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Myotonic Dystrophy Protein Kinase Is Involved in the Modulation of the Ca²⁺ Homeostasis in Skeletal Muscle Cells

Ad A.G.M. Benders,* Patricia J.T.A. Groenen,† Frank T.J.J. Oerlemans,‡ Jacques H. Veerkamp,* and Bé Wieringa†

*Department of Biochemistry and †Department of Cell Biology and Histology, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

Abstract

Myotonic dystrophy (DM), the most prevalent muscular disorder in adults, is caused by (CTG)n-repeats expansion in a gene encoding a protein kinase (DM protein kinase; DMPK) and involves changes in cytoarchitecture and ion homeostasis. To obtain clues to the normal biological role of DMPK in cellular ion homeostasis, we have compared the resting [Ca²⁺], the amplitude and shape of depolarization-induced Ca²⁺ transients, and the content of ATP-driven ion pumps in cultured skeletal muscle cells of wild-type and DMPK[−/−] knockout mice.

In vitro-differentiated DMPK[−/−] myotubes exhibit a higher resting [Ca²⁺], than do wild-type myotubes because of an altered open probability of voltage-dependent L-type Ca²⁺ and Na⁺ channels. The mutant myotubes exhibit smaller and slower Ca²⁺ responses upon triggering by acetylcholine or high external K⁺. In addition, we observed that these Ca²⁺ transients partially result from an influx of extracellular Ca²⁺ through the L-type Ca²⁺ channel. Neither the content nor the activity of Na⁺/K⁺ ATPase and sarcoplasmic reticulum (SR) Ca²⁺-ATPase are affected by DMPK absence.

In conclusion, our data suggest that DMPK is involved in modulating the initial events of excitation-contraction coupling in skeletal muscle. (J. Clin. Invest. 1997. 100:1440-1447.) Key words: excitation-contraction coupling • ion channels • ion pumps • knockout mouse • cultured myotubes

Introduction

Myotonic dystrophy (DM) follows an autosomal dominant inheritance, and is characterized by a wide variety of symptoms, including increased excitability and delayed relaxation of muscle, muscle weakness and wasting, ocular cataract, sensorineural deafness, cardiac conduction defects, hyperventilation, testicular atrophy causing male sterility, and endocrine dysfunction (1). Clinical manifestation of this rather frequent disorder is caused by expansion of an unstable CTG-repeat in the 3' untranslated region of a gene encoding the myotonic dystrophy protein kinase (DMPK), with the age of onset and the severity of the disease being correlated to the extent of expansion (2,4).

Contradictory results have been obtained with regard to the effects of abnormally long CTG repeats on DMPK mRNA and protein levels in patient tissues (5-8). In addition, the study of transgenic mouse models with altered DMPK levels (9,10) has not provided the answer on the question whether over- or underexpression of this protein is involved in disease etiology. Homozygous DMPK[−/−] mice exhibit only minor changes in neck muscle fibres at older age, whereas animals carrying multiple copies of the DMPK transgene show hypertrophic cardiomyopathy and enhanced neonatal mortality as the only features.

Typically, muscle fibers and/or cultured skeletal muscle cells of DM patients exhibit a decreased resting membrane potential (11-13) and increased basal cytosolic Na⁺ and Ca²⁺ concentrations (14-16). These features may be attributed to anomalies in the functioning of voltage-operated Na⁺ channels (17,18) and Ca²⁺ channels (15,16) and/or to a reduced content of Na⁺/K⁺-ATPase and sarcoplasmic reticulum (SR) Ca²⁺-ATPase (19,20). In addition, the persistence of an apamin-receptor, i.e. Ca²⁺-activated K⁺ channel, has been demonstrated (21).

Many of these ion channels and ion pumps are key players in the excitation-contraction (E-C) coupling mechanism of skeletal muscle in vivo, a cascade of events in which, sequentially, acetylcholine receptors (AChR) of the neuromuscular junction, sarcolemmal tetrodotoxin-sensitive voltage-operated Na⁺ channels (TTXR), T-tubular dihydropyridine receptors (DHPR), and finally ryanodine receptors (RyR), i.e. Ca²⁺ release channels in the terminal cisternae of the SR, are involved (22). E-C coupling of vertebrate skeletal muscle is thought to occur by a mechanical coupling, in which intramembrane charge movements and associated conformational changes of the voltage-sensing DHPR activate the RyR without entry of external Ca²⁺ through DHPR (23-25). Before relaxation, the disturbed ion concentrations are restored to resting levels by ATP-driven ion pumps, i.e., Na⁺/K⁺-ATPase and SR Ca²⁺-ATPase.

This whole process is subject to complex regulation in which local luminal and cytosolic Ca²⁺ and ATP levels are in-
involved. It is also influenced by protein phosphorylation and ion channel-modulating proteins like FK506, triadin, or calsequerin. Since dysregulation of ion fluxes is a likely determining factor in the abnormal cellular functions in DM, and since DMPK-mediated protein phosphorylation could play a role, we examined if and how DMPK deficiency affects the depolarization behavior and Ca2+ homeostasis in cultured skeletal muscle cells in the absence or presence of specific inhibitors of the voltage-operated ion channels or the SR Ca2+ release channel. We also determined the activity and content of SR Ca2+-ATPase and Na+/K+-ATPase in cultured muscle cells and skeletal muscle of wild-type and DMPK[-/-] mice. Although the morphological and physiological effects of DMPK absence at the animal level are surprisingly mild, we demonstrate here, that DMPK deficiency has conspicuous consequences at the cellular level. Our finding of abnormally operating TTXRs and DHPRs gives evidence for a relation between DMPK activity and ion homeostasis in skeletal muscle.

Methods

Materials. The acetoxymethyl ester of Fura-2 (Fura-2/AM) was purchased from Molecular Probes Europe (Leiden, The Netherlands); acetylcholine chloride (ACh), tetrodotoxin (TTX), ryanodine and ionomycin were from Sigma (St. Louis, MO, USA); and nifedipine from Bayer (Leverkusen, Germany). Sources of other materials were described previously (20, 26).

DMPK-deficient mice. Homozygous DMPK-deficient (DMPK[-/-]) mice were generated by targeted mutagenesis and genotyped by PCR analysis as described (9). DMPK[-/-] animals from the F3 generation (on a mixed C57BL/6/129/OLA 50·50% background) were used. Wild-type mice were from the C57BL/6 inbred strain, and from a 129/OLA mixed background were taken as controls.

Cytosolic Ca2+ concentration measurement in cultured skeletal muscle cells. Hind leg muscles of 3-10-d-old wild-type and DMPK[-/-] mice were dissociated, and the isolated satellite cells were allowed to proliferate for 2 d, and were then cultured under differentiation-promoting conditions for 4 d on collagen-coated glass coverslips (8·25 mm) in serum-containing media, as described for human muscle cells (26). The free cytosolic Ca2+ concentration ([Ca2+]i) and the effects of depolarization induced by the addition of 20 µM ACh or 125 mM KCl on [Ca2+]i were measured in single cells with Fura-2 using conventional fluorescence video microscopy (26). For calibration, Ca2+- saturated or Ca2+-free dye was set to 2 µM ionomycin in the presence of 1.8 mM Ca2+ (pH 7.5) or 20 mM EGTA (pH 8.0), respectively. Under these conditions the myotubes remain attached to the coverslip. Although the calibration parameters of wild-type and DMPK[-/-] skeletal muscle cells did not differ significantly, we used these parameters strictly separated for calculation of [Ca2+]i to rule out cell-type-dependent artefacts (27). The half-increase (τi) and half-decay time (τd) of the depolarization-triggered Ca2+ responses were analyzed as described (26, 28).

SR Ca2+-ATPase and Na+/K+-ATPase. The activity and concentration of SR Ca2+-ATPase were examined in homogenates of cultured muscle cells (differentiated for 4 d) and hind leg skeletal muscle of adult mice by measuring Ca2+-dependent ATP hydrolysis and steady-state phosphorylation, respectively (20). The activity of Na+/K+-ATPase was assayed as the K+-dependent, ouabain-sensitive 3-O-methylfluorescein phosphatase (3-O-MFPase) activity and the content of Na+/K+-ATPase was derived from the binding capacity of [3H]ouabain (20).

Other procedures. Creatine kinase (CK) activity of cultured muscle cells (differentiated for 4 d) was determined with the CK N-acetylcysteine-activated monotest (26). For determining the percentage CK muscle-specific isoenzyme MM (CK-MM), this isoenzyme was separated from the other CK isoenzymes by gel electrophoresis (29). The protein content was assayed according to Lowry et al. (30) with BSA as a standard.

Statistics. Data represent means±SD. Statistical analysis was performed by means of the unpaired Student’s t test, and significance was set at P<0.05.

Results

General characteristics of cultured wild-type and DMPK[-/-] skeletal muscle cells. Upon differentiation, in vitro skeletal muscle cells derived from wild-type and DMPK-deficient mice exhibit a similar morphological appearance. Typically, the diameter of these cells is ~10-20 µm, whereas their length varies between 500 and 700 µm. Also, the total CK activity of wild-type (1.93±0.59 U/mg protein; n = 11) and mutant cells (1.84±0.84 U/mg protein; n = 5) and the percentage of CK-MM, a measure for the overall maturation grade, are the same for both cell types (wild-type: 33.8±10.1; n = 11, and DMPK[-/-]: 37.1±5.8; n = 5). Spontaneous contractions of wild-type and DMPK[-/-] myotubes are occasionally observed in culture, and occurred frequently upon agonist-induced depolarization in both cell types.

Ca2+ homeostasis of wild-type skeletal muscle cells. The resting [Ca2+]i of differentiated wild-type mouse skeletal muscle cells in culture is 120 nM (Table 1). Depolarization of the sarcolemma of these cells either by 20 µM ACh or by 125 mM KCl, induces Ca2+ transients with an amplitude ([Ca2+]i,max) of ~0.8 µM (Fig. 1) and a τi and τd of ~3 and 5 s, respectively. The provoked Ca2+ responses are completely prevented by inhibition of the SR Ca2+ release channel (RyR) using 10 µM ryanodine (Fig. 2, B and C). Thus, Ca2+ release from the SR is entirely responsible for the increase of the [Ca2+]i, indicating that the wild-type myotubes possess a skeletal muscle type of E-C coupling mechanism (28, 31). As anticipated, also nifedipine (5 µM), a blocker of DHPR, impedes the depolarization-induced Ca2+ transients (Fig. 3, B and C). Inhibition of TTXR by 5 µM tetrodotoxin only partially suppresses depolarization by ACh (Fig. 4 B). This inhibition is incomplete due to the coexistence of TTX-sensitive and TTX-resistant volt-

Table 1. Ca2+ Transients Induced by ACh or KCl in Cultured Skeletal Muscle Cells From Wild-type and DMPK[-/-] Mice

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Parameter</th>
<th>Wild-type</th>
<th>DMPK[-/-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>[Ca2+]i,initial</td>
<td>122±7</td>
<td>185±24*</td>
</tr>
<tr>
<td></td>
<td>t&lt;sub&gt;i&lt;/sub&gt;</td>
<td>(170)</td>
<td>(63)</td>
</tr>
<tr>
<td>20 µM ACh</td>
<td>[Ca2+]i,peak</td>
<td>775±131</td>
<td>511±124*</td>
</tr>
<tr>
<td></td>
<td>t&lt;sub&gt;i&lt;/sub&gt;</td>
<td>3.0±0.6</td>
<td>6.6±1.9*</td>
</tr>
<tr>
<td></td>
<td>t&lt;sub&gt;d&lt;/sub&gt;</td>
<td>4.6±0.8</td>
<td>8.4±2.1*</td>
</tr>
<tr>
<td></td>
<td>(84)</td>
<td>(38)</td>
<td></td>
</tr>
<tr>
<td>125 mM KCl</td>
<td>[Ca2+]i,peak</td>
<td>881±164</td>
<td>552±130*</td>
</tr>
<tr>
<td></td>
<td>t&lt;sub&gt;i&lt;/sub&gt;</td>
<td>3.2±0.7</td>
<td>6.1±1.7*</td>
</tr>
<tr>
<td></td>
<td>t&lt;sub&gt;d&lt;/sub&gt;</td>
<td>4.8±0.7</td>
<td>7.9±2.8*</td>
</tr>
<tr>
<td></td>
<td>(86)</td>
<td>(25)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means±SD of the number of muscle cells examined (listed between parentheses) from at least four individual cultures. The values of [Ca2+]i are expressed in nM, and the half-increase (t<sub>i</sub>) or half-decay time (t<sub>d</sub>) in s. Parameters of DMPK[-/-] and wild-type cells differ with *P < 0.01.
Figure 1. Depolarization-induced Ca\(^{2+}\) transients in cultured skeletal muscle cells derived from wild-type (solid line) and DMPK\([-/-]\) mice (dashed line). Depolarization was generated by 20 \(\mu\)M ACh (A) or 125 mM KCl (B) as marked by the bars. Traces show the average [Ca\(^{2+}\)], after superimposing all appropriate experiments as listed in Table I.

Figure 2. Effects of ryanodine on resting [Ca\(^{2+}\)] (A) and depolarization-provoked Ca\(^{2+}\) responses (B and C) in cultured wild-type and DMPK\([-/-]\) muscle cells. Muscle cells were incubated with 10 \(\mu\)M ryanodine for 30 min before depolarization (B and C). Traces show the mean [Ca\(^{2+}\)], after superimposing all relevant experiments as given in Table II. Ryanodine does not normalize the higher resting [Ca\(^{2+}\)] of DMPK\([-/-]\] cells (A), and only partially inhibits depolarization-induced Ca\(^{2+}\) transients (B and C).
Figure 3. Effects of nifedipine on resting [Ca\(^{2+}\)]\(_i\) (A) and depolarization-generated Ca\(^{2+}\) transients (B and C) in cultured wild-type and DMPK\([-/-\)] muscle cells. Muscle cells were incubated with 5 \(\mu\)M nifedipine for 30 min before depolarization (B and C). For other details, see legend to Fig. 2. Nifedipine completely normalizes the higher resting [Ca\(^{2+}\)]\(_i\) of DMPK\([-/-\)] cells (A).

Figure 4. Effects of tetrodotoxin on resting [Ca\(^{2+}\)]\(_i\) (A) and depolarization-elicted Ca\(^{2+}\) responses (B and C) in cultured wild-type and DMPK\([-/-\)] muscle cells. Muscle cells were preincubated with 5 \(\mu\)M tetrodotoxin for 30 min before depolarization (B and C). For other details, see legend to Fig. 2. Tetrodotoxin partially normalizes the higher resting [Ca\(^{2+}\)]\(_i\) of DMPK\([-/-\)] cells (A).
Table II. Effects of E-C Coupling Inhibitors on ACh- and KCl-induced Ca2+ Transients in Cultured Skeletal Muscle Cells From Wild-type and DMPK[-/-] Mice

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Stimulus</th>
<th>Parameter</th>
<th>Wild-type</th>
<th>DMPK [-/-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ryanodine (10 μM)</td>
<td>ACh</td>
<td>[Ca2+]b, b</td>
<td>119±21</td>
<td>182±38*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Ca2+]m, b</td>
<td>119±21</td>
<td>251±31*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(40)</td>
<td></td>
<td>(21)</td>
</tr>
<tr>
<td>KCl (50 μM)</td>
<td>ACh</td>
<td>[Ca2+]b, b</td>
<td>120±24</td>
<td>179±39*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Ca2+]m, b</td>
<td>120±24</td>
<td>248±31*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(28)</td>
<td></td>
<td>(17)</td>
</tr>
<tr>
<td>Nifedipine (5 μM)</td>
<td>ACh</td>
<td>[Ca2+]b, b</td>
<td>120±23</td>
<td>127±33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Ca2+]m, b</td>
<td>120±23</td>
<td>127±33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(42)</td>
<td></td>
<td>(19)</td>
</tr>
<tr>
<td>KCl (50 μM)</td>
<td>ACh</td>
<td>[Ca2+]b, b</td>
<td>121±20</td>
<td>125±29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Ca2+]m, b</td>
<td>121±20</td>
<td>125±29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(52)</td>
<td></td>
<td>(32)</td>
</tr>
<tr>
<td>Tetrodotoxin (5 μM)</td>
<td>ACh</td>
<td>[Ca2+]b, b</td>
<td>120±23</td>
<td>155±36*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Ca2+]m, b</td>
<td>378±56</td>
<td>351±67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(29)</td>
<td></td>
<td>(23)</td>
</tr>
<tr>
<td>KCl (50 μM)</td>
<td>ACh</td>
<td>[Ca2+]b, b</td>
<td>116±25</td>
<td>153±40*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Ca2+]m, b</td>
<td>826±196</td>
<td>719±234*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(48)</td>
<td></td>
<td>(45)</td>
</tr>
</tbody>
</table>

Values expressed in nM, are means±SD of the number of muscle cells (listed between parentheses) from at least three individual cultures. Concentrations of ACh and KCl are 20 μM and 125 mM, respectively. Parameters of DMPK[-/-] and wild-type cells differ with \*P < 0.01, and \textsuperscript{1}P < 0.05. In the presence of tetrodotoxin, \( \tau _{g} \) of the KCl-evoked Ca2+ responses in wild-type and mutant cells are 3.1±0.9 s and 4.2±1.3 s (P < 0.01), whereas \( \tau _{d} \) are 4.9±1.1 s and 5.5±1.5 s (P < 0.05), respectively.

Discussion

The focus of interest in DM research has slowly drifted from the behavior of the unstable (CTG), repeat to the actual function(s) of individual genes in the mutant chromosome 19q area. Three candidate genes for the DM locus have now been characterized: DMR-N9, DMPK, and DMAHP (34, 35). As the expanding repeat in DM actually interrupts the gene for DMPK, most efforts in explaining disease etiology have been directed towards the involvement of the product(s) of this single gene. The results presented in this paper provide novel evidence that there is a specific relationship between DMPK activity and Ca2+ homeostasis in skeletal muscle cells. Although results from DMPK-cDNA transfection studies in BC3H1 cells pointed to a function of DMPK in the myogenic pathway (36), we conclude from study of the close-to-natural context presented here, that the sole absence of DMPK does not appear to interfere with the growth and differentiation profiles of muscle-derived satellite cells in vitro. Neither the morphological appearance, nor the maturation grade, or the ability to contract, differed between wild-type and mutant cells. Moreover, our observations are in accordance with the absence of any gross morphological and structural modifications in vivo in mice that lack or overexpress the DMPK gene (9, 10).

The behavior of Ca2+ homeostasis in cultured muscle cells depends on DMPK activity. Estimates for resting [Ca2+]i values of mouse skeletal muscle cells in culture clearly depend on the cell origin, the Ca2+ indicator used for assaying, and the calibration procedure applied. All we can say is that our value for the resting [Ca2+]i in cultured muscle cells of wild-type mice, based on the in vivo calibration of Fura-2, falls well within the range of values published for cultured mouse myotubes (37) and myoﬁbers in vivo (38). Since inhibition of the SR Ca2+ release channel by ryanodine effectively blocks depolarization-induced Ca2+ efflux in cultured wild-type cells (Fig. 2, B and C), these cells, like those of rats and humans, must possess a skeletal muscle type of E-C coupling mechanism, rather than a cardiac muscle type, in which Ca2+ release is provoked by an influx of external Ca2+ (28, 31). Moreover, it suggests that the contribution of a Ca2+ influx through the ion-unselective AChR (39), the dihydropyridine-insensitive fast-activated voltage-operated T-type Ca2+ channel (40), and/or the slow-activated voltage-dependent L-type Ca2+ channel, i.e., DHPR (41), during depolarization is negligibly small, and/or is ineffective to induce Ca2+ release from the SR. This does not rule out that Ca2+ entry via the DHPR may play a role in long-lasting depolarizations in wild-type cells, but usually this process is slower than the charge movement in DHPR and the associated activation of the RyR (41).

The situation in mutant myotubes is clearly different in that absence of DMPK augments the resting [Ca2+]i, and has dampening effects on the release and sequestration of Ca2+ upon depolarization. Application of inhibitors showed that the phenomenon of higher resting [Ca2+]i, and the altered excitability are somehow linked to the mode of action of voltage-dependent Ca2+ and Na+ channels. It is unlikely that a recently described Ca2+-specific leak channel is involved, as the situation...
Cultured muscle cells

DMPK[-/-]  Wild-type

Feature that is inconsistent with the observed high \([\text{Ca}^{2+}]_j\) leads to an increase of the \([\text{Ca}^{2+}]\) current (45), and we would expect absence of phosphorylation to reduce this current, a feature that is inconsistent with the observed high \([\text{Ca}^{2+}]_j\). Thus, although the cell model is clearly too simplistic, our findings do not provide direct answers to this question, as the effects of CTG expansion on DMPK expression still have not been clearly resolved. The cell model is clearly too simplistic, our findings do not provide direct answers to this question, as the effects of CTG expansion on DMPK expression still have not been clearly resolved.

Relevance for disease etiology? Since \([\text{Ca}^{2+}]_j\) is a crucial regulator of many physiological processes, elevated \([\text{Ca}^{2+}]_j\) could potentially have profound consequences in vivo. Can we extrapolate our in vitro findings and relate abnormal \([\text{Ca}^{2+}]_j\) homeostasis to disease manifestation in DM patients? We realize that our knockout mouse and cell models do not provide a direct answer to this question, as the effects of CTG expansion on DMPK expression still have not been clearly resolved. The cell model is clearly too simplistic, our findings do not provide direct answers to this question, as the effects of CTG expansion on DMPK expression still have not been clearly resolved.

Table III. SR Ca\(^{2+}\)-ATPase and Na\(^+\)/K\(^+\)-ATPase in Muscle and Cultured Muscle Cells of Wild-type and DMPK[-/-] Mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Muscle</th>
<th>Wild-type</th>
<th>DMPK[-/-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR Ca(^{2+})-ATPase activity (mU/mg protein)</td>
<td>76.2 ± 7.4 (5)</td>
<td>75.9 ± 5.0 (5)</td>
<td>75.0 ± 4.5 (3)</td>
</tr>
<tr>
<td>SR Ca(^{2+})-ATPase content (pmol/mg protein)</td>
<td>94.9 ± 8.0 (3)</td>
<td>94.7 ± 5.8 (3)</td>
<td>94.7 ± 5.8 (3)</td>
</tr>
<tr>
<td>Molecular activity (min(^{-1}))</td>
<td>802 ± 14 (5)</td>
<td>801 ± 9 (3)</td>
<td>800 ± 8 (8)</td>
</tr>
<tr>
<td>Na(^+)/K(^+)-ATPase activity (mU/mg protein)</td>
<td>0.57 ± 0.06 (5)</td>
<td>0.56 ± 0.08 (3)</td>
<td>0.93 ± 0.09 (7)</td>
</tr>
<tr>
<td>Na(^+)/K(^+)-ATPase content (pmol/mg protein)</td>
<td>6.3 ± 0.6 (5)</td>
<td>6.2 ± 0.2 (3)</td>
<td>6.2 ± 0.2 (3)</td>
</tr>
<tr>
<td>Molecular activity (min(^{-1}))</td>
<td>91 ± 7 (5)</td>
<td>89 ± 11 (3)</td>
<td>92 ± 8 (5)</td>
</tr>
</tbody>
</table>

Values are mean±SD of the number of muscles or muscle cell cultures (given between parentheses). The activity and content of SR \(\text{Ca}^{2+}\)-ATPase were determined by \(\text{Ca}^{2+}\)-dependent ATP hydrolysis and steady-state phosphorylation, respectively. The \(\text{K}^{+}\)-dependent, ouabain-sensitive hydrolysis of 3-O-MFP and the binding capacity of ouabain were used as a measure for the activity and content of Na\(^+/K\(^{+}\)-ATPase, respectively.

Since \([\text{Ca}^{2+}]_j\) is a crucial regulator of many physiological processes, elevated \([\text{Ca}^{2+}]_j\) could potentially have profound consequences in vivo. Can we extrapolate our in vitro findings and relate abnormal \([\text{Ca}^{2+}]_j\) homeostasis to disease manifestation in DM patients? We realize that our knockout mouse and cell models do not provide a direct answer to this question, as the effects of CTG expansion on DMPK expression still have not been clearly resolved. The cell model is clearly too simplistic, our findings do not provide direct answers to this question, as the effects of CTG expansion on DMPK expression still have not been clearly resolved.

Thus, although the cell model is clearly too simplistic, our finding of similar disturbances in \([\text{Ca}^{2+}]_j\) homeostasis in muscle cells of both DM patients and DMPK[-/-] mice lends indirect support to the contention that the DM mutation results in a redistribution of DMPK mRNA and protein isoforms, in line with the molecular explanations provided by others (8, 60, 61). In conclusion, our study provides new insight in the relation between DMPK activity and \([\text{Ca}^{2+}]_j\) responsive-
ness in skeletal muscle. The suggestion that DMPK modulates the movement of gating charge and/or the activity of voltage-gated ion channels opens up new possibilities for studies into the molecular etiology of DM.

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References


