann functions with parameters given in Table 1. (D) Recovery from inactivation was measured at -100 mV. Na^+ channels were inactivated by a 36 ms prepulse to 0 mV from a holding potential of -100 mV, and repolarised to -100 mV recovery potential with increasing duration prior to the test pulse to 0 mV. The time course of recovery from inactivation was best described by a single exponential. Lines represent exponential fits. The values of the recovery time constant τ_r are given in the text and Table 2 ($n = 5-7$).

3.1.4. Recovery from inactivation

Recovery from inactivation determines the refractory period after an action potential and therefore limits the maximal firing rate of nerve and muscle cells. Recovery was determined as follows: in order to inactivate all sodium channels, cells were depolarised for 36 ms to 0 mV, then repolarised with increasing duration to -100 mV prior to the test pulse to 0 mV. The time course of recovery from inactivation was well described with a single exponential yielding the time constant τ_r . τ_r was faster for both mutants than for the WT (Fig. 2D, Table 2, τ_r at -100 mV in ms: WT: 3.06 ± 0.13 versus R1448H: 2.13 ± 0.18 versus

M1360V: 2.72 ± 0.09 ms; for R1448H versus WT, $P < 0.01$; $n = 5-7$, for R1448H versus M1360V $P < 0.05$). The faster recovery from inactivation promotes the development of myotonia by shortening the refractory period after a muscle action potential.

3.2. Effects of mexiletine

3.2.1. Mexiletine block

Mexiletine did not significantly affect the time course of inactivation and recovery from inactivation, and steady-state activation for either of the investigated channels

Table 2

Recovery from inactivation and recovery from mexiletine block for WT and mutant channels

	WT	n	R1448H	n	M1360V	n
<i>Control solution</i>						
τ_r (ms)	3.06 ± 0.13	6	2.13 ± 0.18	7	2.72 ± 0.09	6
<i>Mexiletine 0.1 mM</i>						
τ_r (ms)	2.91 ± 0.16	3	2.19 ± 0.19	4	2.63 ± 0.20	5
τ_{mex} (ms)	246 ± 58		519 ± 24*		351 ± 32	
A (τ_{mex}) %	17.0 ± 1.5		22.0 ± 0.7		28.0 ± 2.2	
<i>Mexiletine 0.5 mM</i>						
τ_{mex} (ms)	395 ± 22	4	470 ± 66	3	461 ± 63	3
A (τ_{mex}) %	51.0 ± 1.5		50.3 ± 4.6		27.7 ± 5.5	

Under mexiletine a second exponential yielding a slow time constant τ_{mex} (ms) was necessary to fit the data. Time constants of recovery from inactivation, τ_r , time constants of recovery from mexiletine block, τ_{mex} , and relative amplitudes of τ_{mex} are shown as mean values ± SEM. At a concentration of 0.1 mM, recovery from mexiletine block was significantly slower for R1448H mutation than for WT and M1360V channels. *P < 0.01.

(data not shown). Sodium channel block by mexiletine could be separated into a resting block and a phasic block [13]. The resting block was determined at -100 mV holding potential, as a reduction of the peak current after administration of mexiletine. The block was significantly larger for R1448H channels ($P < 0.05$) when compared to WT and M1360V (Table 3). The larger block may be caused by an increased affinity of mexiletine for the resting state of the R1448H channel. However, it may also reflect the binding of mexiletine to the inactivated state, since some of R1448H channels at -100 mV are already inactivated, due to the left shift of steady-state inactivation. To discriminate between these two possibilities we measured the resting block at -150 mV holding potential where all channels are non-inactivated. At this potential, block was similar for all three clones (tonic block for 0.1 mM versus 1.0 mM at -150 mV in %: WT: 11.0 ± 2.0 versus 56.5 ± 4.7 R1448H: 10.3 ± 2.5 versus 55.7 ± 5.0; n = 3–5) suggesting that the larger block for R1448H at -100 mV is due to the shift of the steady-state inactivation curve.

To investigate the phasic block, a series of 10 depolarising pulses to 0 mV at different frequencies (from 1 up to 100 Hz) was applied and the reduction of the current amplitude between the 1st and 10th depolarising pulse was observed. Under control conditions, i.e. in the absence of mexiletine, none of the channels showed a significant reduction of the peak amplitude at frequencies up to 50 Hz (Fig. 3B). Mexiletine induced a pronounced, concentration-dependent phasic block in all clones (Fig. 3A,B). At a concentration of 0.1 mM, the block was more pronounced for R1448H channels than for M1360V and WT at -100 mV (Fig. 3B), but also at -150 mV holding potential (data not shown). At higher concentrations (0.5 and 1 mM) mexiletine blocked all channels to a similar extent (Fig. 3B). The increased phasic block for R1448H may be either due to a

higher affinity of mexiletine for the inactivated state of the mutant channel or due to a slower recovery from block. We explored both possibilities.

The affinity of mexiletine to the inactivated state was estimated by determining of steady-state inactivation under different concentrations of mexiletine. Boltzmann parameters of steady-state inactivation for WT and mutant channels are summarised in Table 1. Mexiletine caused a concentration-dependent left shift of the midpoint of the inactivation curve, but the extent of the shift was not different between WT and both mutants (Fig. 4A). The left shift is due to a higher affinity of mexiletine to the inactivated state affecting the equilibrium between closed and inactivated channels. In order to calculate the affinity of mexiletine for the inactivated state, we used the following equation: $V_{1/2} = k^* \ln(1/(1 + [\text{mexiletine}]/k_d)) + V_{1/2c}$ [15,21], where $V_{1/2}$ and $V_{1/2c}$ are the midpoints of steady-state inactivation for mexiletine and control solutions respectively, k is the slope of the steady-state inactivation curve, and k_d is the dissociation constant for mexiletine from the inactivated state. Fits revealed that the k_d for R1448H was not significantly different when compared to WT and M1360V channels (Fig. 4B).

3.2.2. Recovery from mexiletine block

As mentioned above mexiletine did not affect recovery from fast inactivation for any mutant, but it introduced a second exponential with a very slow time constant, τ_{mex} , representing the recovery from block (Fig. 4C, Table 2). The pulse protocol used to study recovery from mexiletine block was the same as for recovery from inactivation (see above or legend to Fig. 2). The equation $I/I_{\text{max}} = 1 - (A * \exp(-t/\tau_r) + (1 - A) * \exp(-t/\tau_{\text{mex}}))$ was fitted to the data and this showed that τ_{mex} was significantly larger for R1448H channels when compared to WT and M1360V in 0.1 mM mexiletine ($P < 0.01$, Table 2). This indicates slower recovery from mexiletine block. Increasing the mexiletine concentration to 0.5 mM this difference was no longer significant (Table 2). Since the affinity of mexiletine for the inactivated state of the WT channel and two mutants was not different (see above), the slower recovery from mexiletine block for R1448H explains the increased phasic block for this mutant. In accordance to the phasic block by mexiletine, a significant difference between R1448H and WT channels was only observed at a concentration of

Table 3
Tonic block for WT and mutant channels, holding potential -100 mV

	WT	n	R1448H	n	M1360V	n
Mexiletine 0.1 mM	18.7 ± 1.8	5	28.5 ± 2.5*	6	11.7 ± 2.8	4
Mexiletine 0.5 mM	22.0 ± 2.9	6	44.0 ± 0.8*	5	16.0 ± 2.5	5
Mexiletine 1.0 mM	66.7 ± 2.2	5	79.3 ± 2.2*	7	66.7 ± 3.6	6

Given are mean values ± SEM. Tonic block was increased for R1448H channels, *P < 0.01.

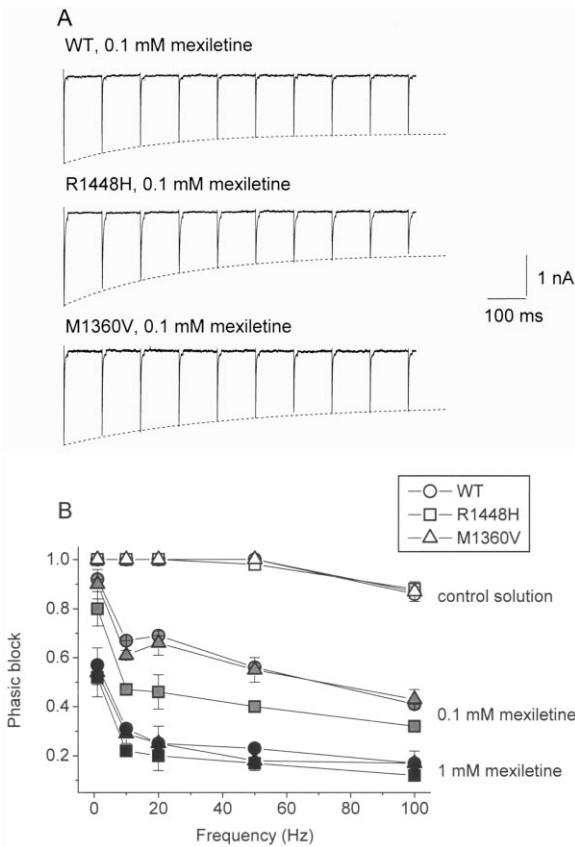


Fig. 3. Phasic block in WT and mutant sodium channels induced by mexiletine. (A) Currents elicited by depolarizations from -100 to 0 mV for 10 ms at 10 Hz frequency; extracellular mexiletine concentration 0.1 mM. Lines represent exponential fits. (B) Mean values of the phasic block at different pulse frequencies in the control solution, 0.1 and 1 mM mexiletine. Phasic block is more pronounced for R1448H channels in 0.1 mM mexiletine.

0.1 mM. At higher concentrations both phasic block and recovery from block were similar for all three clones.

4. Discussion

Mexiletine block was studied on two mutants, R1448H and M1360V, in the α -subunit of the human skeletal muscle sodium channel causing either pure paramyotonia congenita (PC), with paradoxical myotonia and cold-induced muscle weakness, or an overlap syndrome, with hyperkalemic paralytic attacks and cold-induced muscle stiffness and weakness. Interestingly, both mutants showed a similar electrophysiological phenotype: slowing of inactivation, left shift and reduced voltage dependence of steady-state inactivation and faster recovery from inactivation, although more pronounced for R1448H, as shown in previous reports [6,16]. Similar alterations of sodium channel gating were found with other PC mutants [6,22–24]. In contrast, HyperPP-causing mutants show a large increase of the persistent current ($\sim 6\%$ [4]), a left shift of the activation

curve, increasing the permanent ‘window current’ [5], and/or a defect of slow inactivation [25,26].

4.1. Mexiletine block

Although both mutants caused a similar electrophysiological phenotype, the effects of mexiletine were different in various aspects. Mexiletine caused a more pronounced resting block for the R1448H mutant, than for WT and M1360V channels at a physiologically relevant holding potential. Phasic block was also increased for the R1448H mutant in comparison to WT and M1360V, at a concentration of 0.1 mM mexiletine which is close to the therapeutic serum concentration of 0.01 mM [27]. The increased phasic block for R1448H is explained by a slower recovery from mexiletine block, since the affinity of the drug for the inactivated state was not changed by the mutation.

Several models have been proposed to explain the complex interactions between inactivation of sodium channels and the binding of local anaesthetics. Most of them predict the stabilisation of the inactivated state after the drug binding resulting in a shift of the steady-state inactivation curve to the left and a decrease of the rate of recovery from inactivation [15,21]. Our observations are in agreement with this model.

Since the recovery from mexiletine block was slower for R1448H one could expect that the affinity of mexiletine for the inactivated state must be increased. This was not the case in our study but can be explained using the model described in Fan et al. (1996) as follows. The affinity is determined by two rate constants, k_{on} and k_{off} . k_{on} and k_{off} determine the entry and the recovery from the blocked state. In the case that both rate constants decrease to a similar extent, the affinity would not change significantly ($k_d = k_{off}/k_{on}$) and time constant of the recovery from block, which is defined as $1/(k_{on} + k_{off})$ would be prolonged.

The R1448H mutant is located in the voltage sensor D4/S4 of the channel which moves in response to changes of the membrane potential and this movement is disturbed by the neutralisation of positive charges such as occur in an arginine to cysteine mutation [6]. The disturbed inward movement of D4/S4 upon membrane hyperpolarization could slow the unbinding of mexiletine, prolong the recovery from block and therefore increase the phasic block.

Another explanation for the large effect of mexiletine on R1448H channels could be an accumulation of mexiletine within the cell due to a reduction of the membrane surface charge. Local anaesthetics are presumed to block sodium channels in their charged form, i.e. with an ammonium group located in the pore and a lipophilic part in the membrane. Protons are known to be able to reach bound drug molecules from the extracellular side [13]. A substitution of the extracellularly located positively charged R1448 to a predominantly neutral histidine could facilitate the access of protons to neutral mexiletine molecules which

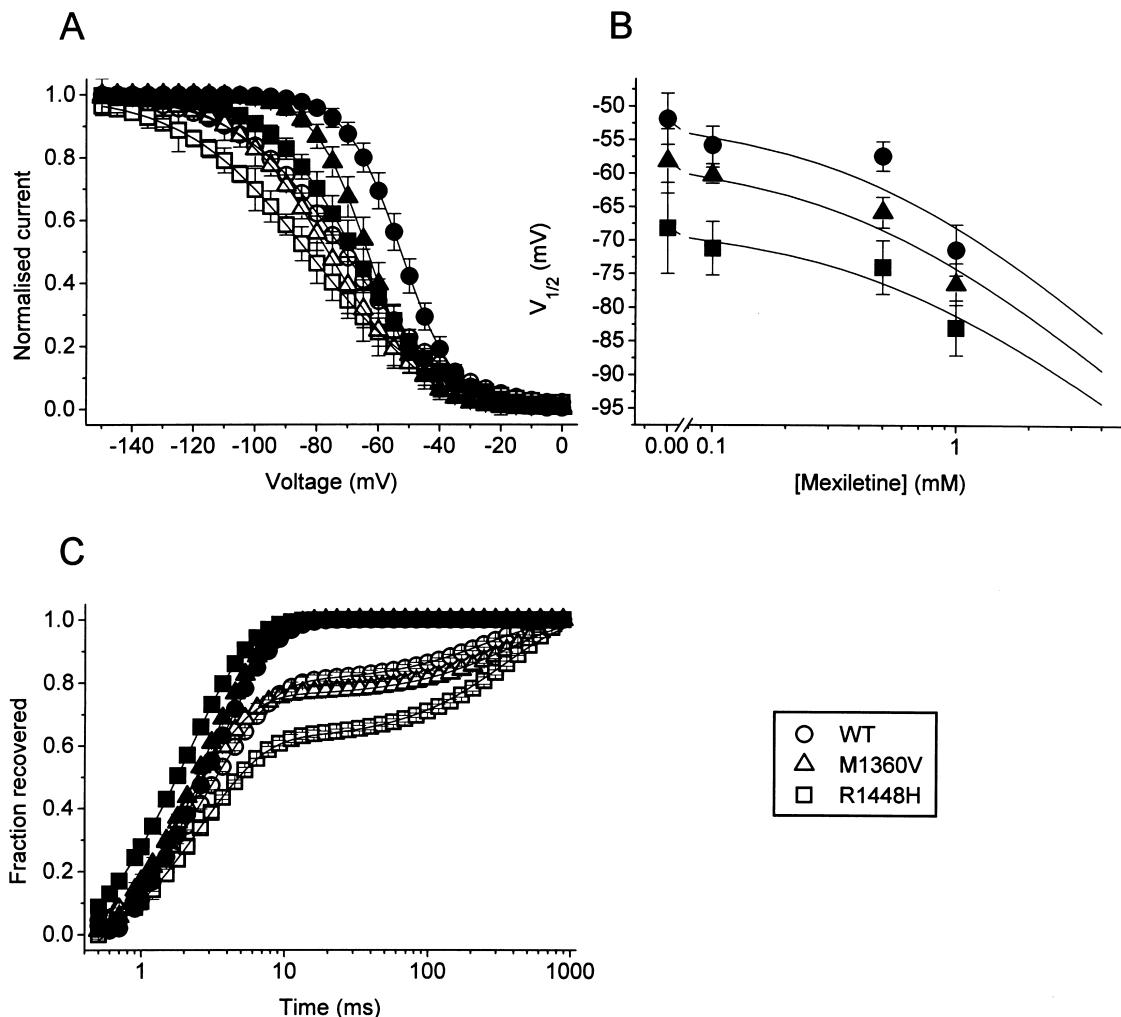


Fig. 4. Steady-state inactivation and recovery from mexiletine block. (A) Steady-state inactivation curves for WT, M1360V and R1448H channels in control solution (closed symbols) and 1 mM mexiletine (open symbols). Lines represent fits of standard Boltzmann functions with parameters given in Table 1. (B) Mexiletine induced a left-shift of the midpoint potential of inactivation ($V_{1/2}$) in wild-type, R1448H and M1360V channels. The relative extent of the shift was similar for all three clones. Symbols are mean values \pm SEM. Lines are fittings by the equation given in the text. k_d was not statistically different for R1448H, WT and M1360V channels. (C) Recovery from inactivation (closed symbols) and recovery from mexiletine block (open symbols) at -100 mV. Mexiletine was continuously present in the bath solution. Pulse protocol was as described in Fig. 2D. The time course of recovery was best described by a sum of two exponentials. Lines represent fits. The values of τ_{rec} and corresponding relative amplitudes are given in the Table 2 ($n = 3-5$).

reach the channel from the cytoplasmic space or through the membrane. This could promote the formation of mexiletine ‘cations’, which are no longer able to escape from the cell and remain trapped in the cell prolonging a blocking effect of mexiletine. Considering that recovery from block is influenced by drug unbinding this would account for the increased value of τ_{rec} and the greater phasic block.

The effects of lidocaine were tested recently on two PC-causing mutants (R1448C, T1313M): for R1448C the phasic block was increased whereas for T1313M it was decreased when compared to the WT channel [15]. T1313M is located in the III–IV interlinker of the sodium channel which is proposed to contain an inactivation particle of the sodium channel blocking the ionic pore from the intracellular side of the membrane. For T1313M Fan and colleagues [15] showed a decreased affinity of mexiletine

for the inactivated state, suggesting an interaction of the inactivation ball and lidocaine molecule. Similarly Bennett and colleagues [28] have shown a decreased affinity of lidocaine for the inactivated state for the IFM–QQQ mutation also located in the III–IV interlinker. In our study, neither the R1448H nor the M1360V mutant affected the affinity of mexiletine for the inactivated state.

4.2. Therapeutic effect of mexiletine

Due to a phasic block of sodium channels, mexiletine is a very effective inhibitor of high frequent trains of action potentials which are the electrophysiological correlate of myotonia. This unspecific effect explains why all forms of myotonia, also the chloride channel diseases myotonia congenita Thomsen and Becker, generally respond well to

treatment with mexiletine [1,29]. Cold-induced muscle weakness in PC patients also responds well to mexiletine whereas the paralytic attacks of HyperPP patients do not [10–12].

The mechanism of weakness in both diseases is probably different. In PC, weakness develops after a period of muscle stiffness in a cold environment. The pathological sodium inward current flows mainly during an action potential, since fast inactivation is slowed but the persistent sodium current is only slightly increased and slow inactivation is normal [1,6,23,24]. This is why weakness develops in the course of prolonged series of action potentials providing enough sodium influx, so that the depolarisation exceeds some point where most sodium channels get inactivated. Such series of action potentials are blocked by mexiletine.

In contrast, weakness and paralysis in HyperPP, only seldom preceded by myotonia, are caused by a large persistent sodium current and impaired slow inactivation [4,25,26]. This induces a substantial and sustained membrane depolarisation which may be reached without repetitive action potentials. The mechanism of mexiletine block is probably not appropriate to reduce the persistent sodium current to a greater degree. Therefore, mexiletine may not be able to prevent paralytic attacks in HyperPP.

Altogether, our results show a pronounced phasic block of mexiletine for R1448H channels thus explaining a very good therapeutic effect of the drug in PC patients carrying this mutation. In agreement with the extent of phasic block in electrophysiological experiments, several of our patients carrying the R1448H or the R1448C mutation respond very well to mexiletine therapy. In contrast, two patients with the T1313M mutation showed a slight or no response to mexiletine, confirming the importance of the phasic block for the clinical efficacy of mexiletine.

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