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Deficiency of Na^+/K^+-ATPase and sarcoplasmic reticulum Ca^{2+}-ATPase in skeletal muscle and cultured muscle cells of myotonic dystrophy patients

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Since defective regulation of ion transport could initiate or contribute to the abnormal cellular function in myotonic dystrophy (MyD), Na^+/K^+-ATPase and sarcoplasmic reticulum (SR) Ca^{2+}-ATPase were examined in skeletal muscle and cultured skeletal muscle cells of controls and MyD patients. Na^+/K^+-ATPase was investigated by measuring ouabain binding and the activities of Na^+/K^+-ATPase and K^+-dependent 3-*O*-methylfluorescein phosphate (3-*O*-MFPase). SR Ca^{2+}-ATPase was analysed by e.l.i.s.a., Ca^{2+}-dependent phosphorylation and its activities with ATP and 3-*O*-methylfluorescein phosphatase (3-*O*-MFP). In MyD muscle the K^+-dependent 3-*O*-MFPase activity and the activity and concentration of SR Ca^{2+}-ATPase were decreased by 40%. In cultured muscle cells from MyD patients the activities as well as the concentration of both Na^+/K^+-ATPase and SR Ca^{2+}-ATPase were reduced by about 30–40%. The ouabain-binding constant and the molecular activities, i.e. catalytic-centre activities with ATP or 3-*O*-MFP, of Na^+/K^+-ATPase and SR Ca^{2+}-ATPase were similar in muscle as well as in cultured cells from both controls and MyD patients. Thus the decreased activity of both ATPases in MyD muscle is caused by a reduction in the number of their molecules. To check whether the deficiency of ATP-dependent ion pumps is a general feature of the pathology of MyD, we examined erythrocytes from the same patients. In these cells the Ca^{2+} uptake rate and the Ca^{2+}-ATPase activity were lower than in controls, but the Ca^{2+}-ATPase concentration was normal. Thus the reduced Ca^{2+}-ATPase activity is caused by a decrease in the molecular activity of the ion pump. The Na^+/K^+-ATPase activity is also lower in erythrocytes of MyD patients. It is concluded that the observed alterations in ion pumps may contribute to the pathological phenomena in the muscle and other tissues in patients with MyD.

INTRODUCTION

Myotonic dystrophy (MyD) is the most important hereditary muscle disease in humans. Clinical manifestations include increased excitability, delayed relaxation, weakness and wasting of muscle and abnormalities of various non-muscle systems. Recently the gene mutation underlying MyD has been identified as an expansion of polymorphic CTG repeats [1–3]. However, the molecular basis of the disease has not yet been resolved. Experimental and clinical data suggest that MyD arises from structural and functional alterations of membranes.

Muscle fibres of MyD patients show a decreased resting membrane potential [4] and an increased intracellular Na^+ concentration [5] compared with controls. These observations are consistent with a reduced number of ouabain-binding sites [6], a raised Na^+ conductance at rest [7], an altered inactivation of Na^+ channels [8] and an abnormal M-wave during exercise [9]. Renaud et al. demonstrated the presence of an apamin receptor (Ca^{2+}-activated K^+ channel) in MyD muscle which is absent from normal human muscle [10]. Moreover, MyD patients show an excessive increase in plasma K^+ after ischaemic exercise [11]. However, the association between these observations and the electrophysiological properties of the MyD muscle remains to be established.

In cultured muscle cells from MyD patients, a decreased resting membrane potential [12,13] and a delayed activation and inactivation of Na^+ channels [14] are observed. Furthermore, we measured a raised cytosolic Ca^{2+} concentration caused by changed voltage-operated Ca^{2+} channels, which are active under conditions in which they are normally present in the inactive state in controls [15].

Divergent changes are reported for the activity and stoichiometry of Na^+/K^+-ATPase, the activity of Ca^{2+}-ATPase, the Ca^{2+} permeability and the physical state of the plasma membrane in erythrocytes of MyD patients [7]. Monocytes and fibroblasts of MyD patients displayed changes in insulin binding, but not in the insulin receptors [16–18]. Receptors of other systems as kidney, smooth muscle, lymphocytes and polymorphonuclear leucocytes also show a changed response to various stimuli [19–21]; however, the occurrence of some of these changes has been questioned [22].

Defective regulation of ion transport could initiate or contribute to the abnormal cellular function in MyD. During an action potential the cytosolic Na^+ concentration in skeletal muscle rises, whereas the intracellular K^+ concentration decreases. This depolarization of the sarcolemma provokes Ca^{2+} release from the sarcoplasmic reticulum (SR) into the cytosol, which causes muscle contraction. After excitation of skeletal muscle, ATP-driven ion pumps restore the disturbed ion homeostasis. Na^+/K^+-ATPase of the plasma membrane re-establishes the Na^+ and K^+ gradients by K^+ uptake from the blood plasma into the resting muscle and Na^+ release [23]. SR Ca^{2+}-ATPase transports Ca^{2+} from the cytoplasm into the lumen of the SR [24].

Abbreviations used: Ca^{2+}-ATPase, Ca^{2+}-dependent Mg^{2+}-ATPase (EC 3.6.1.38); CK-MM, creatine kinase muscle-specific isoenzyme MM; MyD, myotonic dystrophy; Na^+/K^+-ATPase, $Na^+ + K^+$-dependent ATPase (EC 3.6.1.37); SR, sarcoplasmic reticulum; 3-*O*-MFPase, 3-*O*-methylfluorescein phosphatase; 3-*O*-MFP, 3-*O*-methylfluorescein phosphate.

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Previously we compared different parameters of Na^+/K^+-ATPase and SR Ca^{2+}-ATPase in human skeletal muscle and cultured human muscle cells [25]. Immunocytochemical localization of Na^+/K^+-ATPase and SR Ca^{2+}-ATPase in highly differentiated cultured muscle cells revealed a cross-striated appearance, indicating the development and subcellular organization of a T-tubule system and SR respectively, which resembles the *in vivo* situation [25].

To check whether the reinstatement of ion homeostasis is generally affected in MyD muscle we investigated the activities and the contents of both Na^+/K^+-ATPase and SR Ca^{2+}-ATPase in skeletal muscle as well as in cultured muscle cells from MyD patients. Muscle cells at different maturation grades were cultured [26] to examine the possibility of a maturation-related disturbance of ion transport in MyD muscle. Erythrocyte Ca^{2+}-ATPase and Na^+/K^+-ATPase were also analysed as examples of ATP-dependent ion pumps in non-muscle cells.

MATERIALS AND METHODS

Materials

Calmodulin was obtained from Sigma, St. Louis, MO, U.S.A., and $^{45}CaCl_2$ was from Amersham International, Amersham, Bucks., U.K. Goat anti-(rabbit renal Na^+/K^+-ATPase) was a gift from Dr. J. J. H. H. M. de Pont (Department of Biochemistry, University of Nijmegen, The Netherlands). Rabbit anti-(rat SR Ca^{2+}-ATPase) was raised against a mixture of slow- and fast-type SR Ca^{2+}-ATPase [27]. Other materials were purchased as reported earlier [25].

Patients

Eleven unrelated patients (nine male, two female) with MyD varied in age between 19 and 58 years. All patients had electromyographic evidence of myotonia, displayed characteristic clinical features of MyD [28] and had an excessive K^+ release into the blood upon a standardized ischaemic forearm exercise [11]. Controls displayed no metabolic or electrophysiological disorders. This study was approved by the Ethical Committee of the University of Nijmegen.

Muscle cell cultures and homogenates

Muscle samples from m. quadriceps, rectus abdominis, gluteus, erector trunci or biceps were dissociated and isolated satellite cells were grown on fetal calf serum- or Ultroser G-containing growth medium [26]. The cells fused and subsequently differentiated on horse serum- and Ultroser G-containing medium respectively. Cultures were harvested 7–20 days after the onset of fusion. Homogenates were prepared in a glass homogenizer with a tight-fitting Teflon pestle (clearance 50 μm) rotating at 1000 rev./min. To exclude sealed vesicles, the homogenates were freeze–thawed four times [25].

Na^+/K^+-ATPase and SR Ca^{2+}-ATPase

Na^+/K^+-ATPase was investigated by measuring ouabain binding and the activities of Na^+/K^+-ATPase and K^+-dependent 3-*O*-methylfluorescein phosphatase (3-*O*-MFPase) as described previously [25]. SR Ca^{2+}-ATPase was examined by an e.l.i.s.a., and by measuring Ca^{2+}-dependent phosphorylation and its activities with ATP and 3-*O*-methylfluorescein phosphate (3-*O*-MFP) [25].

Erythrocyte Ca^{2+}-ATPase and Na^+/K^+-ATPase

The amount of Ca^{2+}-ATPase was quantified by an e.l.i.s.a., as published before [27], using calmodulin coating and rabbit anti-(human erythrocyte Ca^{2+}-ATPase). Erythrocyte inside-out vesicles free of calmodulin and haemoglobin were prepared according to Steck [29]. The initial $^{45}Ca^{2+}$ uptake rate into these vesicles was measured as described by Van Corven et al. [30] in the presence of 0.1 μM $^{45}CaCl_2$ and 20 nM calmodulin, with or without 3 mM ATP, during 1 min at 37 °C. Erythrocyte Ca^{2+}-ATPase activity was determined as Ca^{2+}-dependent ATPase activity. Vesicle protein (50 μg) was incubated in 100 mM imidazole/100 mM KCl/5 mM $MgCl_2$/0.5 mM EGTA/5 mM ATP (pH 7.4) in the absence or presence of 0.54 mM $CaCl_2$ at 37 °C. After 2 h the reaction was stopped and the system analysed for inorganic phosphate [31].

Erythrocyte Na^+/K^+-ATPase activity was measured in saponin-permeabilized vesicles as ouabain-sensitive ATPase activity, as published by Hanahan and Ekholm [32]. After 2 h of incubation at 37 °C, inorganic phosphate was measured [31].

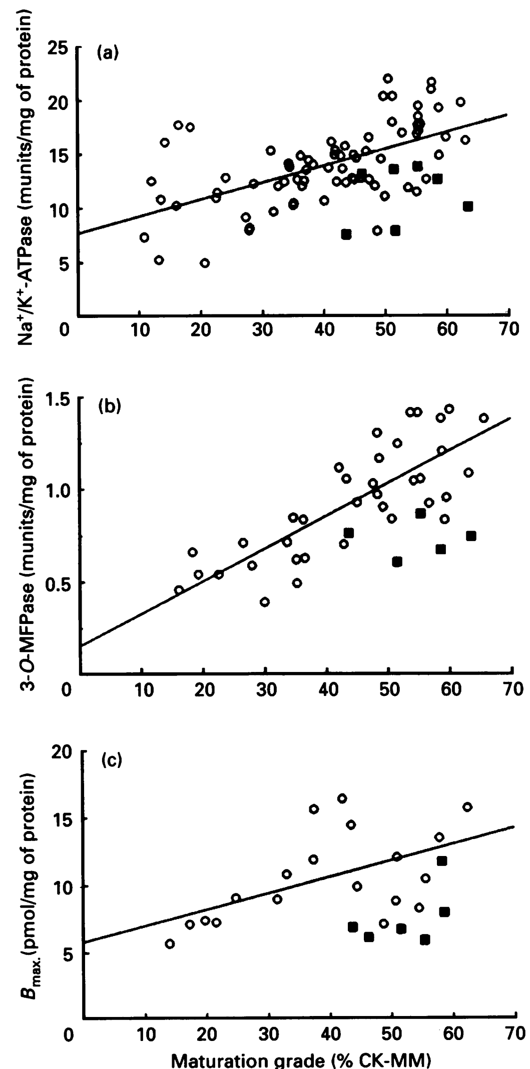

Figure 1 Activities of Na^+/K^+-ATPase (a) and K^+-dependent 3-*O*-MFPase (b), and the maximum number of ouabain-binding sites (c), in cultured muscle cells of controls (○) and MyD patients (■)

The maturation grade of the cells is expressed as percentage CK-MM. The lines represent the linear-regression curves of the control values ($P < 0.01$).

Table 1 Na^+/K^+-ATPase in muscle and cultured muscle cells of controls and MyD patients

Values are means ± S.D. of the numbers of individuals given between parentheses. Units of activity represent μmol of ATP or 3-*O*-MFP hydrolysed/min at 37 °C. The maturation grade (percentage CK-MM) of cultured control and MyD muscle cells varied between 43.7 and 63.5% (activities of Na^+/K^+-ATPase and K^+-dependent 3-*O*-MFPase) or 43.7 and 58.6% respectively (B_{max}). Parameters significantly different from controls are indicated by $^*P < 0.01$ $^{**}P < 0.05$. N.D., not detectable.

Parameter	Muscle		Muscle cultures	
	Controls	MyD	Controls	MyD
Na^+/K^+-ATPase (munits/mg of protein)	N.D.	–	16.1 ± 3.3 (32)	11.2 ± 2.7* (7)
K^+-dependent 3-*O*-MFPase (μunits/mg of protein)	399 ± 62 (18)	245 ± 49* (9)	1108 ± 204 (19)	727 ± 98* (5)
B_{max} (ouabain binding) (pmol/mg of protein)	4.1 ± 0.8 (5)	–	10.6 ± 2.6 (8)	7.5 ± 2.2** (6)
K_d (ouabain binding) (nM)	257 ± 82 (5)	–	245 ± 54 (8)	213 ± 33 (6)
Molecular activity (ATP) (min^{-1})	–	–	1579 ± 461 (8)	1768 ± 432 (5)
Molecular activity (3-*O*-MFP) (min^{-1})	101 ± 21 (5)	–	110 ± 30 (8)	108 ± 28 (4)

Other procedures

For the determination of the percentage of creatine kinase MM (CK-MM), as a measure of the maturation grade of muscle cultures, this isoenzyme was separated from other CK isoenzymes by anion-exchange chromatography and analysed as reported [33]. The protein content was assayed according to Lowry et al. [34] with BSA as standard. Na^+/K^+-ATPase and SR Ca^{2+}-ATPase were immunocytochemically studied in cryosections of muscle [25]. For fibre type analysis, myofibrillar ATPase was histochemically investigated [35].

Statistics

Data represent means ± S.D. Statistical analysis was performed by using the unpaired Student's *t* test, and significance was set at $P < 0.05$.

RESULTS

General characteristics of muscle

The non-collagen protein content varied in control muscle between 130 and 160 mg and in MyD muscle between 110 and 150 mg per g wet wt. The fibre-type composition of muscle depended on the muscle type. In controls, m. quadriceps and biceps exhibited 34–51 % type I fibres, m. rectus abdominis 35–57 %, m. gluteus 41–72 % and m. erector trunci 33–84 %. In MyD muscle, the type I fibres in m. quadriceps and biceps ranged from 31 to 59 %, and in m. erector trunci from 62 to 87 %.

Na^+/K^+-ATPase

Na^+/K^+-ATPase activity is hardly detectable in homogenates of muscle biopsies, because of an excess of Mg^{2+}-ATPase activity. The K^+-dependent hydrolytic cleavage of the artificial substrate 3-*O*-MFP and its inhibition by ouabain can be used as a measure of Na^+/K^+-ATPase activity in muscle [36]. Muscle fibre-type composition and aging (14–58 years) had no effect on the K^+-dependent 3-*O*-MFPase activity (results not shown). The maximal ouabain-binding capacity, i.e. the number of Na^+/K^+-ATPase molecules, of normal human skeletal muscle (360 ± 70 pmol/g wet wt.; $n = 5$) agrees with earlier data [37–40]. The latent Na^+/K^+-ATPase activity is maximally exposed in freeze–thawed homogenates of cultured cells [25]. The Na^+/K^+-ATPase and K^+-dependent 3-*O*-MFPase activities and the number of Na^+/K^+-ATPase molecules increased in cultured muscle cells when their maturation grade increases (Figure 1), and are always higher than in muscle biopsies (Table 1). The dissociation constant and the molecular phosphatase activity did not differ in skeletal muscle and cultured muscle cells (Table 1).

The K^+-dependent 3-*O*-MFPase activity was lowered by 40 % in MyD muscle (Table 1). Due to a lack of sufficient biopsy material the ouabain-binding capacity of MyD muscle could not be determined. The cytochemical distribution of Na^+/K^+-ATPase is not disturbed in cryosections of MyD muscle (results not shown). In cultured muscle cells from MyD patients the activities of Na^+/K^+-ATPase and K^+-dependent 3-*O*-MFPase and the number of ouabain-binding sites were significantly reduced (30–40 %) compared with control cells of the same maturation grade (Figure 1; Table 1). The dissociation constant and the molecular activities (catalytic centre activities with ATP or 3-*O*-MFP) did not differ. These results imply that the decrease in Na^+/K^+-ATPase activity in cultured MyD muscle cells is caused by a lowered Na^+/K^+-ATPase content. The decrease in K^+-dependent 3-*O*-MFPase activity in MyD muscle also indicates a decreased ATP-dependent ion transport.

SR Ca^{2+}-ATPase

SR Ca^{2+}-ATPase was examined only in m. quadriceps and biceps. This ion pump is maximally activated at a free Ca^{2+} concentration of about 5 μM, and thapsigargin (1 μM), a specific inhibitor of SR Ca^{2+}-ATPase, inhibits 90 % of the Ca^{2+}-dependent phosphatase activity and phosphorylation [25]. Thus the measured parameters represent quite well the parameters of the SR Ca^{2+} pump. The content of SR Ca^{2+}-ATPase can be quantified by Ca^{2+}-dependent phosphorylation and e.l.i.s.a.. The former assay assesses the total concentration of SR Ca^{2+}-ATPase under steady-state conditions, where all enzyme molecules are in a phosphorylated state, since dephosphorylation of the phosphoenzyme is the rate-limiting step in fast- as well as slow-twitch muscle [37]. The latter assay also measures total SR Ca^{2+}-ATPase, since an antiserum raised against a mixture of fast- and slow-type SR Ca^{2+}-ATPase is used [25]. The activity and the concentration of SR Ca^{2+}-ATPase showed slight but significant

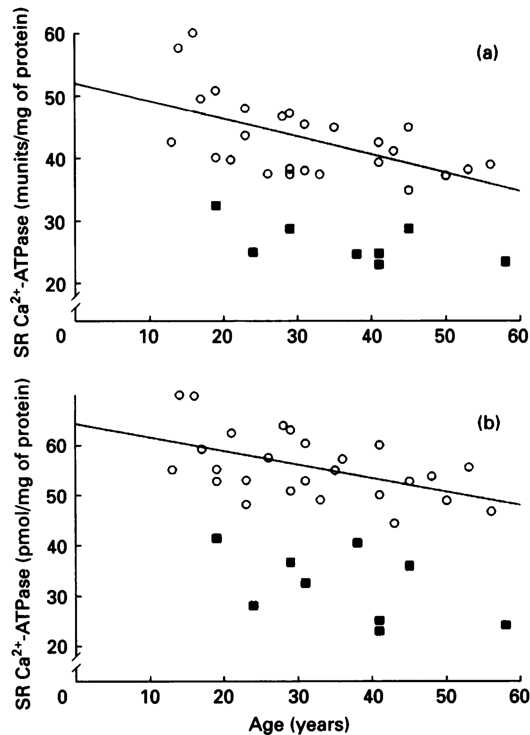

Figure 2 Effect of aging on the SR Ca^{2+}-ATPase activity (a) and content (b) of control (○) and MyD (■) muscle

Other details are given in the legend to Figure 1.

age-related decreases in human muscle (Figure 2). The content of SR Ca^{2+}-ATPase in adult human skeletal muscle (6.7 ± 1.3 nmol/g wet wt.; $n = 20$) is comparable to previously published data [37,40]. The activities of SR Ca^{2+}-ATPase and Ca^{2+}-dependent 3-*O*-MFPase, and the content of SR Ca^{2+}-ATPase, rose in cultured muscle cells as the maturation grade increased (Figure 3), but remained lower than in muscle biopsies (Table 2). The molecular activities of SR Ca^{2+}-ATPase with ATP or 3-*O*-MFP as substrates were similar in muscle and cultured cells (Table 2).

The activity as well as the content of SR Ca^{2+}-ATPase were reduced by about 35–40% in both muscle (Figure 2; Table 2) and cultured muscle cells (Figure 3; Table 2) of MyD patients compared with controls of the same age or maturation grade respectively. No dislocation of SR Ca^{2+}-ATPase was detected in MyD muscle cryosections (results not shown). The molecular activities of SR Ca^{2+}-ATPase did not differ from the control values (Table 2). These observations indicate that the decrease in SR Ca^{2+}-ATPase activity is due to a decrease in the content of SR Ca^{2+}-ATPase.

Erythrocyte Ca^{2+}-ATPases and Na^+/K^+-ATPase

The Ca^{2+}-dependence of the Ca^{2+}-ATPase of erythrocytes can be determined using Ca^{2+}-(0.5 mM)EGTA buffers. The free Ca^{2+} concentration is measured in the presence of vesicle protein with the Ca^{2+} probe Fura-2 [41]. Erythrocyte Ca^{2+}-ATPase was maximally activated at 6 μM free Ca^{2+}, and the K_m value of 0.9 μM was in agreement with reported data [42]. The ion pump was nearly completely inhibited by 5 μM vanadate. In erythrocytes of MyD patients the Ca^{2+} uptake and Ca^{2+}-ATPase activity were

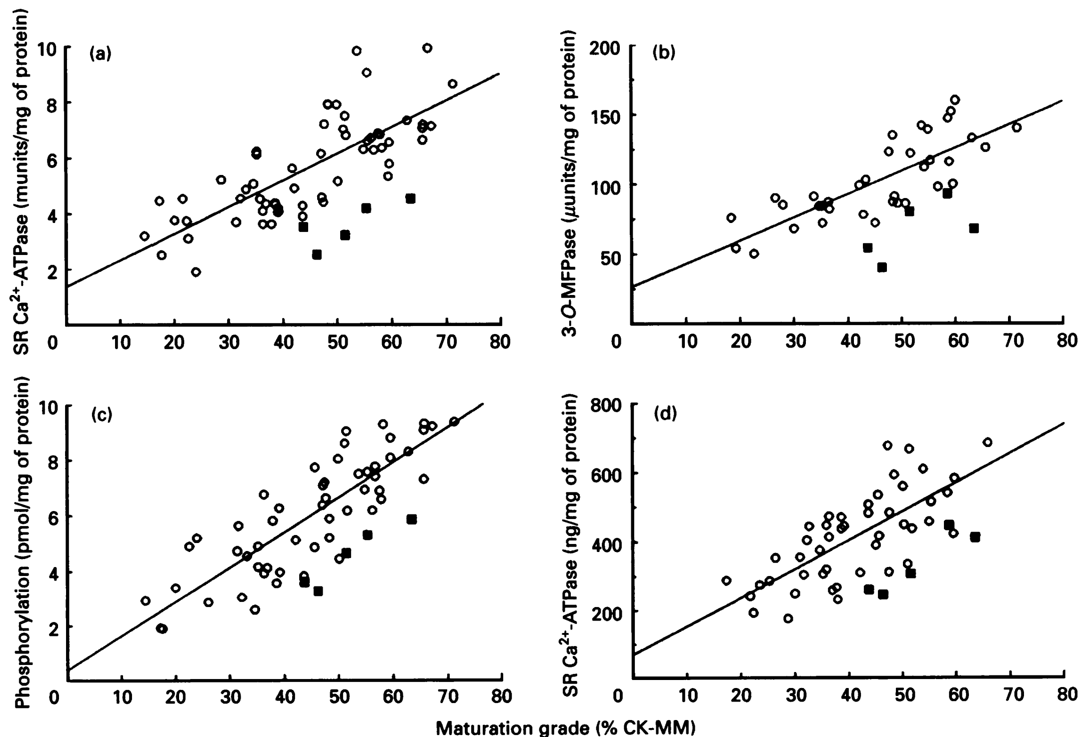

Figure 3 Activities of SR Ca^{2+}-ATPase (a) and Ca^{2+}-dependent 3-*O*-MFPase (b), and the concentration of SR Ca^{2+}-ATPase, as quantified by phosphorylation (c) or e.l.i.s.a. (d), of cultured control (○) and MyD (■) muscle cells

Parameters are related to the maturation grade of the cells. For other details, see the legend to Figure 1.

Table 2 SR Ca^{2+}-ATPase in muscle and cultured muscle cells of controls and MyD patients

Values are means ± S.D. of the numbers of individuals given between parentheses. The age of the controls and MyD patients ranged from 19 to 58 years. The maturation grade (percentage CK-MM) of cultured control and MyD muscle cells varied between 43.7 and 63.5%. Parameters significantly different from controls are indicated by: $^{*}P < 0.01$. For other details, see the legend to Table 1.

	Muscle		Muscle cultures	
Parameter	Controls	MyD	Controls	MyD
SR Ca^{2+}-ATPase (munits/mg of protein)	41.4 ± 4.3 (22)	26.3 ± 3.3* (8)	6.35 ± 1.41 (26)	3.75 ± 0.79* (5)
Ca^{2+}-dependent 3-*O*-MFPase (μunits/mg of protein)	1019 ± 131 (18)	628 ± 163* (9)	112 ± 28 (19)	67 ± 21* (5)
SR Ca^{2+}-ATPase content (ng/mg of protein)	6039 ± 1001 (19)	3685 ± 887* (8)	496 ± 104 (20)	325 ± 92* (5)
Phosphorylation (pmol/mg of protein)	54.3 ± 5.4 (22)	32.0 ± 7.1* (9)	7.02 ± 1.39 (26)	4.53 ± 1.10* (5)
Molecular activity (ATP) (min^{-1})	792 ± 107 (20)	848 ± 132 (8)	916 ± 237 (23)	796 ± 105 (5)
Molecular activity (3-*O*-MFP) (min^{-1})	18 ± 2 (16)	18 ± 4 (9)	17 ± 4 (14)	14 ± 2 (4)

Table 3 Ca^{2+}-ATPase and Na^{+}/K^{+}-ATPase in erythrocytes of controls and MyD patients

Values are means ± S.D. of eight controls and eight MyD patients. Units of activity represent μmol of ATP hydrolysed/min at 37 °C. Values significantly different from controls are indicated by: $^{*}P < 0.01$.

Parameter	Controls	MyD
$^{45}Ca^{2+}$ uptake (nmol/min per mg of protein)	9.22 ± 2.44	5.76 ± 1.19*
Ca^{2+}-ATPase (munits/mg of protein)	8.84 ± 1.78	5.30 ± 0.91*
Ca^{2+}-ATPase (μg/mg of protein)	1.64 ± 0.38	1.64 ± 0.23
Na^{+}/K^{+}-ATPase (munits/mg of protein)	14.1 ± 2.1	9.7 ± 2.0*

40 % lower than in controls whereas the content of Ca^{2+}-ATPase was unchanged (Table 3). Assuming a molecular mass of the Ca^{2+}-ATPase of 140 kDa [42], the molecular activities can be calculated. The ion pump in control erythrocytes was estimated to transport 790 Ca^{2+} ions/min and hydrolyse 760 ATP molecules/min, versus 490 Ca^{2+} ions/min and 450 ATP molecules/min in MyD erythrocytes. Our results imply that the reduction in the activity of the erythrocyte Ca^{2+}-ATPase in MyD is caused by a decreased molecular activity of the ion pump. However the stoichiometry of the erythrocyte Ca^{2+} pump is unaffected in MyD. The Na^{+}/K^{+}-ATPase activity was also lower in erythrocytes of MyD patients than in those of controls (Table 3).

DISCUSSION

Myotonia is a transient uncontrollable muscle tension that occurs during muscle contraction which is caused by trains of repetitive action potentials in response to a contraction, and as a result the skeletal muscles are unable to relax normally [7]. All myotonias appear to be due to an abnormality of the muscle itself, since they persist after section or blocking of the motor nerve and after curarization [42]. Several mechanisms have been proposed to explain the different types of myotonias. A reduced chloride conductance underlies myotonia congenita [43]; however, it seems to be insignificant in MyD [7]. In primary hyperkalaemic periodic paralysis, primary hypokalaemic paralysis and MyD an increased permeability to Na^{+} appears to be responsible for the myotonia [44]. A tight control of the Na^{+}/K^{+} transport is essential for the maintenance of optimal muscle function [23]. The capacity for muscle performance is evidently related to the Na^{+}/K^{+}-ATPase concentration [45]. The lowered K^{+}-dependent 3-*O*-MFPase activity is in agreement with the decreased concentration of ouabain-binding sites, as measured in microsomal fractions of MyD muscle [6]. This decrease can explain the raised intracellular Na^{+} concentration [5] and the lower resting membrane potential [4]. Furthermore, it might contribute to the abnormally high increase in the plasma K^{+} concentration upon muscular exercise in MyD and the reduced amount of work performed by MyD patients [11]. In cultured MyD muscle cells the decrease in the activity of the Na^{+}/K^{+} pump is due to a lowered concentration of Na^{+}/K^{+}-ATPase, and is probably the cause of the decreased resting membrane potential in these cells [12,13].

SR Ca^{2+}-ATPase constitutes about 90 % of the total protein content in the SR membrane [46]. Human skeletal fast-twitch muscle fibres have a higher content of SR Ca^{2+}-ATPase than slow-twitch fibres [25]. The decreases in the activity and concentration of SR Ca^{2+}-ATPase in human skeletal muscle upon aging is associated with a selective atrophy of fast-twitch fibres [47,48]. In muscle as well as in cultured cells of MyD patients the decrease in the activity of the SR Ca^{2+}-ATPase is caused by a reduction of the SR Ca^{2+}-ATPase content. This is the first time that a change at the protein level has been detected in an intracellular membrane of MyD muscle. Ultrastructural modifications in the SR have been shown previously (reviewed in [49]). The reduced Ca^{2+} uptake of the SR may contribute to the abnormal relaxation of MyD muscle and reinforce the myotonia. Moreover, in combination with an additional type of Ca^{2+}-activated K^{+} channel (apamin receptor [10]), it could cause the excessive increase in plasma K^{+} [11], which in its turn can induce myotonia [50,51].

The reductions in the activity as well as the content of both ion pumps investigated were of the same order of magnitude in the adult muscle and cultured muscle cells of MyD patients. The differences seem to be specific, because neither the fibre-type distribution nor the protein content of the examined MyD muscle are changed compared with control muscle. Fibre type I

predominance was not observed in MyD muscle, in contrast to previously published data [35,52]. The activities and content of Na^+/K^+-ATPase and SR Ca^{2+}-ATPase in highly matured cultured MyD muscle cells are comparable with those in less mature control cells. A differentiation-related disturbance of membranes might be the origin of the measured alterations in the muscle of MyD patients. This is also suggested by morphological observations in neonatal MyD muscle [53] and the persistent expression of two membrane antigens [54] and the apamin receptor [10] in MyD muscle, in contrast to the situation in normal human muscle.

To check whether ATP-driven ion pumps are generally affected, Ca^{2+}-ATPase and Na^+/K^+-ATPase were examined in erythrocytes from MyD patients. The Ca^{2+}-ATPase activity in erythrocytes of controls agreed with earlier published data [55,56]. The lowered activity of the Ca^{2+}-ATPase in MyD erythrocytes is provoked by a diminished molecular activity of the ion pump. Thus, in MyD, the plasma membrane Ca^{2+}-pump is altered in a different way in erythrocytes compared with the ion pumps investigated in muscle. Luthra et al. also found a decrease in Ca^{2+}-ATPase activity of erythrocytes from MyD patients [55], but others have observed no change or an increase [7]. The reduced Na^+/K^+-ATPase activity in MyD erythrocytes can explain their increased intracellular Na^+ concentration [57].

In conclusion, skeletal muscle and cultured muscle cells from MyD patients show decreased Na^+/K^+-ATPase and SR Ca^{2+}-ATPase activities due to a decrease in the content of these proteins. A maturation-related disturbance of membranes may play a role in MyD. The detected changes may contribute to the characteristics of the pathology in muscle and other tissues.

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