Abnormal Na channel gating in murine cardiac myocytes deficient in myotonic dystrophy protein kinase

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Departments of 1Biomedical Engineering, 2Internal Medicine (Cardiovascular Division), and 3Molecular Physiology and Biological Physics, and 4Cardiovascular Research Center, University of Virginia Health Systems, Charlottesville, Virginia 22908; and 5Institute for Genetic Medicine, University of Southern California School of Medicine, Los Angeles, California 90033

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Lee, Hwa C., Manoj K. Patel, Dilawar J. Mistry, Qingcai Wang, Sita Reddy, J. Randall Moorman, and J. Paul Mounsey. Abnormal Na channel gating in murine cardiac myocytes deficient in myotonic dystrophy protein kinase. Physiol Genomics 12: 147–157, 2003. First published November 26, 2002; 10.1152/physiolgenomics.00095.2002.—DMPK is a serine/threonine kinase implicated in the multisystem disease myotonic muscular dystrophy (DM). Skeletal muscle Na channels exhibit late reopenings in Dmpk-deficient mice and peak current density is reduced, implicating DMPK in regulation of membrane excitability. Since complete heart block and sudden cardiac death occur in the disease, we tested the hypothesis that cardiac Na channels also exhibit abnormal gating in Dmpk-deficient mice. We made whole cell and cell-attached patch clamp recordings of ventricular cardiomyocytes enzymatically isolated from wild-type, Dmpk+/−, and Dmpk−/− mice. Recordings from membrane patches containing one or a few Na channels revealed multiple Na channel re-openings occurring after the macroscopic Na current had subsided in both Dmpk+/− and Dmpk−/− muscle, but only rare re-openings in wild-type muscle (>3-fold difference, P < 0.05). This resulted in a plateau of non-inactivating Na current in Dmpk-deficient muscle. The magnitude of this plateau current was independent on the magnitude of the test potential from −40 to 0 mV and was also independent of gene dose. Macroscopic Na current density was similar in wild-type and Dmpk-deficient muscle, as was steady-state Na channel gating. Decay of macroscopic currents was slowed in Dmpk−/− muscle, but not in Dmpk+/− or wild-type muscle. Entry into, and recovery from, inactivation were similar at multiple test potentials in wild-type and Dmpk-deficient muscle. Resting membrane potential was depolarized, and action potential duration was significantly prolonged in Dmpk-deficient muscle. Thus in cardiac muscle, Dmpk deficiency results in multiple late re-openings of Na channels similar to those seen in Dmpk-deficient skeletal muscle. This is reflected in a plateau of non-inactivating macroscopic Na current and prolongation of cardiac action potentials.

sodium ion channel; action potential; protein kinase; cardiac myocytes

GENETIC STUDIES OF THE HUMAN DISEASE MYOTONIC DYSTROPHY (DM) led to the discovery of a novel serine/threonine protein kinase, dystrophia myotonica protein kinase (DMPK) (1, 7, 13, 16, 19). DM is a multisystem disease with its primary effects in skeletal and cardiac muscle. The symptoms include progressive weakness and myotonia (inability to relax a contracted muscle group), heart block, and ventricular arrhythmias. Patients with DM are deficient of DMPK mRNA (6, 8, 12, 37), but a Dmpk-deficient mouse model reproduces only a partial DM phenotype, with skeletal muscle weakness and heart block (5, 33, 34). These mice also recapitulate the characteristic skeletal muscle Na channel defect of DM (10, 25), suggesting that DMPK is a significant regulator of skeletal muscle Na channels.

Phosphorylation of Na channels by protein kinases is recognized as an important mechanism for modulation of Na currents (2, 4, 11, 22, 27, 31, 39). Coexpression of DMPK mRNA with μ1 skeletal muscle Na channels in Xenopus oocytes leads to a reduction of Na current amplitude that is dependent on the presence of a phosphorylation site in the inactivation gate (26). In Dmpk-deficient murine skeletal myocytes, there were frequent late Na channel openings on prolonged depolarization that resulted in a plateau of late Na current (25). There was also a reduction in macroscopic Na current amplitude (25, 32). The effect of DMPK on cardiac Na channels is not known. Interestingly, coexpression of DMPK mRNA with cardiac muscle Na channels in Xenopus oocytes had no effect on macroscopic Na currents (9), suggesting an important isoform difference in the response of cardiac and skeletal muscle Na channels to DMPK. The effects of DMPK coexpression on single cardiac Na channel gating were not reported (9).

We tested the effects of Dmpk deficiency on cardiac Na channel gating and resting and action potential characteristics in isolated murine cardiomyocytes. Cardiac muscle Na channels were studied and analyzed under identical conditions as we have previously reported for Dmpk-deficient skeletal muscle Na channels. We find that Dmpk deficiency has no effect on peak cardiac Na current or equilibrium Na channel...
gating, but the single-channel gating defect characteristic of Dmpk deficiency in skeletal muscle is recapitulated, and there is an associated prolongation of action potential duration (APD).

MATERIALS AND METHODS

Mice Deficient in Dmpk

The DMPK–/– 129SV mouse model that we studied has been described previously (5, 20, 25, 32–34). Homozygous Dmpk+/– and heterozygous Dmpk+/– mice were studied at >60 wk of age, when the Na channel phenotype in skeletal muscle is most profound. Control recordings were obtained from wild-type littermates.

Enzymatic Isolation of Mouse Cardiac Muscle Cells

Mice were killed by cervical dislocation after general anesthesia with intraperitoneal pentobarbital. The heart was rapidly excised and transferred to ice-cold, oxygenated Tyrode solution containing (in mmol/l) 145 NaCl, 5.4 KCl, 0.5 MgCl2, 1.8 CaCl2, 0.6 Na2HPO4, 5.0 HEPES, and 10 glucose, pH 7.4 (NaOH). The aorta was cannulated using a blunt 21-gauge needle, and the heart was Langendorff-perfused (37°C, 5 ml/min) first with standard Tyrode solution to wash out blood and then with nominally Ca-free Tyrode solution. Dissociation was accomplished by perfusion with collagenase (0.2 mg/ml, Worthington type II) and protease (0.05 mg/ml, Sigma type XIV) in Ca-free Tyrode solution. The enzyme solution was washed out with 0.18 mmol/l Ca Tyrode solution and the heart was then removed from the Langendorff perfusion apparatus and cells were released by sharp dissection in 0.18 mmol/l Ca Tyrode solution. Quiescent, Ca-tolerant, rod-like cells with clear cross striations were used for electrophysiological recordings.

Solutions

Action potential recordings were made in standard Tyrode solution; the pipette solution contained (mmol/l) 110 potassium aspartate, 20 KCl, 1 MgCl2, 10 HEPES, 0.1 sodium GTP, 5 potassium ATP, and 5 EGTA, pH 7.2 (KOH). Whole cell Na currents were recorded in low-Na Tyrode solution containing (in mmol/l) 2 NaCl, 138 CsCl, 1 MgCl2, 0.5 CaCl2, 0.5 CoCl2, 5.0 HEPES, and 10 glucose, pH 7.4 (NaOH); the pipette solution contained (in mmol/l) 120 NaCl, 5 KCl, 1 MgCl2, and 0.5 EGTA, pH 7.4 (KOH). The solution contained (in mmol/l) 120 NaCl, 5 KCl, 1 MgCl2, 2.5 CaCl2, 0.5 Na2HPO4, 10 HEPES, and 11.0 glucose, pH 7.2 (NaOH), with 1 CoCl2, 5 TEA-Cl, 5 4-AP, and 2 9-AC to block K and Ca currents.

Cell-attached patch clamp recordings were made in a depolarizing bath solution containing (in mmol/l) 160 KCl, 10 HEPES, 1 MgCl2, and 0.5 EGTA, pH 7.4 (KOH). The pipette solution contained (mmol/l) 120 NaCl, 5 KCl, 1 MgCl2, 2.5 CaCl2, 0.5 Na2HPO4, 10 HEPES, and 11.0 glucose, pH 7.2 (NaOH), with 1 CoCl2, 5 TEA-Cl, 5 4-AP, and 2 9-AC to block Ca, K, and Cl currents.

Electrophysiological Recordings

Our techniques for recording membrane potentials and Na currents from isolated mammalian muscle cells have been reported (20, 25). Briefly, recordings were made at room temperature (23 ± 0.1°C) using an amplifier (Axopatch model 200A; Axon Instruments, Foster City, CA) and pCLAMP (Axon) hardware and software. For action potential and macroscopic currents the electrode resistance was 1–1.5 MΩ. Series resistance compensation was 85 to 95%, and only cells with <100 pA current at the holding potential (~120 mV) were used for recording.

Action potentials were induced under current clamp (Axopatch 200A amplifier using the Ifast circuit) using current stimuli (0.45 ms, 6–12 nA). The stimulus intensity was adjusted to keep the latency between 5.5 and 6.5 ms. Although it is possible for action potential characteristics to be distorted when action potential recordings are made using patch clamp amplifiers in current clamp mode (18), it is unlikely that any conclusions drawn here will be affected, because all measurements were made using the same amplifier in the same current clamp mode.

Membrane potential signals were filtered at 10 kHz and sampled at 33 kHz. Ten consecutive action potentials were recorded at 1 Hz from each cell in whole cell current clamp mode. The last five action potentials were averaged, and resting membrane potential, overshoot, rise time, rate of rise, and the APD at repolarization to potentials of −30 mV (APD−30) and −70 mV (APD−70) were measured (Strathclyde Electrophysiology Software V3.1).

Macroscopic Na currents were recorded from a holding potential of −120 mV. Currents were filtered at 5 kHz and sampled at 66 kHz. Conductance and channel availability as a function of voltage were determined using standard techniques. Boltzmann functions were fitted to the raw data for each experiment, and the parameters were averaged. Gating kinetics (recovery from inactivation and development of inactivation) were measured over a range of test potentials using standard techniques (32). Decay of Na current was assessed as time to half decay from the peak of the macroscopic current.

Na currents were recorded from patches containing a few channels using standard techniques (25). Microelectrode resistance was 2–5 MΩ. Current signals were filtered at 2 kHz and digitized at 13 kHz. Na currents were measured over 150 ms at test potentials of 0, −20, and −40 mV from a holding potential of −120 mV. Currents were analyzed in Transit (38).

Statistical Analysis

The significance of differences in burst duration distributions (see Fig. 3) was assessed using a two-sample Kolmogorov-Smirnov test. Differences between all other single-channel parameters were assessed using a two-way ANOVA on ranks to measure the effects of test potential and gene dose. Macroscopic current amplitudes and resting and action potential data were analyzed by one-way and two-way ANOVA. Numerical data are given as means ± SE unless stated otherwise.

RESULTS

Effects of Partial and Complete Dmpk Deficiency on Cardiac Single Na Channel Gating

Na channel bursts and persistent late Na current in Dmpk-deficient myocytes. Figure 1 shows the effects of Dmpk deficiency on cardiac single Na channel gating. Figure 1, A–C, shows currents during voltage clamp pulses to −20 mV, and Fig. 1, D–F, shows corresponding ensemble average currents. In wild-type muscle, Na channel openings were concentrated early in the depolarizing clamp pulse and correspond to the macroscopic current. Late Na channel openings were rare, and the ensemble average current returned rapidly to zero. By contrast, in Dmpk-deficient muscle there were
frequent late openings that persisted throughout the depolarization, and this resulted in a plateau of non-inactivating Na current. The Na channel bursts occurred at a similar frequency in Dmpk+/− and Dmpk−/− muscle, and as a result the size of the plateau current was similar.

When data from multiple patches were assessed, plateau current was significantly larger in Dmpk+/− and Dmpk−/− muscle compared with wild type muscle, comprising overall 0.4 ± 0.06% of peak in wild-type, 2.1 ± 0.4% in Dmpk+/−, and 2.8 ± 0.6% in Dmpk−/− muscle (P < 0.05, ANOVA; Table 1), but there was no difference between Dmpk+/− and Dmpk−/− muscle (P = not significant [NS], ANOVA). Plateau current was independent of test potentials (Table 1; P = NS, ANOVA).

A possible explanation for the plateau current was that Dmpk deficiency induced a second population of non-inactivating Na channels. To investigate this possibility, unitary current amplitude and mean open time were measured during the macroscopic current (over the first 10 ms of the depolarizing clamp pulses), and during the plateau current (between 10 and 110 ms). The unitary current amplitude was similar for early and late Na channel openings and was unaffected by Dmpk deficiency (Table 2; P = NS for the comparison between early and late openings, and for the comparisons between Dmpk-deficient and wild-type cells, ANOVA). The mean open time was also similar in wild-type and Dmpk-deficient muscle over these two epochs (Table 2; P = NS, ANOVA). These data are consistent with the idea that Dmpk deficiency exerts its action through a change in gating of native cardiac Na channels rather than through induced expression of a physically unrelated Na channel.

Fig. 1. A–C: representative current traces at a test potential of −20 mV from cell-attached patches containing one or a few Na channels in wild-type (A), Dmpk+/− (B), and Dmpk−/− (C) myocytes. D–F: corresponding ensemble averages of idealized current recordings from 12–14 patches, normalized so that peak currents are the same. The currents are scaled to emphasize the non-inactivating persistent component of the current. Up to 100 depolarizing clamp pulses of 150-ms duration were studied from each patch.
Dmpk+/-, and Dmpk−/- muscle. Figure 2, A–C, shows examples of these measurements at test potentials of −40, −20, and 0 mV. We assessed Po over 100 consecutive depolarizations in the 100-ms epoch beginning 10 ms after the onset of the trace, when the macroscopic current would have subsided. Po was obtained by correcting for the number of channels in the patch, assessed from the number of overlapping channel openings early in the traces at strong depolarization, and data from multiple patches were pooled. The shaded bars of the histograms represent traces where the probability of channel activity exceeded 0.1. This increase in channel activity was associated with an increase in the proportion of prolonged bursts of Na channel openings in Dmpk-deficient muscle. Burst duration histograms from all patches at each test potential were pooled for wild-type, Dmpk+/- and Dmpk−/- muscle exhibited more than a threefold increase in openings per channel, an effect that was evident at all test potentials (P < 0.05 for each comparison, ANOVA) but independent of the magnitude of the test potential (P = NS for comparisons between test potentials, ANOVA).

This increase in channel activity was associated with an increase in the proportion of prolonged bursts of Na channel openings in Dmpk-deficient muscle. Burst duration histograms from all patches at each test potential were pooled for wild-type, Dmpk+/-, and Dmpk−/- cells, and empirical cumulative distribution functions (ECDFs) were constructed (Fig. 3, A–C). The ECDFs for Dmpk+/- and Dmpk−/- muscle were significantly different from wild-type muscle at all test potentials (P < 0.05 by two-sample Kolmogorov-Smirnov test), but Dmpk+/- and Dmpk−/- muscle were

Table 1. Single-channel properties

<table>
<thead>
<tr>
<th>Animals (cells)</th>
<th>Wild Type</th>
<th>Dmpk+/−</th>
<th>Dmpk−/−</th>
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<tbody>
<tr>
<td>Test potential</td>
<td>mV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−40</td>
<td>3(12)</td>
<td>3(14)</td>
<td>3(14)</td>
</tr>
<tr>
<td>−20</td>
<td>3(6)</td>
<td>3(6)</td>
<td>3(6)</td>
</tr>
<tr>
<td>0</td>
<td>3(6)</td>
<td>3(6)</td>
<td>3(6)</td>
</tr>
<tr>
<td>% Persistent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Po &gt; 0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Po &gt; 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Openings/</td>
<td>30.2 ± 10.6</td>
<td>28.1 ± 5.4</td>
<td>9.1 ± 4.1</td>
</tr>
<tr>
<td>channel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of</td>
<td>0.22 ± 0.02</td>
<td>0.2 ± 0.04</td>
<td>0.15 ± 0.07</td>
</tr>
<tr>
<td>longer bursts</td>
<td></td>
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</table>

Values are means ± SE and were derived from a 100-ms epoch starting 10 ms after depolarization. One hundred consecutive depolarizations from each patch were analyzed. The number of channels per patch was estimated from number of overlapping openings at the onset of the clamp pulse. There were no significant differences in the number of channels per patch between the groups. P > 0.1 represents probability that opening probability (Po) will be greater than 10% in the epoch of interest. P > 0 represents probability that Po will be greater than zero. Openings/channel were derived from Transit (36). The proportion of long Na channel bursts was derived from empirical cumulative distribution functions (ECDFs) as described in the text. Holding potential was −120 mV.

Table 2. Single-channel characteristics

<table>
<thead>
<tr>
<th>Single-Channel Current, pA</th>
<th>Wild type</th>
<th>Dmpk+/−</th>
<th>Dmpk−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10 ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−40 mV</td>
<td>1.0 ± 0.09</td>
<td>1.11 ± 0.03</td>
<td>1.02 ± 0.13</td>
</tr>
<tr>
<td>−20 mV</td>
<td>0.96 ± 0.15</td>
<td>1.10 ± 0.20</td>
<td>1.07 ± 0.15</td>
</tr>
<tr>
<td>0 mV</td>
<td>0.72 ± 0.06</td>
<td>0.50 ± 0.15</td>
<td>0.78 ± 0.34</td>
</tr>
<tr>
<td>10–110 ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−40 mV</td>
<td>1.05 ± 0.07</td>
<td>1.02 ± 0.03</td>
<td>1.06 ± 0.15</td>
</tr>
<tr>
<td>−20 mV</td>
<td>0.84 ± 0.05</td>
<td>0.98 ± 0.03</td>
<td>0.94 ± 0.05</td>
</tr>
<tr>
<td>0 mV</td>
<td>0.75 ± 0.04</td>
<td>0.78 ± 0.06</td>
<td>0.74 ± 0.11</td>
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</table>

<table>
<thead>
<tr>
<th>Mean Open Time, ms</th>
<th>Wild type</th>
<th>Dmpk+/−</th>
<th>Dmpk−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10 ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−40 mV</td>
<td>0.93 ± 0.15</td>
<td>0.69 ± 0.10</td>
<td>0.72 ± 0.06</td>
</tr>
<tr>
<td>−20 mV</td>
<td>1.0 ± 0.11</td>
<td>1.10 ± 0.09</td>
<td>0.91 ± 0.11</td>
</tr>
<tr>
<td>0 mV</td>
<td>0.54 ± 0.08</td>
<td>0.80 ± 0.15</td>
<td>0.62 ± 0.13</td>
</tr>
<tr>
<td>10–110 ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−40 mV</td>
<td>0.92 ± 0.15</td>
<td>0.67 ± 0.05</td>
<td>0.70 ± 0.06</td>
</tr>
<tr>
<td>−20 mV</td>
<td>1.01 ± 0.08</td>
<td>1.01 ± 0.07</td>
<td>0.96 ± 0.08</td>
</tr>
<tr>
<td>0 mV</td>
<td>0.50 ± 0.10</td>
<td>0.81 ± 0.10</td>
<td>0.59 ± 0.11</td>
</tr>
</tbody>
</table>

Values are means ± SE. Single-channel current amplitude and mean open time measured early (0–10 ms) and later (10–100 ms) in the voltage clamp pulse at various test potentials. There were no significant differences between early and late openings or between wild-type and Dmpk-deficient myocytes (P = not significant, ANOVA).
not different from each other. This suggests that the populations of Na channel bursts come from different distributions in wild-type and Dmpk-deficient muscle.

Inspection of the ECDFs suggested two populations of bursts: short bursts that mainly correspond to single Na channel openings, and a population of long bursts. We constructed ECDFs from each individual patch and fit them to a sum of two-exponentials model (Fig. 3). The time constants of the fits were 0.9 ± 0.2 and 10.9 ± 0.7 ms overall and were independent of gene dose and test potential. Dmpk-deficient muscle exhibited a two-fold increase in the proportion of the longer time constant compared with wild-type muscle, indicating that there were more long bursts in Dmpk-deficient muscle (Table 1). For example, at test potential of −20 mV, the proportions were 0.42 ± 0.06 for Dmpk+/−, 0.41 ± 0.03 for Dmpk−/−, and 0.2 ± 0.04 for wild type. This difference was significant across all test potentials (P < 0.05, ANOVA for each comparison; Table 1), but there was no significant difference between Dmpk+/− and Dmpk−/− muscle or between test potentials (P = NS, ANOVA for all comparisons).

Effects of Partial and Complete Dmpk Deficiency on Macroscopic Cardiac Na Channel Gating

No change in macroscopic Na current density or equilibrium gating. Figure 4, A–C, shows representative families of macroscopic whole cell Na currents recorded from wild-type, Dmpk+/−, and Dmpk−/− muscle. The currents were recorded at a low external Na concentration (2 mmol/l) to optimize voltage con-

Fig. 2. Frequency histograms normalized for sample size of opening probability (Po) from all patches at test potentials of −40 mV (A), −20 mV (B), and 0 mV (C) for wild-type (top), Dmpk+/− (middle), and Dmpk−/− (bottom) myocytes. The left-most bar in each panel represents traces with no openings after 10 ms, and the shaded area represents traces where Po exceeds 0.1. Compared with wild type, cells with partial and complete Dmpk deficiency exhibited 3- and 4-fold increases in late openings, respectively, but there was no apparent effect of test potential on the frequency of late openings. The horizontal axis has a logarithmic scale with bin width 10^2.25.
Fig. 3. Empirical cumulative distribution functions of Na channel burst durations at -40 mV (A), -20 mV (B), and 0 mV (C) derived from multiple patches. These are the cumulative distributions normalized for sample size. The maximum distance between any pair is the basis for the Kolmogorov-Smirnov two sample test. The lines are sums of two exponential functions.

Fig. 4. A–C: representative families of whole cell Na currents recorded from wild-type (A), Dmpk+/− (B), and Dmpk−/− (C) muscle recorded in symmetric [Na] at 2 mmol/l. D–F: superimposed representative whole cell Na currents recorded at -10 mV and an external [Na] of 135 mmol/l, before and after tetrodotoxin (20 μmol/l). The currents are shown on an expanded scale to emphasize the late plateau current in Dmpk-deficient muscle. G: the peak current density-voltage relationship was similar in all three groups. H: macroscopic Na current half decay time was prolonged in Dmpk−/− myocytes compared with Dmpk+/− and wild-type myocytes (P < 0.05, ANOVA). I: equilibrium gating relationships. Data points are means ± SE of individual experiments; smooth lines are least square fits to a Boltzmann function of the form 1/(1 + exp(V - V0.5/k)), where k is a slope factor and V0.5 is the potential at half-maximal activation. For channel availability as a function of voltage in wild-type muscle, the V0.5 and slope factors were, respectively, -86.5 ± 1.3 mV and -5.3 ± 0.2. In Dmpk+/− muscle the corresponding values were, respectively, -87.0 ± 1.1 mV and -5.3 ± 0.2, and in Dmpk−/− muscle these were -86.9 ± 1.3 mV and -5.7 ± 0.2, respectively. For conductance as a function of voltage, the values were, respectively, -45.9 ± 0.7 mV and 5.2 ± 0.2 for wild-type muscle, -48.0 ± 1.0 mV and 4.9 ± 0.1 for Dmpk+/− muscle, and -45.0 ± 0.7 mV and 5.0 ± 0.1 for Dmpk−/− muscle. Data were derived from 22–33 cells.
trol. The pipette Na concentration was also 2 mmol/l, leading to the observed reversal potential near 0 mV. The peak current amplitude was larger in the Dmpk−/− cell, but this did not translate to an increase in current density because cell capacitance was >40% larger in Dmpk−/− cells when compared with Dmpk+/− and wild-type cells. The mean cell capacitance was 134.5 ± 5.3 pF for wild-type, 138.2 ± 8.7 pF for Dmpk+/−, and 190.7 ± 11.5 pF for Dmpk−/− cells (P < 0.05 for the comparison of Dmpk−/− cells with either Dmpk+/− or wild type; one-way ANOVA). Data from multiple patches confirmed the absence of an effect of Dmpk deficiency on cardiac macroscopic inward current density (Fig. 4G). There were no significant differences in equilibrium gating relationships in Dmpk-deficient muscle. Figure 4I shows that channel availability and conductance as a function of voltage were essentially identical in wild-type and Dmpk+/− and Dmpk−/− muscle.

The plateau of Na current on prolonged depolarization that was evident in the ensemble average recordings in Fig. 1 above is difficult to appreciate in the whole cell Na current recordings (Fig. 4, A–C), which were obtained at low, symmetrical Na concentrations. Figure 4, D–F, shows superimposed Na currents recorded at −10 mV in the presence and absence of tetrodotoxin (20 μmol/l) using a bath [Na] of 135 mmol/l. A plateau current was evident in Dmpk-deficient muscle but not in wild-type muscle. This amounted to 2.9 ± 0.8 pA (n = 7) in wild-type muscle, compared with 147 ± 19 pA (n = 4) in Dmpk+/− muscle and 179 ± 21 pA (n = 8) in Dmpk−/− muscle (P < 0.05 for the comparison between wild type and Dmpk-deficient muscle; P = NS for the comparison between Dmpk+/− and Dmpk−/− muscle, ANOVA).

Macroscopic Na channel gating kinetics. Entry into inactivation and recovery from inactivation were each measured at multiple conditioning potentials (Fig. 5, A–F). Neither parameter showed any systematic variation with altering Dmpk-gene dose. Compared with wild type, decay rate of macroscopic current was slower in Dmpk−/− myocytes (Fig. 4H), an effect that was not apparent in Dmpk+/− muscle.

Effects of Partial and Complete DMPK Deficiency on Cardiac Muscle Resting and Action Potentials

Resting and action potential characteristics were measured from epicardial left ventricular free wall myocytes. Dmpk deficiency induced a small but significant depolarization of the resting membrane potential (P < 0.05, ANOVA; Table 2), which amounted to >4

![Fig. 5. Kinetics of recovery from and entry into inactivation in wild-type (A and D), Dmpk+/− (B and E), and Dmpk−/− (C and F) muscle. Data points represent means ± SE of individual experiments. The smooth lines are least square fits to a function of the form 1 – A exp (–t/τ) (for A–C) and A exp (–t/τ) (for D–F), where A is a constant, and τ is the time constant. There were no significant differences in time constants of either recovery from or entry into inactivation between wild-type and Dmpk-deficient muscle. Data were derived from 24–33 cells for each group.](http://physiolgenomics.physiology.org/)

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Table 3. Resting and action potential properties

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>Dmpk+/−</th>
<th>Dmpk−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>RMP, mV</td>
<td>−75.6  ±0.5</td>
<td>−72.8 ±0.9*</td>
<td>−71.2 ±1.1*</td>
</tr>
<tr>
<td>APD−30, ms</td>
<td>7.5 ± 0.9</td>
<td>6.4 ± 1.0</td>
<td>10.9 ± 2.1</td>
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<tr>
<td>APD−70, ms</td>
<td>26.0 ± 2.2</td>
<td>50.2 ± 7.0 †</td>
<td>64.8 ± 7.5 †</td>
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<tr>
<td>Overshoot, mV</td>
<td>57.4 ± 2.0</td>
<td>59.2 ± 4.2</td>
<td>53.2 ± 4.5</td>
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<td>Rise time, ms</td>
<td>0.33 ± 0.01</td>
<td>0.31 ± 0.02</td>
<td>0.35 ± 0.01</td>
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</table>

Values are means ± SE. RMP, resting membrane potential. APD−30 and APD−70, times to repolarization to −30 mV and −70 mV, respectively. *Statistically significant by one-way ANOVA on ranks (P < 0.05). †Statistically significant difference between Dmpk-deficient muscle and wild type, but no difference between Dmpk+/− and Dmpk−/− muscle by two-way ANOVA.

mV in Dmpk−/− muscle. APD−30 was not affected by Dmpk deficiency, but APD−70 was prolonged in Dmpk+/− and Dmpk−/− muscle (P < 0.05 for the difference between Dmpk-deficient and wild-type muscle, and P = NS for the difference between Dmpk+/− and Dmpk−/− muscle, by two-way ANOVA; Table 3; Fig. 6). Other action potential characteristics were similar in wild-type and Dmpk-deficient muscle (Table 3).

**DISCUSSION**

We report a characterization of the effects of Dmpk deficiency on macroscopic and single Na channel gating, and membrane and action potentials in isolated murine cardiac ventricular myocytes. Our major findings are 1) Dmpk deficiency resulted in a Na channel abnormality comprising frequent, long bursts of Na channel reopenings during sustained depolarization resulting in a plateau of non-inactivating late Na current; 2) the gating abnormality was independent of the degree of reduction of Dmpk dosage in that the Na channel defect was identical in Dmpk−/− and Dmpk+/− muscle; 3) macroscopic Na channel gating and current density were unaffected by Dmpk deficiency; and 4) Dmpk deficiency resulted in a small but significant depolarization of resting potential, and prolongation of APD.

**Late Persistent Na Current with Prolongation of APD**

Dmpk-deficient cardiac muscle exhibited persistent Na current, and we can think of two general explanations for this result. The first is that Dmpk deficiency leads to expression of a population of voltage-independent Na channels. Testing this idea requires a molecular study of Na channel isoforms in Dmpk-deficient mice, a topic for future work. The second is that Dmpk deficiency causes the fast inactivation process to fail, a hallmark of the slow mode Na channel gating that is a normal albeit rare feature of cardiac and skeletal muscle Na channels (see, e.g., Refs. 14, 17, 23, 30). We suggest that Dmpk deficiency makes this mode of gating more likely. By analogy with Na channel mutations that produce the long QT syndrome, which also cause persistent late Na current (3, 28), we anticipated Dmpk deficiency, too, would result in prolongation of APD. For example, in cardiomyocytes isolated from genetically engineered mice with a LQT3 mutation (ΔKPQ), APD was prolonged without effect on the resting potential, action potential amplitude, and upstroke velocity (28). Repolarization of the action potential to −70 mV (close to the resting potential of these cells) was approximately doubled in Dmpk+/− and Dmpk−/− muscle, and this degree of prolongation is consistent with the defect of Na channel inactivation gating we have demonstrated here.
DMPK may regulate other cardiac ion channels, and effects on these potential substrates may also affect APD. This is a credible idea in that DMPK includes among its substrates the β-subunit of the dihydropyridine receptor Ca channel (36) and phospholemman, a membrane substrate for kinases that forms ion channels and may be involved in regulation of cell volume (21, 24, 29). The effects of Dmpk deficiency on these, and potentially other as yet unidentified DMPK substrates on the electrophysiology of the cardiac cell remain to be studied.

The depolarization of the resting membrane potential in Dmpk-deficient myocytes could be the result of the Na channel defect, but this could also be caused by other as yet unidentified electrophysiological defects resulting from Dmpk deficiency. It would be of interest to assess the effect of Na channel blockade with tetrodotoxin on resting potential. A hyperpolarization in Dmpk-deficient cells would be consistent with the idea that the Na channel defect was responsible for the depolarization.

Why Did Dmpk+/− and Dmpk−/− Animals Exhibit a Similar Na Channel Defect?

DMPK levels are predicted to drop to zero in Dmpk−/− muscle, and to a minimum of 50% of normal in Dmpk+/− cells. As loss of one allele in most genes is usually insufficient to produce pathology, it is unclear why the wild-type Dmpk allele in Dmpk+/− cells is unable to maintain the normal phenotype. This, however, is the same effect found previously in skeletal muscle of Dmpk-deficient mice. The relationship between Dmpk gene dose and Na channel phosphorylation may, however, be nonlinear. A possible mechanism is that when DMPK levels fall below a relatively sharp threshold, an all-or-none effect may be observed. If this were the case, then both heterozygous and homozygous mutant animals would show a similar phenotype if the Dmpk threshold level required for normal function is more than 50% of the wild-type levels. Interestingly, mice partially deficient in Six5, the gene upstream of Dmpk, exhibit a reduction of Dmpk mRNA levels to 75% of normal (35), and these mice do not exhibit the skeletal muscle Na channel defect characteristic of Dmpk deficiency (20). The effects of partial deficiency of Six5 on cardiac Na channels have not been reported, but if effects of Six5 deficiency on cardiac and skeletal muscle Na channels are similar, this observation suggests that the threshold of Dmpk deficiency required to produce the Na channel defect is between 75% and 50% of normal.

Comparison of the Effects of DMPK in the Xenopus Oocyte Expression System and the Dmpk-Deficient Mouse

The effects of Dmpk deficiency on Na channels have now been characterized for skeletal and cardiac isoforms both in isolated Dmpk-deficient mouse muscle, and in Na channels coexpressed with DMPK in Xenopus oocytes (9, 25, 26, 32). In Xenopus oocytes, the effect of DMPK coexpression on skeletal muscle Na channels was severalfold. Current amplitude was reduced, an effect that was dependent on a phosphorylation site in the cytoplasmic linker between transmembrane domain III and IV of the channel (9, 26). Na channel density, as assessed from binding of the highly selective Na channel ligand saxitoxin (STX), was unaffected (Miller JR, Mounsey JP, and Moorman JR, unpublished observations). Macroscopic equilibrium gating was unaffected, current decay was enhanced, and single-channel opening probability was reduced (26). The results in Dmpk-deficient skeletal muscle were consistent with the Xenopus oocyte expression data in that Na channel density (as assessed from STX binding) was unaffected, single-channel opening probability was increased, and equilibrium gating was unaffected (25, 32). The current amplitude results were, however, opposite in that both a reduction of DMPK activity (in Dmpk-deficient mice) and enhancement of DMPK activity (in Xenopus oocytes) resulted in a reduction in current amplitude.

Macroscopic cardiac Na current amplitude, current decay, and equilibrium gating were unaffected by DMPK coexpression in Xenopus oocytes, and, apart from the slight slowing of macroscopic Na current decay in Dmpk−/− muscle, Dmpk deficiency was without effect on macroscopic cardiac Na currents. The effects of DMPK coexpression on cardiac Na single-channel gating in Xenopus oocytes have not been reported.

Thus the major effects of DMPK on Na channel gating in the Xenopus oocyte expression system and the Dmpk-deficient mouse are consistent, with the exception of the effects of DMPK coexpression on skeletal muscle Na current density in Xenopus oocytes. Single-channel gating is abnormal in both cardiac and skeletal muscle from Dmpk-deficient mice, and a consistent abnormality is present in Xenopus oocyte-expressed skeletal muscle Na channels. The reasons for the expression system-dependent differences in channel gating are not clear but might be linked to differences in other cell signaling systems between amphibian and mammalian cells.

The observation that effects of Dmpk deficiency on single Na channel gating were similar in cardiac and skeletal muscle channels, whereas effects on macroscopic currents were different, suggests the possibility that these effects were the result of interaction of DMPK with different sites in the Na channel molecule. By this hypothesis, the single-channel effect would be the result of interaction of DMPK with a site common to both isoforms, whereas the effect on macroscopic current would result from interaction with a site unique to the skeletal muscle isoform.

In conclusion, DMPK is a novel regulator of cardiac Na channels. Dmpk deficiency leads to failure of Na channel inactivation with a plateau of non-inactivating Na current. The membrane potential was depolarized in Dmpk-deficient cells, and the time course of action potential repolarization was prolonged. Potential regulators of DMPK remain unidentified, and the in vivo...
consequences of DMPK activation remain to be determined.

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