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The origin of (+)-tubocurarine resistance in dystrophic mice

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1 Intracellular recording, twitch responses and radio-ligand binding techniques were used to study the causes of resistance to (+)-tubocurarine (curare) of extensor digitorum longus (EDL) muscles from dystrophic mice (129 ReJ/strain).

2 The indirectly evoked twitch response of muscles from dystrophic mice was more resistant to block by curare than the twitch response of muscles from normal littermates. The IC_{50} (concentration producing 50% inhibition of stimulus-evoked contractions) values for the curare block of muscle twitch were $0.78 \pm 0.03\,\mu M$ and $1.32 \pm 0.05\,\mu M$ (mean ± 95% confidence limits) for muscles from normal and dystrophic mice, respectively.

3 There was no difference between muscles from normal and dystrophic mice in the number of α-bungarotoxin binding sites per endplate.

4 The amplitudes of both spontaneous miniature endplate potentials (m.e.p.ps) in unblocked preparations and of evoked endplate potentials (e.p.ps) in 1.91 μM curare were greater in muscles from dystrophic mice than in muscles from normal mice. The ratio dystrophic/normal was greater for the e.p.p. amplitudes than for the m.e.p.p. amplitudes.

5 The quantum content of e.p.ps in magnesium-blocked and in cut-fibre preparations was greater in muscles from dystrophic mice than in muscles from normal littermates. Calculation of the binomial parameters n and p in the cut-fibre preparations indicated that this increased quantum content was caused by an increase in the value of p.

6 It is concluded that at least part of the increased resistance to curare of the indirectly evoked twitch response of muscles from dystrophic mice is due to an increase in the quantum content of e.p.ps in these muscles.

Introduction

It has been reported that dystrophic mice are relatively insensitive to injections of (+)-tubocurarine (curare) and that higher concentrations of curare are needed to induce neuromuscular paralysis in hind-limb muscles from dystrophic mice, than in muscles from normal littermates (Baker *et al.*, 1960; Baker & Sabawala, 1963). However, Harris & Ribchester (1979a) were unable to demonstrate any difference in the effects of curare on indirectly evoked twitch responses of hemidiaphragms from normal and dystrophic mice. Thus the decreased sensitivity to curare may be found only in limb muscles and could be explained by an increase in the safety factor of neuromuscular transmission or to a difference in sensitivity to any presynaptic effects of curare. An increased safety factor could be brought about by: (1) an increase in the number of acetylcholine (ACh) receptors on the postsynaptic membrane or in the output of transmitter from the nerve terminal; (2) a decrease in the activity of acetylcholinesterase, the postsynaptic threshold potential or the muscle fibre resting membrane potential (RMP); (3) an increased postsynaptic input resistance.

The present study was designed first to confirm the curare-resistance of extensor digitorum longus (EDL) muscles from dystrophic mice and secondly to investigate the mechanism(s) responsible for this change. A preliminary account of these findings has been given to the Physiological Society (Kelly *et al.*, 1984a,b).

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Methods

Experiments were carried out on extensor digitorum longus (EDL) muscle preparations from male dystrophic mice of Bar Harbor 129 ReJ strain and their phenotypically normal littermates; no distinction was made between homozygous and heterozygous littermates. Dystrophic mice were identified by their characteristic posture. The animals were killed when they were 8 weeks old, at which age the body weight was 25.6 ± 2.1 g (mean ± s.d.) and 17.2 ± 2.5 g in 17 normal and 14 dystrophic mice, respectively. Muscle weights were 4.4 ± 0.8 mg ($n = 8$) in dystrophic mice and 6.9 ± 0.8 mg ($n = 10$) in normal littermates.

Twitch response

Nerve-muscle preparations were removed from animals anaesthetized with ether and isolated preparations were placed in a perspex bath containing 20 ml of physiological saline of the following composition (mM): NaCl 137, $NaHCO_3$ 12, NaH_2PO_4 1, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1 and D-glucose 25. The saline was gassed with a mixture of 95% O_2 and 5% CO_2 and the temperature was maintained at 33 ± 1°C. The proximal tendon of the EDL was pinned firmly to the base of the bath and the distal tendon attached to a Statham (UF1) tension transducer. The motor nerve was stimulated at a frequency of 0.1 Hz via a suction electrode with pulses of 0.05 ms duration and supramaximal voltage. Twitch responses were recorded isometrically with the Statham transducer and the output from the transducer was suitably amplified and displayed on a pen recorder (Devices, MX2). The resting length of the muscle was adjusted to produce maximum twitch tension.

Electrophysiological recording

Muscles were pinned onto Sylgard 184 (Dow Corning) in a perspex bath and perfused with physiological saline at 33 ± 1°C. Resting membrane potentials, endplate potentials (e.p.ps) and spontaneous miniature endplate potentials (m.e.p.ps) were recorded intracellularly with glass capillary microelectrodes filled with 3M KCl. Miniature e.p.ps and e.p.ps were displayed on an oscilloscope (Tetronix 5000) and the potentials were analysed on-line with an analogue to digital converter and a Z-80 based S100 microcomputer (North Star Horizon). The presence of m.e.p.ps or e.p.ps with a rise time to amplitude ratio of less than 1.1 mV ms^{-1} was used to indicate the focal location of the microelectrode at the endplate. Between 50 and 200 m.e.p.ps were recorded from each muscle fibre and approx. 10 fibres were sampled from each muscle. Spontaneous and evoked e.p.p. amplitudes were corrected for non-linear summation (Martin, 1955) and to a standard resting membrane potential of −74 mV (cf. Kelly, 1978) and the mean amplitude and frequency of m.e.p.ps was calculated at each endplate.

Endplate potentials were recorded either in physiological saline containing (+)-tubocurarine chloride (curare), or in physiological saline in which the calcium concentration was reduced to 0.55 mM and the magnesium concentration raised to 2.45 mM (magnesium-blocked preparations). Cut-fibre preparations (Barstad, 1962; Hubbard & Wilson, 1973) were also used, in which case Martin's correction for non-linear summation was empirically trimmed by a factor of 0.7 (cf. Discussion, McLachlan & Martin, 1981) so that corrected e.p.p. amplitudes would not exceed the reversal potential of approximately −5 mV in these preparations (Banker *et al.*, 1983). Motor nerves were stimulated via a suction electrode by use of an isolated stimulator unit (Digitimer DS2). Stimuli were of 0.05 ms duration and five times threshold intensity. In curare-blocked preparations, trains of e.p.ps elicited by stimulating the nerve at either 10 Hz or 50 Hz were recorded from endplate regions of muscle fibres. Two trains of 100 e.p.ps were usually recorded from each muscle fibre. In each train, the mean amplitude of the last 90 e.p.ps and the ratio of each of the first 6 e.p.ps to the first e.p.p. were calculated. In cut-fibre and magnesium blocked preparations m.e.p.p. and e.p.p. amplitudes were analysed as in the curare experiments and the mean quantum content was also determined directly by dividing mean e.p.p. amplitude by mean m.e.p.p. amplitude.

Statistical analysis of the results

Unless otherwise stated, the results are routinely presented as mean ± s.d. The differences between two

Table 1 Characteristics of the indirectly evoked twitch response of extensor digitorum longus (EDL) muscles from eight week old normal and dystrophic mice

	Normal	*Dystrophic*
Relative twitch tension (g mg^{-1} wet tissue)	0.22 ± 0.09 (10)	*0.12 ± 0.03 (8)
Total twitch duration (ms)	51.10 ± 8.32 (10)	48.0 ± 12.28 (8)
Time to peak (ms)	9.90 ± 1.26 (10)	9.42 ± 1.75 (8)
Half relaxation time (ms)	8.98 ± 2.24 (10)	8.98 ± 1.67 (8)

Values are presented as the mean ± s.d. with the number of muscles in parentheses.
*$P < 0.05$.

means were analysed either by Student's t test or, where a normal distribution could not be assumed, by the Mann-Whitney test. A probability level $P < 0.05$ was considered statistically significant. Statistical analysis of the twitch response data enabled the calculation of the mean dose of curare inducing 50% block (IC_{50}) and its 95% confidence limits by analysis of variance and determining the regression parameters of the linear portion of the log dose-response curves (cf. Documenta Geigy, pp. 176–179). By definition, the true value of IC_{50} has only a 5% probability of being outside the statistically derived 95% confidence limits, and it therefore follows that if the 95% confidence limits of two estimates do not overlap then the probability is considerably greater than 95% that the two estimates are indeed different.

Results

Effect of curare on twitch response

In the absence of curare, EDL muscles from dystrophic mice developed less tension in response to indirect stimulation than did muscles from normal littermates. As this difference was also evident when the tension was expressed per mg muscle wet weight (Table 1), it was not merely a reflection of the smaller mass of dystrophic muscles. There was no significant difference in twitch duration, time to peak, or half relaxation time between muscles from normal and dystrophic animals (Table 1).

The indirect twitch responses of EDL muscles from dystrophic mice were more resistant to curare than muscles from normal littermates, as may be seen from the shift to the right of the log dose-response curve in Figure 1. The calculated IC_{50} values for curare, together with their 95% confidence limits (see Methods), were $0.78 \pm 0.03\,\mu M$ (12 points, 3 animals) and $1.32 \pm 0.04\,\mu M$ (11 points, 3 animals) in normal and dystrophic muscles, respectively.

[^{125}I]-α-bungarotoxin binding

In order to determine whether a difference in the number of ACh receptors might contribute to the curare-resistance of dystrophic EDL muscles, the number of α-bungarotoxin binding sites per endplate was measured using a dissociated fibre technique (Robbins *et al.*, 1980). The results show no difference between normal and dystrophic muscle fibres (Table 2).

Endplate potentials in curarized muscles

The amplitudes of evoked endplate potentials (e.p.ps), recorded intracellularly from muscles in which neuromuscular transmission had been blocked with $1.91\,\mu M$ curare, were greater in muscles from dystrophic mice than in muscles from normal littermates (Table 3). The mean amplitude of spontaneous m.e.p.ps in unblocked EDL muscles was greater in

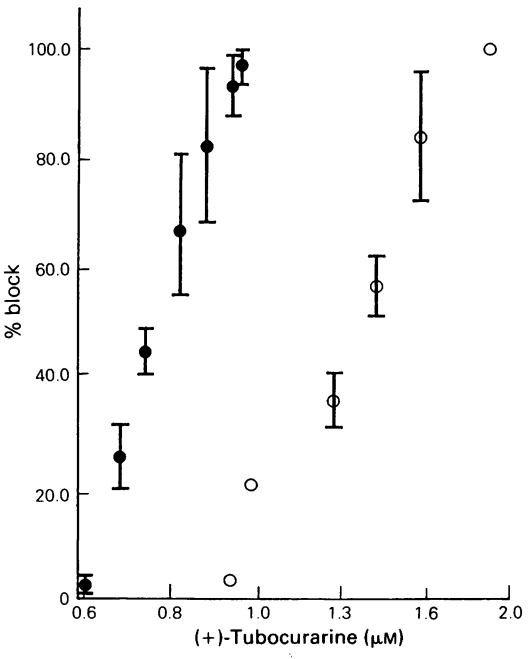

Figure 1 The effect of (+)-tubocurarine on the twitch response of indirectly stimulated extensor digitorum longus (EDL) preparations from normal (●, $n = 3$) and dystrophic (○, $n = 3$) mice. The vertical bars indicate ± 1 s.e. of mean where this exceeds the diameter of the points. The concentration of (+)-tubocurarine is plotted on a logarithmic scale.

Table 2 Binding of [^{125}I]-α-bungarotoxin at junctional and extrajunctional regions of dissociated muscle fibres from EDL muscles of dystrophic mice and their normal littermates

	Number of binding sites ($\times 10^6$)	
	Endplate	*Extrajunctional*
Normal	34.4 ± 2.1	5.5 ± 1.2
Dystrophic	39.1 ± 4.0	6.0 ± 1.5

For each muscle the average number of binding sites per endplate (or equivalent extrajunctional length of fibre) was obtained from 30–50 muscle fibres. The values are presented as the mean ± s.e.mean of those averages from 11 normal and 11 dystrophic muscles.

Table 3 Evoked transmitter release in curarized normal and dystrophic EDL muscles

		Normal	*Dystrophic*
Amplitude of first e.p.p. (mV)		1.69 ± 0.08 (28/3)	*4.04 ± 1.48 (27/3)
10 Hz	Plateau e.p.p. amplitude (mV)	0.84 ± 0.35 (28/3)	*2.05 ± 1.04 (27/3)
	Plateau / first e.p.p.	0.51 ± 0.04 (28/3)	0.51 ± 1.04 (27/3)
50 Hz	Plateau e.p.p. amplitude (mV)	0.71 ± 0.27 (28/3)	*1.58 ± 0.75 (20/3)
	Plateau / first e.p.p.	0.44 ± 0.05 (28/3)	*0.36 ± 0.09 (20/3)

Amplitudes corrected for nonlinear summation and to a standard RMP (see Methods).
Values are presented as the mean ± s.d. with the number of muscle fibres per number of muscles in parentheses.
*$P < 0.05$.

dystrophic than in normal muscles, being 0.84 ± 0.46 mV ($n = 75$) and 0.56 ± 0.27 mV ($n = 96$), respectively.

The ratio of the mean amplitude of plateau e.p.ps evoked at 10 Hz in dystrophic muscles to the mean amplitude of plateau e.p.ps in normal muscles was 2.2, whereas the ratio of mean m.e.p.p. amplitudes in the corresponding unblocked muscles was only 1.5. If it is assumed that curare produces the same proportional reduction of quantum size in muscles from dystrophic mice as in muscles from normal littermates, then these results suggest that transmitter output is increased in nerve terminals in dystrophic mice. Another indication of a presynaptic difference between normal and dystrophic muscles is the significant difference in the ratio, mean plateau e.p.p. amplitude/amplitude of first e.p.p., between muscles from normal and dystrophic mice stimulated at 50 Hz (Table 3) suggesting a steeper rundown of e.p.ps in muscles from dystrophic mice. When the differences between the amplitudes of each of the first six e.p.ps and the mean plateau e.p.p. amplitude were expressed as percentages of the first e.p.p. and then these normalized values were plotted against e.p.p. number, a good fit was obtained to an exponential decline (Pearson's $r > 0.85$). At 10 Hz the decay constants calculated from a least-squares regression were -0.7 ± 0.21 ($n = 23$) and -0.48 ± 0.12 ($n = 25$) in dystrophic and normal muscles, respectively. The corresponding values at 50 Hz were -0.49 ± 0.10 ($n = 19$) and -0.30 ± 0.08 ($n = 27$).

Magnesium-blocked preparations

The quantum content of e.p.ps cannot be obtained directly in a curare-blocked preparation because m.e.p.ps are absent and it has been reported that curare affects transmitter release (e.g. Wilson, 1982). Therefore neuromuscular transmission was blocked by placing muscles in low-calcium, high-magnesium saline (see Methods) and then recording m.e.p.ps and e.p.ps in the same muscle fibres. The quantum contents of e.p.ps evoked at 1, 10 or 50 Hz were then calculated and it was found that the values obtained at each stimulus frequency were greater in muscles from dystrophic mice than in muscles from normal littermates (Table 4).

Cut-fibre preparations

In magnesium-blocked preparations the output of transmitter is very much lower than in unblocked muscle and it may be that the larger quantum content in the dystrophic EDL under such conditions reflects a difference in the calcium sensitivity of the release process rather than a difference in transmitter release under the more normal conditions. For this reason, it was decided to study transmitter release in the cut-fibre preparation by recording e.p.ps evoked at 10 Hz and m.e.p.ps from the same muscle fibres. The results show that plateau e.p.p. amplitudes and quantum content were all greater in the dystrophic muscles (Table 5).

Histograms of plateau e.p.p. amplitudes recorded at individual endplates were plotted and, using the method of Miyamoto (1975), theoretical binomial and Poisson distributions were fitted to the observed distribution of amplitudes. In all cases it was found that the binomial distribution gave the best fit to the observed distribution (Chi-square test), and thus it

Table 4 Quantum content in magnesium blocked EDL muscles from normal and dystrophic mice

Stimulus frequency (Hz)	*m* *Normal*	*Dystrophic*
1	2.65 ± 1.15 (34/5)	*6.09 ± 3.87 (15/6)
10	2.69 ± 1.23 (38/5)	*5.25 ± 3.57 (30/6)
50	4.14 ± 1.83 (36/5)	*6.92 ± 4.58 (27/6)

Values are presented as the mean ± s.d. with the number of muscle fibres per number of muscles in parentheses. Quantum content was determined by the direct method (see Methods).
*$P < 0.05$.

Table 5 Evoked transmitter release in cut EDL muscle fibres from normal and dystrophic mice

	Normal	*Dystrophic*
Plateau e.p.p. amplitude (mV)	13.64 ± 4.60 (34/5)	*19.98 ± 5.60 (32/6)
Quantum content (m)	36.25 ± 13.81 (34/5)	*53.57 ± 19.19 (32/6)

Amplitudes corrected for nonlinear summation and to a standard RMP. Quantum content determined by the direct method (see Methods). Values are presented as the mean ± s.d. with the number of muscle fibres per number of muscles in parentheses.
*$P < 0.05$.

was possible to calculate values of the binomial parameters n and p in normal and dystrophic muscles (cf. Miyamoto, 1975; Glavinović, 1979). The mean values of these parameters are given in Table 6, from which it may be seen that the value of p is greater in dystrophic muscles, whereas there is no difference in the value of n between the two groups.

Discussion

The reduction in twitch tension found in the present study is a feature characteristic of dystrophic muscle (Sandow & Brust, 1958; Harris & Wilson, 1971) indicative of degeneration of muscle fibres. When the total twitch duration, time to peak, or half relaxation time were compared in EDL muscles from 8 week old dystrophic mice and normal littermates no significant differences were found (Table 1), and although the variation in these parameters between muscles was considerable, in no case was the value of either parameter in dystrophic muscle greater than the maximum for normal muscle. There have been numerous reports that the time to peak and half relaxation time of directly evoked twitch responses are prolonged in dystrophic mouse hind-limb muscles (Sandow & Brust, 1958; Hinterbuchner *et al.*, 1966; Sabbadini & Baskin, 1976), but it may be argued that the discrepancy between our results and the findings of others are due to differences in the experimental procedures, the muscles and/or the ages of the animals used.

Table 6 Binomial parameters (m, n and p), in cut-fibre EDL muscles from normal and dystrophic mice

	Normal	*Dystrophic*
Quantum content (m)	39.92 ± 13.27 (17/2)	*56.39 ± 14.75 (15/3)
Number of quanta available for release (n)	65.60 ± 28.06 (17/2)	62.16 ± 16.23 (15/3)
Probability of release (p)	0.66 ± 0.18 (17/2)	*0.91 ± 0.07 (15/3)

Values are presented as the mean ± s.d. with the number of muscle fibres per number of muscles in parentheses.
*$P < 0.05$.

The decreased sensitivity of EDL muscles from dystrophic mice to curare (Figure 1) is consistent with the findings of Baker & Sabawala (1963), who studied the peroneus longus muscle of the mouse. Therefore, in murine dystrophy there seems to be a reduced effect of curare in hind-limb muscles, although in other dystrophic muscle preparations the sensitivity to curare may not differ from normal (see Harris & Ribchester, 1979a). Baker & Sabawala (1963) suggested that one explanation for this resistance to curare in hind-limb muscles from dystrophic mice could be an increase in the number of acetylcholine receptors on the postsynaptic membrane. It has been reported that there are more junctional (Howe *et al.*, 1976) and extrajunctional (Howe *et al.*, 1977) receptors on the postsynaptic membrane of dystrophic EDL muscles, but our results (Table 2) and the results from other binding studies (Marusyk & Monckton, 1976; Matthews-Bellinger, 1980) are not consistent with this suggestion.

Another possible explanation for the curare resistance of dystrophic muscle, suggested by Beaulnes *et al.* (1966), is that in murine muscular dystrophy there is a presynaptic abnormality that increases the amount of acetylcholine released by the nerve terminal in response to a nerve impulse. Support for such an explanation can be found in the following results obtained in this study: (i) e.p.p. quantum content in both cut-fibre and magnesium-blocked preparations was greater in dystrophic mice; (ii) the initial rundown of e.p.ps in curarized preparations was greater in dystrophic mice, and (iii) the ratio (dystrophic/normal) of e.p.p. amplitudes in curarized preparations was larger than the corresponding ratio of m.e.p.p. amplitudes in unblocked preparations. Other workers have reported that quantum content is not significantly different in EDL muscles from normal and dystrophic mice (Carbonetto, 1977; Harris & Ribchester, 1979b) but in the experiments of Harris & Ribchester (1979b) the quantum content of e.p.ps was calculated from the coefficient of variation of e.p.p. amplitudes in curarized preparations, and this method of estimating quantum content assumes that transmitter release is described by Poisson statistics. However, although there is evidence to suggest that transmitter release follows Poisson statistics when release is depressed by lowering the calcium/magnesium concentration-ratio in the bathing solution (del Castillo & Katz, 1954;

Elmqvist & Quastel, 1965), a number of studies at vertebrate and invertebrate neuromuscular junctions, including the present one, have demonstrated that a binomial distribution fits the results better when release is not depressed (Johnson & Wernig, 1971; Bennet *et al.*, 1975; Miyamoto, 1975; Glavinović, 1979). Under these conditions the estimation of quantum content based on a Poisson distribution seriously overestimates its value (Wilson, 1977). The disparity between Carbonetto's (1977) results and our own may be explained by the different experimental conditions used; for example, he used muscle preparations bathed in solutions containing neostigmine and 15 mM Mg^{2+}.

An increase in quantum content in EDL muscles from dystrophic mice, coupled with the lower resting membrane potential of dystrophic muscle fibres (Harris & Ribchester, 1979b) and the increase in m.e.p.p. amplitude, would all contribute to the increase in safety factor reported in the dystrophic EDL (Harris & Ribchester, 1979b). However, curare is claimed to have a presynaptic effect (e.g. Wilson, 1982), so a further contributing factor to the difference in curare resistance could be a different presynaptic effect of curare on nerve terminals in normal and dystrophic mice.

It is possible to calculate a value for the relative safety factors of neuromuscular transmission by combining the data obtained in these experiments with values for threshold potential obtained by Harris & Ribchester (1979b) in the following formula:

$$\text{safety factor} = \frac{\text{m.e.p.p. amplitude} \times \text{quantum content}}{\text{corr. (RMP} - \text{Threshold)}}$$

where corr. (RMP – Threshold) is the difference between the resting membrane potential and the threshold potential after this difference has been corrected for non-linear summation of unit depolarization (cf. Kelly & Roberts, 1977). Using values of quantum content in the cut fibre preparations, the ratio of the safety factors between muscles from dystrophic and normal mice is 4.2 and 4.4 for plateau e.p.ps and first e.p.ps of trains, respectively. This relative increase in safety factor in muscles from dystrophic mice is greater than that reported by Harris & Ribchester (1979b) and also greater than the curare IC_{50} ratio (1.69) determined from the results of the present experiments. Two explanations could account for these differences: (1) the threshold values used to calculate safety factor were those determined by Harris & Ribchester (1979b) and so small differences between the values they obtained and the true threshold potentials for e.p.ps in our preparation could cause marked differences in the absolute values of safety factor and hence in any relative difference between normal and dystrophic muscle preparations; (2) any presynaptic effect of curare on quantum content which was less in dystrophic muscle preparations would tend to reduce differences in the curare IC_{50} ratio caused by differences in the safety factor.

Quantum content is thought to be the product of the number of quanta available for release (n) and the probability (p) that a quantum will be released by a stimulus (del Castillo & Katz, 1954). Thus an increase in quantum content could be due to an increase in n and/or p. When n and p were calculated from our cut-fibre results no significant difference was found between the mean value for n between normal and dystrophic EDL muscles but the mean value for p was significantly greater in dystrophic muscles (Table 6). McLachlan (1978) postulated that the probability of release of transmitter is dependent upon calcium influx during nerve terminal activation and on the concentration of residual calcium in the motor nerve terminal, so an increase in p in dystrophic EDL muscles could be a consequence of an increase in residual calcium in the motor nerve terminal and/or increased calcium influx during the nerve action potential. However, Bennett *et al.* (1975) found that when $[Ca^{2+}]_o$ was varied in the range 0.1 to 1.0 mM, p increased as the first power of $[Ca^{2+}]_o$ and n as the third power of $[Ca^{2+}]_o$. Therefore, any abnormality of dystrophic nerve terminals causing an increase in calcium entry during the nerve terminal activation should increase both p and n and the increase in n should be greater than the increase in p. The finding that only p is increased in dystrophic nerve terminals could be explained by an increase in the concentration of free calcium ions in the nerve terminal or by a change in the neuronal membrane which facilitates transmitter release by increasing the probability of fusion of synaptic vesicles with the presynaptic membrane. The intraterminal concentration of free calcium ions in motor nerve terminals is thought to determine the rate of spontaneous transmitter release (Baker, 1972). Therefore, any increase in the concentration of free calcium ions in dystrophic nerve terminals should be reflected by an increase in the rate of spontaneous transmitter release, i.e. an increase in m.e.p.p. frequency. Under control conditions, m.e.p.p. frequency in EDL muscles from dystrophic mice (129/Rej strain) is not significantly different from normal (Kelly *et al.*, 1984b). Furthermore, m.e.p.p. frequency in EDL muscles from normal and dystrophic mice is similarly affected by increasing the extracellular calcium or potassium concentration or following repetitive stimulation of the motor nerve (Kelly *et al.*, 1984b). Thus it appears that nerve terminals in EDL muscles from dystrophic mice can regulate intraterminal free calcium at rest, or under conditions of increased calcium influx, as well as nerve terminals in muscles from normal littermates. It is therefore unlikely that the increase in p found at nerve terminals in dystrophic muscles is the result of an increase in

intraterminal free calcium ion concentration and is more likely to be a consequence of a difference in the neuronal membrane at these nerve terminals.

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