Mechanical and Energetic Properties of Dystrophic (*mdx*) Mouse Muscle

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Abstract The mechanical and energetic properties of extensor digitorum longus (EDL) and soleus muscles of X chromosome-linked muscular dystrophic mutant (*mdx*) mice aged 4-6 weeks were studied and compared with those of the muscles of normal mice. Maximum tetanic tension, the speed of contraction and relaxation, and the heat production of *mdx* soleus muscles were not significantly different from those of the normal muscles. However, in *mdx* EDL muscles, the tension and heat production were significantly reduced, and relaxation was prolonged. To study the cause of these changes in *mdx* EDL muscles, tension and heat production were measured at various muscle lengths greater than optimum for tension. Both the amount of twitch heat and the heat rate for a tetanus were linearly related to the tension and had non-zero intercepts at zero tension, the activation heat. The twitch activation heat and the tension-related heat in tetani of *mdx* EDL muscles were not different from those in normal muscles. On the other hand, the tetanus activation heat of *mdx* EDL muscles was significantly smaller than that of normal muscles. Assuming that the degenerated fibers do not contribute to the active force produced, these results suggest that the amount of Ca^{2+} released in a contraction is not significantly different between normal and *mdx* muscles, but the Ca-ATPase activity of the sarcoplasmic reticulum is reduced in *mdx* EDL, which could cause the slowing of relaxation.

Key words: muscular dystrophy, *mdx* mouse, heat production, muscle mechanics.

An X chromosome-linked dystrophic mutant (*mdx*) mouse described by BULFIELD *et al.* (1984) has been presented as a model for Duchenne muscular dystrophy. However, the *mdx* mutants do not have a progressive muscle disease. The muscles in *mdx* mice degenerate at a particular age and subsequently regenerate (DANGAIN and VRBOVA, 1984). No noticeable paralysis or deficiency of movement

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can be seen in adult *mdx* mice.

DANGAIN and VRBOVA (1984) have shown that contractile properties of *mdx* mouse muscles, at a certain age, are significantly different from those of normal muscle; tetanic tension is much reduced and the half relaxation time of a twitch contraction is prolonged in *mdx* muscles.

The reduction in tension in *mdx* muscles may arise from the following: (1) the number of working crossbridges has decreased due to incomplete activation, (2) the crossbridge properties are modified to produce less tension per crossbridge, and (3) part of the cross sectional area consists of degenerated fibers which cannot produce tension. The process of relaxation after contraction is not a simple process and the rate of relaxation is affected by several factors (for a review GILLIS, 1985).

The present study was undertaken to investigate the cause of this reduction in tension and the slowing of relaxation in *mdx* muscles. Tension and heat production were measured in extensor digitorum longus (EDL) and soleus muscles from 4–6-week-old normal and *mdx* mice. The heats caused by Ca-ATPase of the sarcoplasmic reticulum (SR) and by actomyosin ATPase were evaluated separately by measuring heat at various muscle lengths greater than optimum. Reasons for the reduction in tension and the slowing of relaxation in *mdx* muscles are discussed.

MATERIALS AND METHODS

Materials. The experiments were performed on EDL and soleus muscles from *mdx* (C57BL/10Sc-*mdx*) and control (C57BL/10Sc) mice aged 4–6 weeks. Krebs-Henseleit solution contained in mM: NaCl 118, KCl 4.75, CaCl₂ 2.54, MgCl₂ 1.18, KH₂PO₄ 1.18, NaHCO₃ 24.8, and glucose 10 and was bubbled with 95% O₂/5% CO₂ gas mixture.

Mice were anesthetized by intraperitoneal injection of nembutal (2.5 mg/100 g body weight). The EDL and soleus muscles were dissected from both limbs. Blotted weights (mg) of EDL and soleus muscles of normal mice were 11.5 ± 0.6 ($7.1 \pm 0.7 \times 10^{-4}$, $n=7$) and 8.9 ± 0.6 ($6.9 \pm 0.9 \times 10^{-4}$, $n=4$), respectively. Those of *mdx* mice were 12.9 ± 2.0 ($9.2 \pm 1.8 \times 10^{-4}$, $n=9$) and 5.8 ± 1.1 ($5.4 \pm 0.6 \times 10^{-4}$, $n=5$), respectively. Values are presented as mean $\pm$ S.D. Numbers in parentheses denote the values of the ratio (muscle weight)/(body weight).

Force and heat measurements. The methods used for tension and heat measurements were similar to those described by KOMETANI and YAMADA (1983).

The isometric twitch (P) and the tetanic tension (P), and their time courses, were recorded. The tetanic tension (P_o), which was normalized to the unit cross-sectional area of muscle (see below), at a muscle length equivalent to the *in situ* fully dorsiflexed length (l_o) was compared between normal and *mdx* muscles. The time from the onset to the peak tension in twitches (twitch contraction time, t_o) and the time needed to get a 50% fall of tension from the last stimulus in tetanic contractions (the half relaxation time, $t_{1/2}$) were also compared between normal and *mdx* muscles.

The exact fiber length of these muscles cannot be measured in the living muscle because EDL and soleus muscles are fusiform and fiber length is shorter than muscle length (CLOSE, 1964). The cross-sectional area of the muscles was calculated as blotted weight/ l_0 . The values of the tetanic tension (P) and twitch tension (P_t) per cross-sectional area are thus somewhat overestimated.

An electroplated thermopile (K3) was used for heat measurements. A pair of muscles was mounted on the thermopile. At one end the muscles were tied to a clamp near the bottom end of the thermopile and at the other end were connected to the force transducer. Muscles were stimulated directly via platinum electrodes one located near the top and the other at the bottom of the thermopile. Rectangular pulses of 5 ms duration were used for stimulation, the intensity being typically 6 V. Fused tetanic contractions were produced with 80 Hz stimulation for EDL and 30 Hz stimulation for soleus muscles.

The output of the thermopile was amplified by an Ancom 15c-3a chopper amplifier, low-pass filtered (80 Hz) and recorded on a Nicolet 2090 digital oscilloscope. The heat loss was corrected for by the method of HILL (1965). Stimulus heat was measured at the end of each experiment after poisoning muscles in 20 mM procaine-Krebs-Henseleit solution. Records were corrected for stimulus heat. All the experiments were performed at 20°C.

Heat produced was expressed per unit weight of muscle, and normalized by $P_{\max}$ (the twitch tension per unit cross-sectional area at l_0) or P_0 (the tetanic tension per unit cross-sectional area at l_0). Thus the heat produced becomes a dimensionless quantity. The specific density of muscle was assumed to be 1.

The heat produced in tetanic contractions was fitted to a linear function of time and the steady rate of heat production (h_b) was obtained. Muscles were first stimulated at l_0 and then stretched in 0.5 mm steps to the length where the tetanic tension was about one third of that at l_0 . Muscles were given a rest period of 20 min in Krebs-Henseleit solution after the change of the length.

The twitch and the tetanus activation heats were obtained, following SMITH (1972) and HOMSHER *et al.* (1972), from the intercept on the ordinate of the linear regression line describing the tension-heat relation (see RESULTS and the legend for Fig. 3). The tension-related heat in tetani was obtained from the slope of the linear regression line of the tension-heat relation.

RESULTS

Force production in twitch and tetanus

Figure 1 shows typical records of tension developed by the stimulation of EDL and soleus muscles of normal and *mdx* mice. In EDL, the time course of twitch tension in *mdx* was prolonged and the relaxation from tetanic contraction was slower than that in normal muscle. In soleus, on the other hand, the time courses of tension in both twitch and tetanic contractions in *mdx* were similar to those of normal muscle.

Fig. 1. Typical records of tension in twitch and tetanus contraction of EDL and soleus muscles from normal and *mdx* mice. Upper records are for EDL and lower ones for soleus muscles. Horizontal bars indicate 0.2 s duration. n, normal; m, *mdx* muscle.

Table 1. Tetanic tension (P_o), twitch contraction time (t_c), and half relaxation time ($t_{1/2}$) of EDL and soleus muscles of normal and *mdx* mice at l_o (see METHODS).

		<i>n</i>	P_o (kN/m ²)	t_c (ms)	$t_{1/2}$ (ms)
EDL	Normal	7	193.7 ± 13.8	38.7 ± 2.0	54.4 ± 2.5
	<i>mdx</i>	9	142.9 ± 5.8*	44.0 ± 1.4*	71.4 ± 3.9**
Soleus	Normal	3	159.3 ± 11.9	86.7 ± 3.9	173.6 ± 15.2
	<i>mdx</i>	5	167.7 ± 13.5	86.5 ± 4.7	162.2 ± 4.8

Results are shown as mean ± S.E. of the mean. Asterisks indicate that the mean is significantly different from that in normal muscles (* $p < 0.05$; ** $p < 0.01$). *n*, number of experiments.

Mean values of P_o , t_c , and $t_{1/2}$ of normal and *mdx* muscles are summarized in Table 1. P_o was significantly smaller and t_c and $t_{1/2}$ were significantly longer in *mdx* EDL muscles than in normal muscles. The results show that *mdx* EDL muscles produce less tension and the speed of contraction and relaxation are slower compared with normal muscles. In soleus muscles, P_o , t_c , and $t_{1/2}$ were not significantly different between *mdx* and normal muscles.

Heat production

Figure 2 shows tension and heat production in normal and *mdx* EDL muscles during tetanic contraction. The time course of heat production in both EDL and

Fig. 2. Tension and heat production in tetanic contractions of normal and *mdx* EDL muscles. Tetanus duration was 0.5 s. Blotted weight of normal EDL, 10.3 mg; *mdx* EDL, 12.6 mg.

Table 2. The steady rate of heat production (h_b) in tetani of normal and *mdx* muscles at l_o .

$h_b/(s^{-1})$			
EDL		Soleus	
Normal ($n=7$)	<i>mdx</i> ($n=9$)	Normal ($n=3$)	<i>mdx</i> ($n=5$)
0.670 ± 0.060	$0.525 \pm 0.060^*$	0.093 ± 0.005	0.083 ± 0.008

Values for h_b were normalized by P_o and are shown as mean $\pm$ S.E. of the mean. Asterisk indicates that the mean is significantly different from that in normal muscle ($p < 0.05$).

soleus muscles were nearly linear with time. The steady rate of heat production (h_b) was obtained by fitting records, after correcting for heat loss, to a linear function of time. The h_b thus obtained has been normalized by P_o . Table 2 summarizes averaged results obtained from 7 (normal) and 9 (*mdx*) in EDL and 3 (normal) and 5 (*mdx*) preparations in soleus muscles. The mean value in *mdx* EDL was significantly smaller than that in normal muscle. However, in soleus no significant difference was observed between h_b in normal and in *mdx* muscle.

Activation heats and tension related heat of EDL muscles

Myothermic experiments were performed at various muscle lengths in both *mdx* and normal EDL. When EDL muscles were stretched beyond optimal length, both tension and heat production were reduced. Figure 3 shows typical examples of the relation between tension and heat production in twitch and tetanic contractions, and shows that both twitch heat (h_t) and the heat rate for tetani (h_b) were linearly related to tension. Solid lines in the figure are the linear regression

Fig. 3. Typical examples of the relation between tension and heat production in twitch and tetanic contractions of normal and *mdx* EDL muscles. Twitch heat (h_t) and the steady rate of heat production in tetani (h_b) at various muscle lengths were plotted against tension per cross-sectional area, expressed as fractions of the maximum twitch ($P_{\max}$) and tetanic (P_o) tension. Values for h_t and h_b have been normalized by $P_{\max}$ and P_o , respectively. P_t and P are twitch and tetanus tension at various muscle lengths. Solid lines in the figure denote linear regression lines. Linear regression equations: $h_t = 0.048 + 0.064 P_t / P_{\max}$ ($r = 0.957$) and $h_b = 0.334 + 0.323 P / P_o$ ($r = 0.937$) for normal EDL; $h_t = 0.052 + 0.064 P_t / P_{\max}$ ($r = 0.915$) and $h_b = 0.276 + 0.359 P / P_o$ ($r = 0.940$) for *mdx* EDL. The r is the correlation coefficient for the equation. Closed circles, normal EDL; open circles, *mdx* EDL.

lines. They have non-zero intercepts on the heat axis at zero tension. The intercept of the heat-tension relation in twitches is the twitch activation heat, a_{twitch} , and that in h_b -tension relation in tetani is the tetanus activation heat, a_{tetanus} . The slope of the regression lines of h_b -tension relation is the tension-related heat, b_{tetanus} (HOMSHER

Table 3. Twitch and tetanus activation heats (a_{twitch} and a_{tetanus}) and tension-related heat (b_{tetanus}) in tetanic contraction of EDL muscles.

	<i>n</i>	a_{twitch}	a_{tetanus} (s ⁻¹)	b_{tetanus} (s ⁻¹)
Normal	5	0.047 ± 0.007	0.300 ± 0.038	0.328 ± 0.020
<i>mdx</i>	7	0.041 ± 0.008	0.185 ± 0.033*	0.355 ± 0.049

Results are shown as mean ± S.E. of the mean. Asterisk indicates that the mean is significantly different from that in normal muscle ($p < 0.05$).

et al., 1972). The results in Fig. 3 show that a_{twitch} and b_{tetanus} in *mdx* are similar to those in normal muscle but a_{tetanus} in *mdx* is smaller than that in normal muscle.

Table 3 shows averaged results for a_{twitch} , a_{tetanus} and b_{tetanus} of EDL muscles. Values for a_{twitch} and b_{tetanus} in *mdx* EDL were not significantly different from those in normal muscle. On the other hand, a_{tetanus} in *mdx* EDL was significantly smaller than that in normal muscles.

DISCUSSION

In *mdx* mutant mice it has been reported that degenerated fibers appear at about 3 weeks and regeneration starts at about 4 weeks after birth. Almost all muscle fibers of 4-month-old mice are regenerated fibers (I. Goto, personal communication). DANGAIN and VRBOVA (1984) have reported that tibialis anterior muscles from *mdx* mice aged 3–4 weeks produce significantly less tension than normal muscles, and that the relaxation after contraction in *mdx* muscles is significantly slower than that in normal muscles.

In the present study we have investigated the mechanical and energetic properties of EDL and soleus muscles of normal and *mdx* young mice. The results (Table 1) show that the contractile properties of *mdx* EDL muscles are significantly different from those of normal muscle; the tetanic tension at l_0 was smaller and the speed of both contraction and relaxation were slower in *mdx* than in normal EDL muscles. This is different from DANGAIN and VRBOVA (1984) who have reported that normal and *mdx* muscles at 4–6 weeks show no significant difference in contractile properties. In soleus muscles there was no significant difference in contractile properties between normal and *mdx* muscles (Table 1, 2) in agreement with DANGAIN and VRBOVA (1984).

The difference in the results between ours and DANGAIN and VRBOVA (1984) may be explained as follows. Firstly we compared tension per cross sectional area between normal and *mdx* muscles, while they compared unnormalized values of tension. Values for unnormalized tension in EDL muscles in the present study were 454.1 ± 80.6 ($n = 7$) and 484.1 ± 69.8 mN ($n = 9$) in normal and *mdx* muscles,

respectively. The difference between the two values is not significant. Secondly we used different muscles from those used by DANGAIN and VRBOVA (1984). They have shown that the muscle fiber destruction starts at different ages in different muscles.

The heat produced in twitch and tetanic contractions arises from ATP splitting by both actomyosin ATPase and the SR Ca-ATPase. Thus, the significant difference in h_b of EDL muscles between normal and *mdx* mice (Table 2) cannot simply be related to the abnormality in either the actomyosin ATPase or the SR Ca-ATPase. The contribution from the two can be evaluated separately, however, by measuring heat and tension at various muscle lengths (HOMSHER *et al.*, 1972). The relation between heat and tension is generally linear at muscle lengths greater than optimum for tension, and the heat can be separated into two parts; the tension independent heat, i.e. the activation heat, and the tension dependent heat (Fig. 3). The activation heat in a twitch is thought to be closely related to the amount of Ca released associated with the stimulation. In a tetanic contraction, on the other hand, a_{tetanus} and b_{tetanus} are closely related to the SR Ca-ATPase and actomyosin ATPase activities, respectively (CURTIN and WOLEDGE, 1978; HOMSHER and KEAN, 1978).

The possibility that low tension in *mdx* EDL muscles is due to an incomplete activation of the contractile system may be excluded because the amount of a_{twitch} in *mdx* muscle was not significantly different from that in normal muscle (Table 3). The possibility that some change in the energy transduction by actomyosin reduces the tension developed by *mdx* muscles may also be excluded because the value of b_{tetanus} in *mdx* was not significantly different from that in normal muscles (Table 3). Thus, we may conclude that low tension in *mdx* EDL muscles is mostly caused by the existence of degenerated fibers and that fibers other than degenerated ones can produce tension similar to that in control muscle fibers.

Since twitch contraction time (t_c) is inversely correlated with the actomyosin ATPase activity (BÁRÁNY, 1967), the longer t_c in *mdx* EDL muscles (Table 1) suggests that the actomyosin ATPase activity in *mdx* muscles is reduced. However, the value for b_{tetanus} , a measure of the actomyosin ATPase activity, in *mdx* muscles is not significantly different from that in normal muscles (Table 3). Therefore, the slower relaxation rate in *mdx* muscles is thought to be responsible for the prolongation in t_c .

As noted above, the rate of relaxation in *mdx* EDL muscles was significantly slower than that in normal muscles (Table 1). The rate of relaxation is affected by various factors (for a review GILLIS, 1985) such as myoplasmic parvalbumin concentration (CELIO and HEIZMANN, 1982), sarcomere inhomogeneities during relaxation (EDMAN and FLITNEY, 1982), and the rate of Ca uptake by the SR (OGAWA *et al.*, 1981). Our results in Table 3 suggest that one of the causes of the slowing of relaxation is a reduction in the rate of Ca uptake into the SR, because a_{tetanus} is significantly smaller in *mdx* than in normal muscles. TURNER *et al.* (1988) have shown that the mean half-decay time for Ca transients in *mdx* muscle fibers is significantly longer than that in normal fibers. This may be explained by the low Ca-ATPase activity of SR in *mdx* muscles.

In summary, EDL muscles from 4–6-week-old *mdx* mice produce less tension and relax more slowly than those from normal mice. The contractile properties of soleus muscles of the same age show no significant difference between normal and *mdx* muscles. The reduced tension in *mdx* EDL muscles is mainly due to the existence of degenerated fibers and is probably not due to alterations in the actomyosin ATPase. The slowing of relaxation in *mdx* muscles is most likely caused by a reduction in the Ca-ATPase activity of SR.

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