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Observations on the effects of low frequency electrical stimulation on fast muscles of dystrophic mice

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SUMMARY The deterioration of tibialis anterior (TA) and extensor digitorum longus (EDL) muscles in dystrophic mice (C 57 BL dy/dy) was compared. The effects of chronic electrical stimulation on various characteristic properties of these muscles were also studied. The results indicate that EDL muscles are less affected by the disease than TA. This "selectivity" is difficult to explain since both muscles have similar fibre type composition. TA and EDL muscles that were stimulated for 10-28 days developed greater tetanic tensions than the contralateral muscles, but this effect was apparent only when the muscles were severely affected by the disease, that is the contralateral TA or EDL muscles developed less than 50% of the tension produced by muscles from normal animals. In all EDL muscles, stimulation increased the fatigue resistance. The time course of contraction and relaxation of dystrophic muscles is usually slower than that of normal muscles. The stimulation reduced this slowing effect, so that the stimulated muscles became similar to homologous muscles from normal littermates.

In a previous study we have found that in dystrophic mice long term low frequency electrical stimulation of fast leg muscles had a beneficial effect, in that the stimulated muscles developed more tension than the unstimulated control muscles of the other leg.[1] The mechanism by which this effect is brought about is unknown and could give some clues as to the possible defects that causes degeneration of dystrophic muscles. It is unlikely that it is simply the increased amount of activity imposed on the muscle that affects the remaining, relatively healthy muscle fibres and that the improvement is due to a better performance of these fibres, since it is known that when electrical stimulation of a similar kind is applied to normal muscles of rabbits and rats it does not lead to an increase in the strength of contraction.[2-4] Thus stimulation affects only the tension developed by diseased muscles.

In our previous study we found that after electrical stimulation tibialis anterior (TA) muscles improved more than extensor digitorum longus muscles (EDL). This result was puzzling, since the two muscles have a similar function and similar composition of muscle fibres and motor units.[5-7] The different effect of the electrical stimulation on the two muscles could however be explained if the effect of stimulation was greater in muscles that were rapidly deterioriating, and if EDL were less affected by the dystrophy. In muscles of dystrophic mice the decrease in tension caused by the disease is due mainly to the destruction of many muscle fibres.[8] In spite of the fact that regeneration of muscle fibres is taking place in dystrophic muscles the decrease in the number of muscle fibres is progressive, indicating that the regenerating fibres cannot survive.[9] Previous results showed that the number of muscle fibres is greater in muscles of dystrophic animals that have been stimulated for some time.[1] The greater number could be due either to an increased survival of the diseased muscle fibres or to regeneration of new muscle fibres and their survival.

The present study was designed to assess these possibilities. In addition, an attempt has been made to see whether the diseased muscles are able to alter some of their other properties such as their time course of contraction and relaxation and resistance to fatigue. Increased resistance to fatigue is readily induced by long term electrical stimulation of normal fast muscles and is related to the increase of capillary density and oxidative metabolism in the muscle.[19] If stimulation of diseased muscle brought about similar changes then it may have an additional beneficial effect.

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Methods

Dystrophic mice of the strain C57 BL $2dy^{2J}/dy^{2J}$ aged 3-5 months were used in these experiments. Under ether anaesthesia and using sterile precautions teflon-coated stainless steel wires were implanted either side of the lateral popliteal nerve in one hind leg, the other leg serving as a control. The teflon coating was removed from the ends over a length of 0·5 mm. A fine silk thread was used to attach the wires to the lateral head of the gastrocnemius muscle. The wires were led under the skin and externalised at the neck of the animal, where the ends were attached to two small hooks and sewn to the skin. The animals were left to recover and stimulated daily via the implanted electrodes at 8 Hz for 30 minutes in each hour for 6-8 hours a day. The animals were stimulated in this way for 10-28 days. Some mice with implanted electrodes were left unstimulated for control experiments. At intervals of 9-28 days after the initial operation the animals were anaesthetised with chloral hydrate (1 ml/100 g of body weight of a 3·5% solution). The tendons of TA and EDL muscles were freed, and attached to fine silk threads. The sciatic nerve was dissected and cut centrally and the medial popliteal nerve was cut distally. Small rigid pins were put through the proximal and distal ends of the tibia and the legs were then secured to a rigid table. Contractions were elicited by stimulating the sciatic nerve by supramaximal stimuli. The tendons were attached to strain gauges so as to record isometric contractions. These were displayed on an oscilloscope screen, as well as on to a Devices pen recorder. To test the fatigability muscles were stimulated by trains of 10 pulses at 40 Hz, repeated every second. The changes in tension were expressed as a ratio of the initial tension to that developed by the muscle after 3 minutes of stimulation. In this way a fatigue index was established.

When the recordings were completed the muscles were excised, weighed and frozen in melting isopentane cooled in liquid nitrogen. The experimental and control muscles from each animal were mounted on either side of a pin and aligned with care so that a similar area of each muscle was at the same level. Ten to fifteen μm cross-sections of both muscles were cut simultaneously. Both experimental and control muscles were then processed together. The enzyme succinic dehydrogenase was visualised using the method of Nachlas, Tsou, De Souza, Cheng and Seligman.[11]

Results

COMPARISON OF THE PROGRESS OF DISEASE IN TA AND EDL MUSCLES OF DYSTROPHIC MICE

In fig 1 recordings of maximal tetanic tension of TA and EDL muscles from a normal littermate (a and c) are compared to those from the same muscles of a dystrophic mouse (b and d). It is apparent that the reduction in tension was greater in TA than EDL. In 18 control animals the mean tension developed by TA was 119 ± 5 g and EDL from the same animals developed 27·9 ± 0·1 g, the ratio of

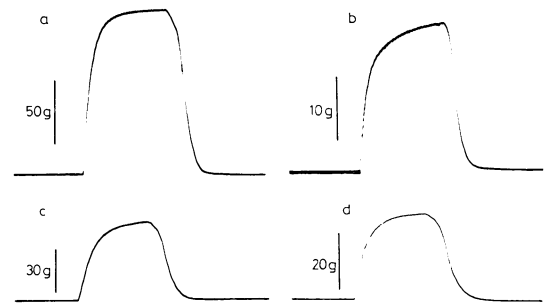

Fig 1 *Records of isometric tetanic contractions from control and dystrophic TA and EDL muscles of mice are shown. a—control TA, b—control EDL, c—dystrophic TA, d—dystrophic EDL. Contractions were elicited by 80 Hz stimulation for 400 ms.*

TA/EDL tensions being 4·5 ± 0·1. The mean tension of TA muscles from 12 3-5 months old dystrophic mice was 56 ± 5 g and that of EDL 20·7 ± 1·7 g. Due to the proportionally smaller tension of TA the ratio of TA/EDL in dystrophic animals was reduced to 2·7 ± 0·2. Since it is unlikely that dystrophic mice have smaller TA muscles at birth, it appears that TA muscles deteriorate at a faster rate than EDL muscles. Single twitches were also recorded and their time course measured. Table 1 summarises the results and shows that the time course of contraction and relaxation was slower in the dystrophic than in the control muscle, thus confirming earlier reports that muscles from dystrophic mice contract and relax more slowly.[12]

EFFECTS OF ELECTRICAL STIMULATION ON TA AND EDL MUSCLES OF DYSTROPHIC MICE

In a previous study it was found that slow frequency stimulation of fast muscles in dystrophic mice was beneficial, in that the stimulated muscles developed more tension than unstimulated controls. This beneficial effect of stimulation was greater in the

Table 1 *Time to peak tension (tpt) and half-relaxation of single twitches from TA and EDL muscles*

	N	*dy Cs*	*dy St*	*dy Cop*	*dy Op*
tpt ms	18* ±0·64 $p < 0·001$	24·1 ±0·9	20·2* ±1·0 $p < 0·01$	26·0 ±6·6	22·2 ±4·5
½ relax. ms.	19·2* ±1·1 $p < 0·0005$	35·8 ±2·4	27·6* ±1·6 $p < 0·01$	32·8 ±2·3	31·3 ±2·3

N = normal littermates, dy Cs = contralateral muscles of stimulated animals, dy St = stimulated muscles, dy Cop = contralateral muscles of sham operated animals, dy Op = muscles from sham operated animals.
*The values are significantly different from dy Cs values,

Table 2 *Tetanic tensions (in g) and fatigue resistance of control and dystrophic muscles. The animals with the stimulated TA and EDL muscles were divided into two groups (a) those where the contralateral TA muscles developed more than 60 g tension and EDL more than 14 g (b) where they developed less. The fatigue resistance is expressed as a % decrease of tetanic tension after 3 minutes stimulation*

		TA					*EDL*		
		N	*dy Cs*	*dy St*	*dy Cop*	*dy Op*	*N*	*dy Cs*	*dy St*
a	Tet tension (g)	119 ±5·1	78 ±2·8	77 ±7·8	59 ·5·6	57·3 ±5·2	27·9 ±1·8	24·57 ·5·9	24·57 ·4·7
b			39·3 ±2·8	56·0* ±5·2 p < 0·01				10·3 ·2·0	18·6* ·5·2 p < 0·02
	Fatigue index						66·7 ±5·0	54·3 ·6·7	22·1* ·2·6 p < 0·005

N = muscles from normal littermates, dy Cs = contralateral muscles to stimulated side from dystrophic mice, dy St = stimulated muscles from dystrophic mice, dy Cop = contralateral muscles of sham-operated dystrophic mice, dy Op = sham-operated muscles from dystrophic mice. · = standard error of the means.
*The values are significantly different from unstimulated control muscles.

TA than EDL muscles. Since EDL seems to be less affected by the disease we tested the possibility that the more severely affected dystrophic muscles responded more readily to treatment by electrical stimulation. The animals were divided into two groups, a severely affected group and a less severely affected group.

As a measure of the severity of the disease the tension developed by the control TA and EDL muscles was used. Animals were considered to be severely affected when the muscles developed less than 50% of the mean tension produced by muscles from normal animals. Table 2 shows that in those animals where the TA muscles developed more than 60 g tension, and EDL more than 14 g the electrical stimulation did not produce a significant increase in tetanic tension, while in animals where the control muscles developed less tension, significant improvement was brought about by slow frequency electrical activity. Although the stimulation did not affect the tension developed by muscles that were not severely dystrophic, it did have an effect on their resistance to fatigue. As in normal animals, low frequency electrical stimulation of dystrophic muscle increased the resistance to fatigue. Figure 2 shows an example from an experiment in which the resistance to fatigue of the chronically stimulated and control EDL muscles was tested. It is apparent that the stimulated EDL muscle fatigued less than the control EDL. Table 2 summarises the results and shows that dystrophic muscles are slightly, but not significantly more resistant to fatigue than muscles from normal littermates, while the fatigue resistance of the stimulated dystrophic muscles was significantly increased compared to normal or dystrophic unstimulated muscles.

Resistance to fatigue is directly correlated to the activity of oxidative enzymes in muscle fibres.[13] This

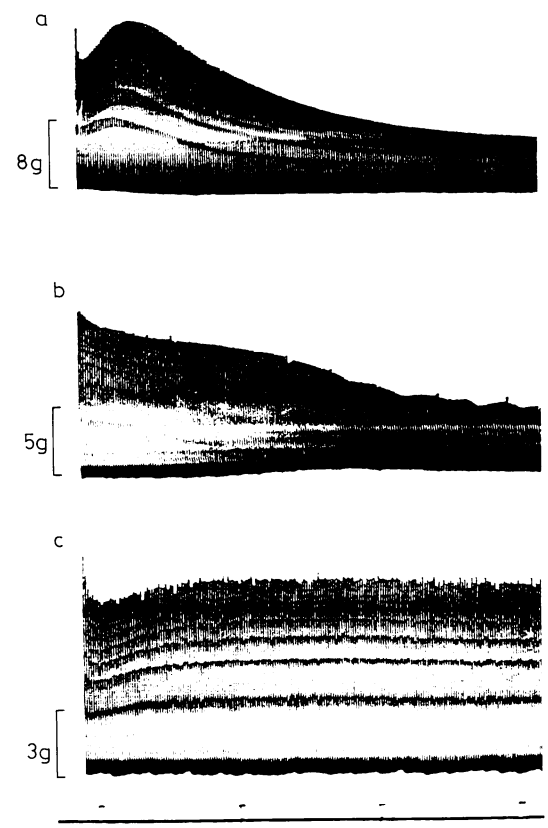

Fig 2 *Records show repeated isometric tetanic contractions of EDL muscles elicited at 40 Hz for 250 ms every second for 3 mins. A—control muscle, B—dystrophic muscle, C—dystrophic stimulated muscle. B and C records were taken simultaneously from the same animal.*

was borne out by our results, where histochemical visualisation of the oxidative enzyme SDH revealed only a smaller proportion of darkly stained fibres in normal EDL muscles, a much higher proportion in muscles of dystrophic mice and the stimulated muscles were almost homogeneously darkly stained. Thus, like normal fast muscles, dystrophic muscles too are able to increase their resistance to fatigue and their content of oxidative enzymes in response to prolonged activity. Not only was the resistance to fatigue altered by electrical activity, but also the time course of contraction. Table 1 shows that the dystrophic muscles contract and relax more slowly than the normal muscles, and that this slowing is much reduced in the stimulated muscles. Table 1 also shows that the time course of contraction and relaxation of dystrophic muscles is unaffected by the sham-operation. Thus stimulation reverses the slowing effect caused by the disease process.

Discussion

The present results suggest that the rate of deterioration of different muscles of dystrophic mice varies. Even muscles that have similar function and composition of muscle fibres, like TA and EDL, are not equally affected by the dystrophic process, in that TA deteriorates more rapidly than EDL. This result cannot be explained satisfactorily by a preferential involvement of one rather than another type of muscle fibre, and other factors must therefore contribute to the faster deterioration of TA. The effect of stimulation was assessed by comparing the tension developed by the stimulated muscle to that of the unstimulated control muscle. Stimulation was considered to be beneficial if the stimulated muscles developed more tension than the unstimulated homologous muscles of the same dystrophic animal. Functional improvement was detected when the muscles were severely affected by the disease, but was not noticeable in less severely affected muscles. This indicates that stimulation did not improve the performance by increasing the strength of the existing muscle fibres, but by favouring the survival of regenerating muscle fibres in the diseased muscle. This suggestion is supported by the finding that in these muscles the number of muscle fibres was increased[1] while the radius of individual muscle fibres was not changed.[14] The presence of regenerating fibres in muscles of dystrophic animals has been described[9] and the progressive decrease of the number of muscle fibres[15] indicates that these regenerating fibres do not survive. Even though in some animals the stimulation had no apparent effect on the tension developed by the muscle, it did induce significant changes. The resistance to fatigue was increased in all cases, and thus a functional improvement of muscle performance had occurred. Another change induced by the stimulation was a small, but significant increase in the speed of contraction and relaxation. This result is contrary to the usual slowing effect of low frequency stimulation on normal fast muscles.

It is interesting that low frequency activity does have a different effect on immature or denervated muscles. Stimulation of fast muscles of developing rats at low frequencies did not prevent the increase in the speed of contraction that is known to take place during the first three weeks of development,[16] and stimulation of denervated muscles of newborn rabbits led to an increase of their speed of contraction.[17] It may be that in developing muscles the low frequency activity acts on a different intracellular compartment and induces its maturation, and only when this is achieved can various activity patterns bring about muscle fibre differentiation. Like denervated or developing muscles, the dystrophic muscles too become slower contracting, and this effect of the disease seems to be reduced by the electrical stimulation. Thus electrical stimulation at low frequencies induces a number of changes in diseased muscles. Our results did not allow us to assess the long term effect of these changes, since we followed the animals for only four weeks.

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