Myotonic dystrophy kinase modulates skeletal muscle but not cardiac voltage-gated sodium channels

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Abstract Altered modulation of skeletal muscle voltage-gated sodium channels by myotonic dystrophy kinase (DMPK) has been proposed as a possible mechanism underlying myotonia in this disease. We examined the effect of a recombinant mouse DMPK on the functional properties of human skeletal muscle (hSkM1) and cardiac (hH1) voltage-gated sodium channels in the *Xenopus* oocyte expression system. Co-expression of DMPK with hSkM1 in oocytes resulted in significantly lower peak sodium current amplitude as compared to cells expressing hSkM1 alone in agreement with a previous report. By contrast, DMPK had no effect on the level of expressed sodium current in cells expressing hH1. Similarly, there were no measurable effects of the kinase on the kinetics or steady-state properties of activation or inactivation. Our findings support the previous observations made with rat muscle sodium channels and demonstrate that the effect of DMPK on sodium channels is isoform specific despite conservation of a putative phosphorylation site between the two isoforms.

Key words: Myotonic dystrophy; Ion channel; Skeletal muscle; Phosphorylation

1. Introduction

Myotonic dystrophy is the most common form of muscular dystrophy affecting adults [1]. Although the syndrome is named for its muscular manifestations (myotonia, muscular wasting, and weakness), the disease involves many systems including skin, central nervous system, eye, and heart. Transmission is autosomal dominant, and the genetic defect is a trinucleotide (CTG) repeat expansion within the 3′-untranslated region of a putative protein kinase gene located on chromosome 19q13.3 [2–4].

Although the genetic basis of the disease has been solved, less is known about the cellular defect responsible for the pleiotropic disease manifestations. Recently, Mounsey et al. reported an effect of the normal myotonic dystrophy gene product (DMPK) on a recombinant skeletal muscle voltage-gated sodium channel [5]. In co-expression experiments performed in *Xenopus* oocytes, DMPK caused a significant reduction in peak sodium channel current amplitude without affecting the time course of channel activation or inactivation. This effect was prevented by a site specific mutation of a conserved protein kinase C (PKC) phosphorylation site (serine 1321), located within the cytoplasmic linker region between repeat domains 3 and 4 (ID3–4). The investigators concluded that altered levels of the kinase in muscle cells of patients with myotonic dystrophy could cause altered sarcolemmal excitability and myotonia. More recently, DMPK has been shown capable of directly phosphorylating sodium channel peptide sequences in vitro [6]. These observations all point to the potential modulation of voltage-gated sodium channels by DMPK as a potential explanation for certain manifestations of the muscular phenotype.

Overt cardiac disease is unusual in myotonic dystrophy, but a majority of patients exhibit subclinical electrocardiographic or echocardiographic abnormalities, and are at increased risk for sudden cardiac death [7]. Myocardial cells express both DMPK, and a unique isoform of voltage-gated sodium channel α subunit, that is distinguished from brain and skeletal muscle channels by its lower sensitivity to the neurotoxin tetrodotoxin. Molecular cloning of the human cardiac sodium channel [8] has revealed a significant amino acid sequence similarity with the skeletal muscle sodium channel including conservation of the protein kinase C phosphorylation site within the ID3–4 region that is the putative target of DMPK.

In this report, we examined the effect of a recombinant mouse DMPK on functional expression of the human cardiac sodium channel α subunit expressed heterologously in *Xenopus* oocytes. Our initial hypothesis was that, like skeletal muscle sodium channels, DMPK would modulate the functional activity of the cardiac isoform and this might help explain the cardiac phenotype of myotonic dystrophy. However, as we report here, DMPK modulates skeletal muscle but not cardiac sodium channels expressed in *Xenopus* oocytes. Therefore, DMPK modulation of sodium channels differs between muscle and heart isoforms and suggests that factors other than a conserved PKC phosphorylation site in the ID3–4 linker may be required for the effect observed in muscle sodium channels.

2. Materials and methods

2.1. Expression of mouse DMPK

Full-length mouse DMPK cDNA [9] was subcloned into the oocyte expression vector pSP64T [10], and junctional regions were sequenced to verify correct assembly. The resulting plasmid, designated as pSP64T-DMK, was made linear by *Xba*I digestion, and used for in vitro transcription using SP6 RNA polymerase in the presence of the methylated 5′-cap analog m^7GpppG. Transcripts were checked for size, purity, and concentration on denaturing agarose/formaldehyde gels.

2.2. Expression of sodium channels in Xenopus oocytes

In vitro transcriptions of human skeletal muscle (hSkM1) and human cardiac (hH1) sodium channel α subunits, and the human sodium channel β_1 subunit were performed as previously described [11]. *Xenopus* oocytes were procured, defolliculated, and microinjected as previously described [12]. In co-expression experiments, sodium channel α subunit cRNA or an equal part mixture of α and β_1 subunit cRNAs was diluted with an equal volume of DMPK cRNA, or nu-

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clease free water. After injection, oocytes were incubated at 18°C in a dilute solution of Leibovitz's L-15 medium (Gibco, Grand Island, NY) enriched with 15 mM 4-(2-hydroxyethyl)-1-piperazine-methanesulfonic acid (HEPES, adjusted to pH 7.6 with NaOH), 1 mM glutamine, and 50 μg/ml gentamycin. Oocytes were used for experiments 2–3 days after injection.

2.3. Electrophysiology

Whole-cell currents were recorded in oocytes using two microelectrode voltage-clamp as previously described [12]. Electrodes were pulled from Prism glass capillaries (Dagan Corp., Minneapolis, MN) and filled with 3 M KCl. Electrode resistances were 1–3 MΩ for voltage recording electrodes and 0.5–1.5 MΩ for current passing electrodes. Experiments were conducted on oocytes from at least two different frogs. Membrane potential was controlled by a Warner oocyte clamp (Warner Instrument Corp., Hamden, CT). A ground metal shield was inserted between the two electrodes to minimize coupling and speed the clamp rise-time. Voltage commands were generated by computer using pCLAMP version 5.5 software (Axon Instruments). Currents were filtered at 2 kHz (−3 dB; 4 pole Bessel filter).

The holding potential was set to −120 mV and Na^+ currents were recorded during test potentials to −20 mV for hSkM1 and −30 mV for hH1. Test voltages were chosen to be at a membrane potential at which most Na^+ channels will open if available, and are not on the negative limb of the current voltage relationship for either hSkM1 or hH1. Analyses were restricted to cells in which the voltage control was adequate. To assess steady-state channel inactivation, two-pulse protocols were employed. Membrane potential was stepped to a voltage between −120 mV and −20 mV for 500 ms, and then peak Na^+ current was measured during a test potential. The prepulse duration used in these experiments was chosen based upon preliminary experiments demonstrating that 500 ms was sufficient to allow steady-state inactivation to occur in both Na^+ channel isoforms prior to the test pulse.

2.4. Data analysis

All measurements were made with pCLAMP. The steady-state inactivation data were fit with a Boltzmann function,

$$I/I_{max} = \left\{1 + \exp[(V - V_{1/2})/k]\right\}^{-1}$$

to determine the membrane potential for half maximal inactivation ($V_{1/2}$) and the slope factor k. The time course of current decay during a voltage step was assessed by measuring the time to half decay ($T_{1/2}$). Results were presented as mean ± standard error of the mean (mean ± S.E.M.).

3. Results

Fig. 1 illustrates the effect of DMPK on hSkM1 sodium channels expressed in oocytes. Representative current tracings obtained at various test potentials are shown for oocytes injected with hSkM1 alone (Fig. 1A), or in oocytes expressing both hSkM1 and DMPK (Fig. 1B). Currents recorded from both groups of oocytes exhibit similar activation and inactivation characteristics, but differ greatly in their peak current amplitudes. Oocytes expressing hSkM1 and DMPK exhibit approximately 50% lower peak current amplitudes measured at a test potential of −10 mV than the corresponding cells injected with hSkM1 alone (hSkM1 alone: 8.4 ± 1.2 μA, $n = 16$; hSkM1+DMPK: 3.2 ± 0.6 μA, $n = 17$; $p < 0.001$, Fig. 1C). There was no detectable effect of the kinase on the time course of inactivation, or in the voltage dependence of steady-state inactivation (data not shown). Also, there was no difference in the extent of current reduction mediated by DMPK when hSkM1 was co-expressed with the human β_1 subunit (data not shown). Our findings are similar to those reported by Mounsey et al. [5], who examined the effect of a recombinant human DMPK on rat skeletal muscle sodium channels expressed in oocytes, suggesting that the mouse

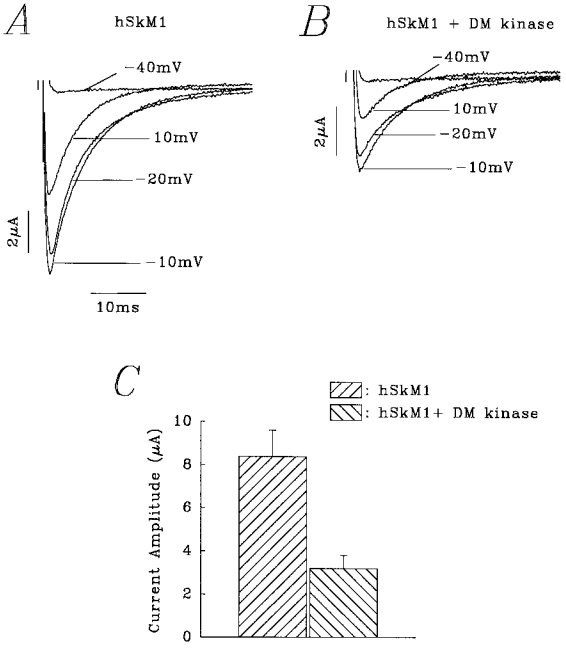

Fig. 1. Effect of mouse DMPK on hSkM1 expressed in oocytes. (A) Representative voltage-clamp recordings made from an oocyte expressing hSkM1 alone. Current tracings obtained at various test potentials from a holding potential of −120 mV are shown. (B) Representative voltage-clamp recordings made from an oocyte expressing hSkM1+DMPK using the same pulse protocol as shown in A. (C) Bar graph comparing peak sodium current measured at a test voltage of −20 mV between oocytes expressing hSkM1 alone or hSkM1+DMPK.

DMPK plasmid used in our study encodes a functional protein.

By contrast, with the hSkM1 isoform, the human cardiac sodium channel, hH1, was not modulated by co-expressed DMPK. Fig. 2 shows representative current tracings obtained from oocytes expressing either hH1 alone (Fig. 2A), or hH1 and DMPK (Fig. 2B). As was observed for hSkM1, DMPK had no effect on the time course of either activation or inactivation. Furthermore, DMPK had no effect on hH1 peak current amplitude measured at any test voltage. Fig. 2C shows a comparison between peak current amplitudes measured at a test potential of −10 mV in oocytes expressing either hH1 alone or the combination of hH1 with DMPK. There was no statistical difference between current amplitudes in cells expression hH1 alone, and cells expressing hH1 with DMPK (hH1 alone: 10.5 ± 1 μA, $n = 11$; hH1+DMPK: 11.2 ± 1 μA, $n = 12$; $p = 0.6$). We examined the time course of sodium current inactivation in both hH1 expressing oocytes and cells expressing hH1 and DMPK. There was no difference between the time required for 50% inactivation at any test voltage (Fig. 3A). In addition, there was no effect of the kinase on the voltage dependence of steady-state inactivation (Fig. 3B). Similar results were obtained from several different batches of oocytes from separate frogs. We performed additional co-expression experiments in the presence of the human β_1 subunit, and similarly found no measurable difference in any functional parameter of hH1. These results, by contrast to the data with hSkM1, demonstrate the lack of effect of DMPK on the cardiac sodium channel isoform.

4. Discussion

The molecular basis of myotonic dystrophy involves a trinucleotide expansion within the 3′ untranslated region of a putative protein kinase gene [2–4]. There is current controversy regarding the effect of this genetic mutation on cellular levels of the protein [13]. Evidence has been presented that the product of the DMPK gene is either increased [14], or decreased [15]. The protein product of the DMPK gene appears to function in vitro as a serine/threonine protein kinase [16], although its natural substrates have not been well defined. Clearly, much more work is needed to define the cellular pathogenesis of this multi-system disease.

In the past few years, evidence that mutations in ion channels, which disrupt their function, can result in abnormalities in muscle membrane excitability. This has been best studied in two distinct forms of congenital myotonia. In paramyotonia congenita and hyperkalemic periodic paralysis, mutations in the human skeletal muscle voltage-gated sodium channel α subunit gene (*SCN4A*, 17q), have been identified and shown to cause significant channel dysfunction [17]. Specifically, mutations in either disorder can disrupt both fast and slow inactivation, and have variable effects on the recovery from inactivation. In a clinically similar but genetically distinct disorder, myotonia congenita, mutations in a skeletal muscle chloride channel gene (*CLCN1*, 7q), have been linked to the pathogenesis of the syndrome [18,19]. It is tempting to speculate that myotonia, which can occur in a wide variety of inherited and acquired neuromuscular diseases, has a limited array of potential pathophysiologic mechanisms. In myotonic dystrophy, a previous hypothesis to explain myotonia, was that there is an indirect effect on sarcolemmal ion channels stemming from abnormalities in the lipid environment of the

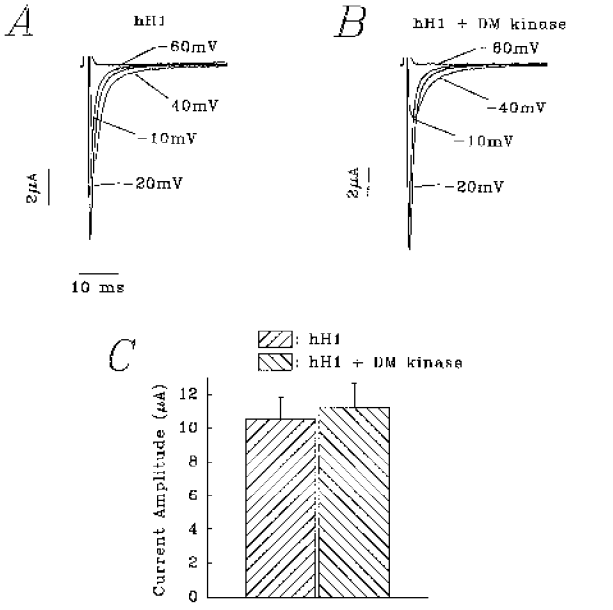

Fig. 2. Effect of mouse DMPK on hH1 expressed in oocytes. (A) Representative voltage-clamp recordings made from an oocyte expressing hH1 alone. Current tracings obtained at various test potentials from a holding potential of −120 mV are shown. (B) Representative voltage-clamp recordings made from an oocyte expressing hH1+DMPK using the same pulse protocol as shown in A. (C) Bar graph comparing peak sodium current measured at a test voltage of −30 mV between oocytes expressing hH1 alone or hH1+DMPK.

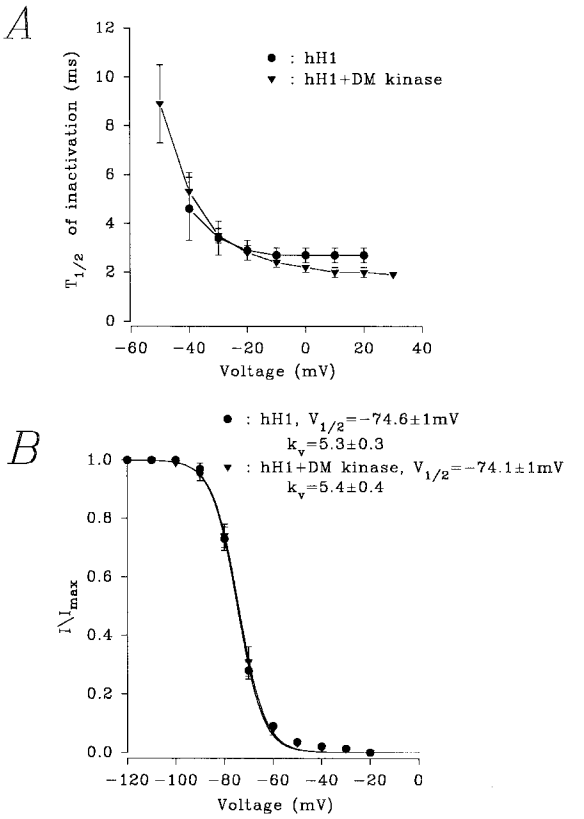

Fig. 3. Effect of DMPK on hH1 inactivation. (A) Voltage dependence of inactivation half-time ($T_{1/2}$) measured in oocytes expressing hH1 alone (●), or hH1+DMPK (▼). (B) Voltage dependence of steady-state inactivation determined for hH1 alone (●), or hH1+DMPK (▼).

muscle cell membrane [20]. One study, attempting to find evidence in support of channel dysfunction, identified abnormalities in the time constants of sodium channel activation and inactivation in myoballs cultured from myotonic dystrophy muscle [21]. Therefore, there are some inconsistencies between this previous study using human muscle tissue, and the effect of DMPK on recombinant sodium channels. In our study, we examined the effect of the wild-type gene product and, therefore we have not excluded the possibility that alterations of sodium channel function, as described by Rüdel et al. [21], occur in the presence of the mutant DMPK transcript. It is possible that in the absence of normal DMPK levels, alterations in muscle sodium channel function result in altered sarcolemmal excitability.

Cardiac involvement in myotonic dystrophy is common but often not clinically apparent [7]. Abnormalities of the electrocardiogram can be found in a majority of patients, and include first degree AV block, intraventricular conduction defects, and a variety of atrial and ventricular arrhythmias. The occurrence of ventricular arrhythmias may help explain an observed increased incidence of sudden death in myotonic dystrophy patients. Furthermore, an altered interaction between DMPK and cardiac ion channels could conceivably be responsible for some of these electrocardiographic abnormalities. It is known that in the congenital long QT syndrome, genetic mutations inducing specific dysfunction of cardiac sodium and potassium channels result in characteristic ventricular arrhythmias and an increased propensity for sudden

death [22]. In contrast, primary myocardial involvement is rare in myotonic dystrophy. Histological evaluation has revealed significant fibrosis, fatty infiltration, and atrophy of the cardiac conduction tissue [23,24] supporting the view that selective involvement of the conduction tissue, rather than a generalized cardiomyopathy, accounts for most of the clinical cardiac problems seen in this disease.

We sought to examine the effect of the wild-type DMPK gene product on the functional characteristics of two recombinant human voltage-gated sodium channels, hSkM1 and hH1. As reported previously by Mounsey et al., DMPK has a profound effect on the level of expressed current in oocytes expressing the muscle sodium channel isoform [5]. This effect is not accompanied by detectable changes in the kinetic properties of the channel, or in the voltage dependence of steady-state inactivation. We obtained similar results whether oocytes expressed the α subunit alone, or in combination with a recombinant human β_1 subunit. These observations provide evidence that the full-length mouse DMPK used in our study encodes a functional protein. By contrast, DMPK had no discernible functional effect on the recombinant human cardiac sodium channel, hH1. This was a surprising result considering that the phosphorylation site considered to be a potential target for DMPK, is conserved in both hSkM1 and hH1. It is conceivable that despite preservation of this site, phosphorylation may exert distinct functional effects on the two different sodium channel isoforms possibly because the effect of the kinase depends upon concomitant phosphorylation of other sites. Alternatively, the amino acid sequence requirements for DMPK phosphorylation may not strictly match that of PKC, and differences between hSkM1 and hH1 within the ID3–4 region or elsewhere may determine the substrate specificity of the kinase. In view of the distinct functional effects exerted by DMPK on hSkM1 and hH1, an experimental approach utilizing chimeras between the two sodium channel isoforms might provide information regarding these substrate determinants.

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