Sternohyoid Muscle Fatigue Properties of *dy/dy* Dystrophic Mice, an Animal Model of Merosin-Deficient Congenital Muscular Dystrophy

ERIK van LUNTEREN AND MICHELLE MOYER

Departments of Medicine (Pulmonary) [E.v.L., M.M.] and Neurosciences [E.v.L.], Cleveland VA Medical Center and Case Western Reserve University, Cleveland, Ohio 44106, U.S.A.

ABSTRACT

Humans with merosin-deficient congenital muscular dystrophy have both sucking problems during infancy and sleep-disordered breathing during childhood. We hypothesized that merosin-deficient pharyngeal muscles fatigue faster than normal muscles. This was tested *in vitro* using sternohyoid muscle from an animal model of this disease, the *dy/dy* dystrophic mouse. Isometric twitch contraction and half-relaxation times were similar for *dy/dy* and normal sternohyoid. However, rate of force loss during repetitive 25-Hz train stimulation was markedly diminished in dystrophic compared with normal sternohyoid muscle. Furthermore, force potentiation, which occurred during the early portion of the fatigue-inducing stimulation, had a longer duration in dystrophic compared with normal muscle (approximately 60 versus 20 s). As a result of these two processes, at the end of 2 min of stimulation, force of dystrophic muscle had decreased by $8 \pm 5\%$ and that of normal muscle by $69 \pm 4\%$ ($p < 0.0001$). The potassium-channel blocker, 3,4-diaminopyridine, increased

force of *dy/dy* sternohyoid muscle during twitch and 25-Hz contractions by $148 \pm 20\%$ ($p < 0.00001$) and $109 \pm 18\%$ ($p < 0.00002$), respectively. During repetitive 25-Hz stimulation, force of 3,4-diaminopyridine-treated dystrophic muscle remained significantly higher than that of untreated muscle, despite the early force potentiation being eliminated and fatigue being accelerated. Thus, merosin deficiency reduces fatigue and prolongs the duration of force potentiation. The latter alterations may partially preserve the integrity of upper airway muscle function, without which the severity of pharyngeal complications (feeding problems, sleep-related respiratory dysfunction) might be even more pronounced in the human merosin-deficient congenital muscular dystrophies. (*Pediatr Res* 54: 547–553, 2003)

Abbreviation

DAP, 3,4-diaminopyridine

Classic congenital muscular dystrophy is caused by a deficiency of merosin (laminin $\alpha 2$). Infants with this disorder present with severe hypotonia postnatally or in the first month of life (1). Furthermore, they may display feeble sucking, which, if severe, necessitates tube-feeding (1). The weakness improves slowly, allowing children to sit unsupported by 2–3 y of age, but most subjects never gain the ability to walk independently. Up to 30% of subjects die of cardiopulmonary complications in the first year of life, and at later stages nocturnal hypoventilation becomes a serious problem (1, 2). The feeding problems during infancy and the sleep-related respiratory disturbances later during childhood suggest that pharyngeal muscle dysfunction may play an important role in the clinical manifestations of this disease.

There are several genetic rodent models of muscular dystrophies, including *dy/dy* and *mdx* mice, which have provided important insight into the pathogenesis and manifestations of the muscular dystrophies (3–12). The *dy/dy* mouse (3–8) lacks merosin, and hence is similar biochemically to the classic congenital muscular dystrophies. This model has weakness of both diaphragm and limb muscles, and hence appears to be a good functional model for the human disorder.

At present there is no information about the extent to which merosin deficiency affects the pharyngeal musculature, other than observations of pharyngeal muscle weakness observed clinically in humans. Limb muscle studies in the *dy/dy* mouse have noted variable effects of merosin deficiency on muscle fatigue. From *in vitro* studies, *dy/dy* hindlimb muscles fatigue less than normal, with alterations being greater for fast-twitch plantaris than slow-twitch soleus muscle (5). In contrast, from *in vivo* studies, volitional forelimb grip fatigues the same or slightly more in *dy/dy* mice than normal (8). Additionally, *dy/dy* mice have much greater clinical weakness and atrophy of hindlimbs than forelimbs, so that during mid to late stages of the disease they achieve locomotion using their forelimbs and

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Correspondence: Erik van Lunteren, M.D., Pulmonary 111J(W), Cleveland VA Medical Center, 10701 East Boulevard, Cleveland, OH 44106, U.S.A.; e-mail: exv4@po.cwru.edu

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dragging their hindlimbs. This apparent caudal-rostral gradient in clinical disease severity suggests that fatigue properties of the skeletal musculature may demonstrate regional variability. Hence fatigue properties of pharyngeal muscles cannot be predicted on the basis of previous limb muscle studies.

The present study had two major purposes. The first was to test whether merosin-deficient pharyngeal muscle has reduced fatigue as described in hindlimb muscles (5) or mildly increased fatigue as noted in forelimb muscles (8). Specifically, we hypothesized, based on the apparent caudal-rostral gradient of disease manifestations in limb muscles, that the sternohyoid would be like the forelimb musculature and have increased fatigue. The second purpose was to examine the effects of potassium-channel blockade with DAP on fatigue of merosin-deficient pharyngeal muscle. We recently found that DAP enhances contractile performance of merosin-deficient diaphragm muscle (13). However, potassium-channel blockers do not affect diaphragm and sternohyoid muscle similarly (14), so our diaphragm data cannot be extrapolated to the pharyngeal muscles. Studies were conducted with muscle from the *dy/dy* mouse model because of the difficulty of obtaining pharyngeal muscles samples from children with this relatively uncommon disease. The sternohyoid muscle, which plays roles in both swallowing and the maintenance of upper airway patency during breathing, was chosen over other pharyngeal muscles because its structural and contractile properties are well delineated (15–17), and its contractile properties (albeit not fatigue properties) were studied previously in the *mdx* dystrophic mouse model (15).

METHODS

Homozygous *dy/dy* male mice [129P1/ReJ-Lama (2 d); $n = 14$] and their phenotypically normal (+/?) controls ($n = 11$) were obtained from Jackson Laboratories (Bar Harbor, ME, U.S.A.) and studied at an average age of 9 wk. According to the original description of this animal model (3), the *dy/dy* disease is first recognizable at 3.5 wk of age; by 2 mo it is well established both clinically (muscle weakness and atrophy, especially of the hindlimbs, kyphosis, low weight) and histologically, and most animals die between 1 and 6 mo of age. The age chosen in the present and previous studies (6, 13) is based on the disease being well established but without the animals being in the terminal stages. All studies were approved by the institutional animal care and use committee. Intraperitoneal urethane was used to induce deep anesthesia (initially 1 g/kg, with additional 0.1–0.2 g/kg as needed). The sternohyoid muscle was removed keeping its bony origin and insertion intact. Muscles were mounted vertically in 37°C oxygenated physiologic solution, allowed to equilibrate, and set at optimal length. Contractions were produced using supramaximal voltages applied via platinum electrodes placed parallel to the muscles. The physiologic solution was composed as follows (in mM): NaCl, 135; KCl, 5; CaCl₂, 2.5; MgSO₄, 1; NaH₂PO₄, 1; NaHCO₃, 15; and glucose, 11, with pH adjusted to 7.35–7.45 while being aerated with 95% O₂–5% CO₂. Studies with the potassium-channel blocker DAP used a single concentration of 0.01 mM. A range of concentrations had previously

been tested on mouse diaphragm, and this concentration was chosen on the basis of reproducibly increasing twitch tension without eliciting multiple contractions in response to a single stimulus (13). Testing of fatigue in the absence and presence of DAP was performed with separate muscle samples, so as to avoid multiple fatigue runs in a single muscle. Only one muscle strip was tested per animal. Sample sizes (for which the number of muscle strips was the same as the number of animals) were as follows: $n = 5$ for normal muscle in the absence of DAP, $n = 6$ for normal muscle with DAP, $n = 7$ for dystrophic muscle in the absence of DAP, and $n = 7$ for dystrophic muscle with DAP.

Force measurements were made with a high-sensitivity isometric transducer, digitized, and recorded on the hard drive of a computer for later analysis. Low-frequency stimulation (0.1 Hz) was applied to the muscles to ascertain stability of each preparation, which also allowed assessment of isometric twitch force, contraction time, and half-relaxation time. Testing of muscle fatigue was done at 25 Hz, using a train duration of 0.33 s with one train per second. To assess rate of fatigue, force values were normalized to twitch force at the onset of each experiment, and expressed as a percent of initial twitch force. This factored out the effects of differences among muscle samples and between normal and dystrophic muscle in absolute force at the onset of repetitive stimulation, making it easier to compare relative force losses in the two groups. During fatigue testing, contraction and half-relaxation times were measured from the first and last twitches of the train, respectively. Statistical analysis was performed with unpaired *t* tests, as well as ANOVA followed in the event of a significant result by the Newman-Keuls test. A *p* value of less than 0.05 (two-tailed) was used as the criterion for statistical significance.

RESULTS

dy/dy mice grow substantially more slowly than normal, and hence their weights were less than those of the control animals (12.3 ± 0.8 versus 23.0 ± 1.4 g). Isometric contraction time did not differ for *dy/dy* dystrophic compared with normal sternohyoid muscle (10.4 ± 0.6 versus 10.5 ± 0.6 ms), nor was half-relaxation time affected significantly (6.2 ± 0.8 versus 7.4 ± 0.7 ms).

Changes in sternohyoid muscle force during repetitive stimulation are depicted in Figures 1 and 2. Both normal and *dy/dy* dystrophic sternohyoid muscle demonstrated an initial force potentiation, as noted previously in normal rat sternohyoid muscle. For normal sternohyoid muscle maximal force was achieved at 10 s, and force remained significantly elevated for 20 s. In contrast, the force of dystrophic muscle increased during the course of 50 s, and was significantly elevated above initial values from 20 to 60 s after the onset of stimulation.

After the transient force increase, force of both normal and diseased sternohyoid muscle decreased, albeit at markedly different rates. At the end of 2 min, force of normal muscle had decreased by $69 \pm 4\%$ from initial values. In contrast, after 2 min of stimulation, force of dystrophic muscle decreased by only $8 \pm 5\%$ from initial values ($p < 0.001$ compared with normal muscle) and by approximately 20% from the peak

Figure 1. Examples of normal and *dy/dy* dystrophic sternohyoid muscle force during 25-Hz train stimulation. Tracings are shown at the onset of stimulation, as well as 1 and 2 min thereafter during ongoing stimulation. Calibration bars: time, 50 ms; force, 0.3 g.

Figure 2. Effect of repetitive 25-Hz train stimulation on force of sternohyoid muscles from normal and *dy/dy* dystrophic animals. Force values are expressed relative to baseline twitch force preceding the 25-Hz stimulation to factor out the effects of substantially lower force values of dystrophic muscle at the onset of stimulation. Values are mean $\pm$ SEM. Significant differences in force for each muscle type relative to force at the onset of stimulation are indicated (*) $p < 0.05$, as are differences between normal and dystrophic muscle (**), $p < 0.05$.

attained during the preceding force potentiation (value at 50 s). As a result, force normalized to initial values was significantly lower for control than dystrophic muscle starting 60 s after the onset of stimulation (Fig. 2).

In normal muscle the isometric contraction time remained constant during repetitive stimulation, whereas the half-relaxation time lengthened progressively during the course of

the first minute (Fig. 3). In contrast, both the contraction and half-relaxation times of dystrophic muscle remained stable during repetitive stimulation.

In response to the potassium-channel blocker DAP, twitch force of *dy/dy* sternohyoid muscle increased by $148 \pm 20\%$ ($p < 0.00001$). DAP also increased force of *dy/dy* sternohyoid considerably at the onset of repetitive 25-Hz stimulation, by $109 \pm 18\%$ ($p < 0.00002$; Fig. 4). However, the extent of subsequent force loss during the 2-min stimulation period was much greater than that seen in the absence of DAP. The rate of force decline was especially rapid during the first approximately 30 s of repetitive stimulation, and then leveled off to a value not dissimilar to that seen without DAP. Also of note was the absence of any early force potentiation in the presence of DAP. Contraction time was lengthened modestly by DAP in dystrophic muscle, although this was attenuated by the end of the stimulation period (Fig. 5). Half-relaxation time was not affected significantly by DAP, and did not change significantly during the course of repetitive stimulation (Fig. 6) despite the considerable force loss in DAP-treated *dy/dy* muscle (Fig. 4).

The effects of DAP were considerably different in normal compared with *dy/dy* sternohyoid muscle. DAP changed twitch force of normal sternohyoid muscle only minimally ($20 \pm 9\%$ increase, $p = 0.08$). During repetitive 25-Hz stimulation, force of normal muscle was increased transiently but not significantly by DAP (Fig. 4). DAP tended to augment the early force potentiation, had no effect on contraction time, and markedly slowed the rate of relaxation in normal sternohyoid muscle (Figs. 5 and 6).

DISCUSSION

Attal *et al.* (15) were the first to report on pharyngeal muscle functional properties in an animal model of muscular dystrophy. However, they examined the *mdx* mouse, which is deficient in dystrophin (the same biochemical defect found in human Duchenne muscular dystrophy). They found that *mdx* dystrophic sternohyoid muscle has an approximately 50% decrement in isometric tetanic force, an approximately 16% reduction in maximal velocity of shortening, and an approximately 66% decrease in peak mechanical power compared with genotypically normal muscle. Values for isometric twitch kinetics were not reported, nor were any studies done to examine effects of *mdx* dystrophy on pharyngeal muscle fatigue.

The manifestations of human merosin-deficient congenital muscular dystrophy differ considerably from human Duchenne muscular dystrophy (18). The two dystrophic diseases also differ considerably in mice. *dy/dy* mice have profound weakness of both limb muscles and diaphragm (3–8), whereas *mdx* mice have marked diaphragm dysfunction but reasonably well-preserved limb muscle function until old age (7–12). Furthermore, *dy/dy* mice gain weight slowly after birth, never reach the size of normal mice, and develop scoliosis and severe hindlimb contractures (8). In contrast, *mdx* mice grow normally in the first few weeks and then gain more weight than control mice (8). Finally, *dy/dy* mice die at a much earlier age than *mdx* mice (8). On the basis of these considerable differences, data on pharyngeal muscle function in *mdx* mice cannot be extrap-

Figure 3. Alterations in speed of isometric contraction and relaxation of normal and *dy/dy* dystrophic sternohyoid muscle during repetitive stimulation. Values for contraction and half-relaxation times are mean $\pm$ SEM. Significant differences in values for each muscle type relative to that at the onset of stimulation are indicated (*), $p < 0.05$.

Figure 4. Effects of DAP on force of normal and *dy/dy* sternohyoid muscle during repetitive 25-Hz train stimulation. Force values are expressed relative to baseline twitch force preceding the 25-Hz stimulation. Significant differences between untreated and DAP muscle strips are indicated (* $p < 0.05$).

olated to the *dy/dy* model, and the same may very well apply to the human homologs.

The major finding of the present study is that merosin deficiency greatly reduced sternohyoid muscle fatigue. Variable effects of merosin deficiency have been reported for fatigue of limb muscles in the *dy/dy* mouse. When tested *in vitro*, fatigue was found to be reduced for *dy/dy* dystrophic compared with normal plantaris and soleus muscles of the hindlimb (5). In contrast, fatigue was noted to be unchanged or increased (depending on age) for volitional forelimb grip when tested in the intact animal (8). Most likely these findings are

related to the entirely different methodological approaches used in the previous studies. However, it is also possible that they reflect differences in forelimb compared with hindlimb muscles. Ever since the original description of the *dy/dy* mouse, it has been well appreciated that hindlimb muscles are more severely affected than forelimb muscles, so that in the mid to late stages of the disease, animals achieve locomotion by using their forelimbs and dragging their hindlimbs (3). Our findings are similar to those of Hayes and Williams (5), indicating reduced muscle fatigue in *dy/dy* dystrophic muscle. Of interest is that fatigue is attenuated more by the *dy/dy*

Figure 5. Effects of DAP on isometric contraction time of normal and *dy/dy* sternohyoid muscle during repetitive 25-Hz train stimulation. Significant differences between untreated and DAP muscle strips are indicated (* $p < 0.05$).

Figure 6. Effects of DAP on isometric half-relaxation time of normal and *dy/dy* sternohyoid muscle during repetitive 25-Hz train stimulation. Significant differences between untreated and DAP muscle strips are indicated (* $p < 0.05$).

genotype in the fast-twitch plantaris muscle than the slow-twitch soleus muscle (5). It is possible that the sternohyoid muscle, comprising a very high proportion of fast fibers and myosins (15, 17), may have a greater reduction of fatigue by merosin deficiency than other respiratory muscles with a slower phenotype.

To test for fatigue, the present study used a stimulation train duration of 0.33 s with one train per second (*i.e.* duty cycle of one third). This paradigm is based on the classic work of Burke

and colleagues (19, 20) and has been used extensively by others (21, 22) and in our previous studies (13, 16, 17, 23, 24). This type of a protocol is reasonable for a muscle that is phasically active mainly during the inspiratory portion of the respiratory cycle, as inspiration is shorter than expiration in most species. A second issue is whether the 25-Hz stimulation frequency is sufficiently high to test for fatigue. Previous studies have found considerable muscle force loss using 20–25 Hz stimulation and a duty cycle of one third for the diaphragm

(13, 16, 23, 24) and sternohyoid muscles (16), as was also the case in the present study for sternohyoid muscle from normal animals. A third issue is whether a stimulation frequency of 25 Hz is physiologically appropriate based on motor unit firing rates during breathing. We are not aware of data on sternohyoid muscle motor unit firing frequencies during breathing. However, for a closely related muscle, the geniohyoid, we previously reported (albeit in an unrelated species, the cat) that the mean inspiratory firing frequency was 23.5 Hz, which was recorded predominantly when the animals were breathing 7% CO₂ in O₂ (*i.e.* when breathing was stimulated) (25). Thus, the stimulation frequency of 25 Hz is a reasonable value for testing fatigue. A higher stimulation rate in all likelihood would induce greater fatigue in the *dy/dy* muscle. On the other hand, this would also accelerate fatigue in the normal muscle, thus the finding of relatively low fatigue in the *dy/dy* sternohyoid muscle would not be changed.

A different methodological issue is that the present study examined fatigue during isometric rather than isotonic contractions. This raises the issue of the manner in which the sternohyoid muscle contracts during breathing. We are only aware of data examining sternohyoid muscle length changes during breathing from a species other than the mouse. In the cat, geniohyoid and sternohyoid muscle length changes were measured during resting breathing and progressive hyperoxic hypercapnia (26). The geniohyoid had progressively greater extents of both neural activation (as reflected by electromyographic activity) and inspiratory shortening as hypercapnia became more severe. In contrast, the sternohyoid muscle changed length minimally despite increasing electromyographic activity, and, if anything, lengthened slightly during inspiration (although on average by no more than 1.5 to 2% at high CO₂ levels). Thus an assessment of fatigue during isometric rather than isotonic contraction would appear to be relevant to the function of this muscle in the intact organism.

There are several potential mechanisms for the low degree of dystrophic sternohyoid fatigue. One possibility is that it is the result of altered neuromuscular junction properties in the *dy/dy* genotype (27). When muscles are stimulated to contract via nerve activation, alterations in neuromuscular transmission are an important potential source of force loss. Slowed depletion of acetylcholine in dystrophic compared with normal neuromuscular junctions could well explain the combination of reduced force loss in the *dy/dy* sternohyoid muscles and the absence of changes in isometric twitch kinetics. However, in the present study sufficiently large stimulation currents were administered to the muscle strips to assure that the muscles were stimulated to contract directly rather than by activation of nerve branches, as evidenced by lack of a force decrease in response to curare (which blocks neuromuscular transmission). Thus effects of merosin deficiency on neuromuscular transmission could not explain the present data.

A second possible explanation for the reduced fatigue of *dy/dy* sternohyoid muscle is that of a change in muscle fiber type proportions. Hayes and Williams (5) found that soleus muscle of *dy/dy* mice had increased proportions of type I fibers (66 *versus* 52%) and decreased proportions of type IIA fibers (33 *versus* 48%) compared with control mice. The fiber pro-

portions or myosin isoform composition of the sternohyoid muscle of *dy/dy* mice have not been described, and hence it is unknown whether merosin deficiency alters these in the same manner as it affects the soleus muscle. In the present study, isometric contraction times were not significantly different for dystrophic compared with normal muscle, although there was a slight (but nonsignificant) trend toward slower rate of relaxation. Normal mouse sternohyoid is composed exclusively of type IIX and IIB myosin heavy chain isoforms (15). The unchanged isometric contraction argues against the appearance of type I (slow twitch) fibers. More likely the *dy/dy* phenotype resulted in a shift toward a greater proportion of fatigue-resistant fibers among the type II (fast twitch) fibers, which needs to be tested directly in future studies.

Two other differences were noted between dystrophic and normal sternohyoid muscles with regard to their behavior during repetitive stimulation. First is that the time course of early force potentiation, which is known to be prominent in normal rat sternohyoid muscle (16), was slower in onset but much longer in duration for dystrophic compared with normal mouse muscle. Force potentiation can occur as a result of an impairment of acetylcholine release being overcome by repetitive nerve firing. As discussed above, the experimental stimulation paradigm using high stimulation currents was such that neuromuscular junction properties should not have affected the present findings. Force potentiation at the level of the muscle has been attributed to a number of factors, most notably calcium fluxes (28). Altered calcium handling has been implicated in some (but not all) studies of dystrophin-deficient dystrophic muscle (29), but whether this accounts for the altered pattern of force potentiation noted here requires further study. We are not aware of any reports of altered force potentiation by the *dy/dy* genotype in any skeletal muscle. It is possible that the slow time course of force potentiation contributed to the reduced rate of fatigue. However, in a subset of muscle strips stimulated for longer durations of time (5 min), the rate of force loss continued to be very slow (data not shown), well after the effects of the force potentiation should have subsided.

The second difference is that the slowing of relaxation rate during repetitive stimulation noted in normal muscle was not present in *dy/dy* dystrophic muscle. This may have been simply owing to the fact that there was very little force loss, rather than being a direct effect of merosin deficiency (although see below for a discussion of force loss and relaxation rate in the presence of DAP). Of interest, reduced (although not absent) slowing of relaxation during repetitive stimulation has been noted in limb muscle of the *mdx* dystrophic mouse (30). In normal muscle the slowing of relaxation rate during fatigue may lead to better fusion of contractions and hence better preservation of force. This could not have contributed to the reduced fatigue of *dy/dy* dystrophic muscle, as stable relaxation rate would not have augmented contractile fusion.

Potassium channels play important roles in regulating skeletal muscle electrophysiologic properties. Most relevant to the present study, potassium-channel opening is responsible for action potential repolarization. Blocking these channels pharmacologically with the aminopyridines (in particular DAP and

4-aminopyridine) prolongs action potential duration, increases intracellular calcium levels, and augments muscle force (13, 23, 24, 31). In normal rat diaphragm, the aminopyridines increase force at low to intermediate stimulation frequencies by up to approximately 150%, and the force increases can be maintained during the course of repetitive 20-Hz stimulation (24). We recently tested the effects of DAP on *dy/dy* dystrophic mouse diaphragm (13). DAP increased force by 111% during twitch stimulation and by 41 to 77% during 5- to 50-Hz stimulation, and these force increases were well maintained during repetitive stimulation.

The effects of DAP on mouse sternohyoid muscle differed in several respects from previous diaphragm findings (13), despite identical methodological approaches. DAP increased twitch force of normal diaphragm by 81%, which was far greater than that noted presently for normal sternohyoid. In addition, during repetitive 25-Hz stimulation, force of normal diaphragm was elevated significantly, whereas this was not the case for normal sternohyoid muscle. Finally, in *dy/dy* diaphragm relaxation time was prolonged by repetitive 25-Hz stimulation in the presence of DAP (albeit less so than in normal diaphragm), whereas this was not the case in *dy/dy* sternohyoid muscle. Whether these differences are caused by differences between muscles in fiber type and myosin type composition, potassium-channel density or subtype distribution, or other properties cannot be determined from the present data.

Focusing directly on the issue of pharyngeal muscle function, the DAP findings of the present study are of interest in several respects. First, this agent altered the time course of force loss during repetitive stimulation, in particular early during the stimulation period. This suggests that there may have been several mechanisms contributing to the force loss, and in particular that the early force loss might have been related to membranous electrophysiologic events. Second, the considerable early force decrease yet stable half-relaxation time in the presence of DAP suggests that stability of relaxation rate may be a direct change induced by merosin deficiency. Relaxation rate is affected by several factors, including membranous potassium and chloride conductances and rate of calcium reuptake by the sarcoplasmic reticulum. In the dystrophin-deficient human and mouse muscular dystrophies several of these systems have been implicated in the disease process, but far fewer studies have examined the merosin-deficient dystrophies to provide insight into which possibility might explain the absent slowing of relaxation with fatigue in the present studies. Lastly, from a potential therapeutic perspective, the present study indicates that even though DAP has limited beneficial effects on normal sternohyoid muscle, the contractile performance of *dy/dy* dystrophic muscle is improved considerably by potassium-channel blockade.

CONCLUSIONS

In conclusion, the present study found that merosin deficiency reduced sternohyoid muscle fatigue and prolonged force potentiation *in vitro*. Functionally this may act as a compensatory mechanism, thereby allowing better preservation of pharyngeal muscle function than would be the case otherwise.

Thus upper airway motor actions may be impaired as a result of weakness, but the degree of dysfunction may not worsen during the course of prolonged activity. These changes are clearly not sufficient to fully overcome the underlying contractile deficits, however, as evidenced by the deficits in feeding-related sucking and sleep-related respiratory disturbances that have been described in human merosin-deficient congenital muscular dystrophy (1, 2).

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