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# Energetic metabolism and fatigability in experimental myotonia

## Metabolismo energético y fatigabilidad en miotonía experimental

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Experimental myotonia was induced in rats by 2,4-dichloro-phenoxyacetic acid (2,4-D). After 4 to 24 h of treatment, the anterior tibialis muscles exhibited increased fatigue at low frequency (30 Hz) nerve stimulation, but they developed normal tension at high-frequency (100 Hz) stimulation. Glycogen content and the activities of glycogen phosphorylase, lactate dehydrogenase and malate dehydrogenase remained normal. The absence of correlation between fatigability and energetic metabolism in this experimental model of myotonia suggests a dysfunction in excitation-contraction coupling.

The myotonias are usually hereditary diseases, characterized by the inability to relax the muscle following their voluntary contraction (Bethlem, 1980). Myotonias may be chemically induced, among other drugs by 2,4-dichlorophenoxyacetic acid (2,4-D). The resulting myotonia is similar to congenital myotonia, in both clinical and electromyographical aspects (Iyer *et al.*, 1977).

Increased fatigue of exercised muscles has been described in several myotonias (Bethlem, 1980). In 2,4-D-induced myotonia, fast muscle are more prone to fatigue than in normal control rats (Ramírez and Soza, 1988). Thus, experimentally induced myotonia provides an attractive model to study the factors underlying muscle fatigue, a phenomenon still poorly understood.

Muscle fatigue produced by stimulation at low frequencies has been mainly attributed to metabolic dysfunctions (Edwards *et al.*, 1977; Hermansen, 1981), among others those induced by failure of energy supply (Idström, 1986; Edwards, 1981) and increased amount of intracellular hydrogen ions (Hermansen, 1981; Sahlin, 1978). Data regarding the level of muscle enzyme activity in myotonic muscle is scant and contradictory (Emery, 1968; Stöhr *et al.*, 1975). Thus, I decided to study the relationship between fatigability and energy supply in a fast muscle (anterior tibialis, AT) from rats treated with 2,4-D. Since fast muscles obtain their energy for contraction mainly from anaerobic glycolysis of endogenous glycogen (Crow and Kushmerick, 1982), the glycogen content of this muscle and the activities of two enzymes involved in anaerobic glycogen metabolism –glycogen phosphorylase (Ph b) and lactate dehydrogenase (LDH)– were measured. Muscle oxidative capacity was also studied by measuring the activity of malate dehydrogenase (MDH).

## METHODS

Adult male Sprague-Dawley rats were injected with single intraperitoneal doses of 2,4-D 200 mg/kg. This dose of 2,4-D caused no rat mortality. The drug was dissolved in ethanol and then added slowly to 0.9% NaCl, under constant stirring and maintaining pH at 7.0-7.2 by adding NaOH. Final concentration of ethanol was 5%. Control animals were injected with the same volume of solvent.

The presence of myotonia was established by conventional electromyography (EMG) as previously described (Ramírez and Soza, 1988). Drug-treated muscles developed characteristic myotonic potentials: high frequency repetitive discharges, that initially increased in frequency and amplitude and then rapidly diminished (Bethlem, 1980). This myotonic activity was already present 10 min after drug injection and persisted for at least 48 h.

Rats were anesthetized with sodium pentobarbitone (40 mg/kg; i.p.) after either 4 or 24 h of injecting the drug or the solvent, to record the isometric contractions of AT muscle through a strain

gauge transducer (Grass, FT.03) and polygraph. Square pulses of 0.2-0.5 ms duration and supramaximal intensity were delivered to the sciatic nerve distally to a crush. The resting length of the muscle was adjusted to obtain maximal twitch tension. Tetanic contractions were evoked by pulses trains at low (30 Hz) or high (100 Hz) frequencies, for 330 ms and repeated every second. The fatigue at 30 Hz stimulation was measured according to Burke *et al.* (1971) by the fatigue index, *i.e.*, the ratio of the tetanic tension at 2 min stimulation over the initial tetanic tension. The contralateral AT muscle was then excised, frozen in liquid nitrogen and stored frozen until the measurement of enzyme activities.

The AT muscles were werghted and homogenized (1:20 w/v) in 0.1M Tris-HCl, pH 8.0, containing 1 mM EDTA and 1 mM mercaptoethanol. The homogenate was centrifuged at 12 000 g for 10 min. The activities of LDH (Dietz and Lubrano, 1967) and MDH (Ochoa, 1955) were measured spectrophotometrically (340 nm) at 25° C in the 12 000 g supernatant fraction. Total glycogen phosphorylase b (Ph b) activity was determined in the homogenate from the amount of inorganic phosphate released from glucose-1-phosphate in presence of AMP, during a 10 min incubation at 37°C, according to Wanson and Drochmans (1972), in a medium supplemented with 0.2 mg/ml of bovine serum albumin. Noncollagen protein was measured both in the homogenate and the supernatant fraction (Lowry *et al.*, 1959). Glycogen content was measured in resting AT muscle from treated and control rats. The muscles were frozen in isopentane precooled with liquid nitrogen and sectioned (10 nm) in a cryostat. Sections of both control and drug-treated muscles were placed on the same slide and their glycogen content estimated by the periodic acid-Schiff reaction.

Values are expressed as means ± SEM's. The Mann-Whitney U test for two independent samples or the Kruskall-Wallis one-way analysis of variance for independent samples (Siegel, 1956) were used to analyse the data, and differences were considered statistically significant when $p < 0.01$.

## RESULTS

The contractile response of the muscle was altered after 2,4-D treatment. At the beginning of the stimulation period, inter-tetanic tension was transiently kept well above the baseline but, as tetanic stimulation continued, this minimal tension reached the baseline and the muscle maximal tetanic tension declined (Fig. 1). After 2 min of stimulation, drug-treated muscle developed less tension than control muscles (Fig. 1).

A decrease in tension output while stimulation is maintained is a clear sign of fatigue. Therefore, resistance to fatigue was measured both in control and 2,4-D-treated AT muscles, by electrical stimulation of the corresponding motor nerves. Drug-treated muscles showed a lower fatigue index than control muscle ($p < 0.01$) that is, were less resistant to fatigue. Muscle fatigability was already increased 4 h after drug treatment (Table 1). No further increase was detected by prolonging the treatment for 24 h ($p > 0.4$).

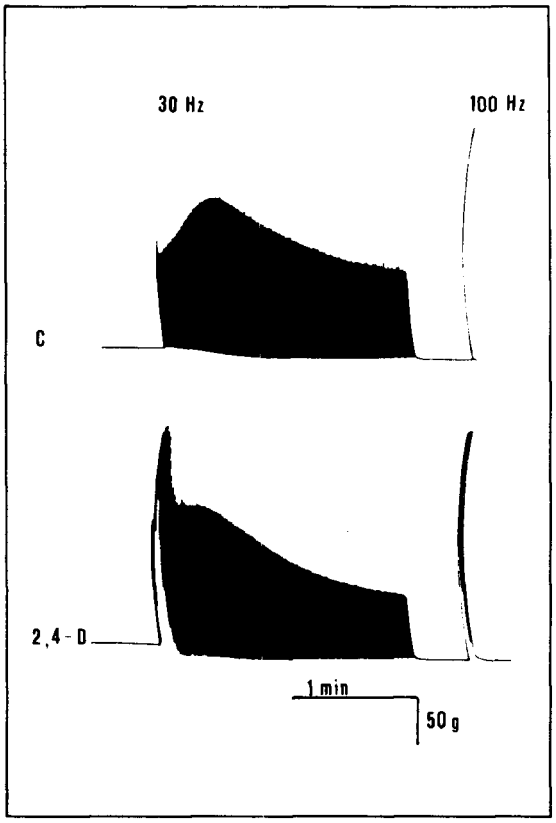


*Fig. 1:* Tetanic contractions of At muscle, evoked by nerve stimulation at 30 Hz (trains of 330 ms every ls), followed by stimulation at 100 Hz. C, control rats; 2,4-D, rats treated for 4 h.

A decrease in tension output could be due to a failure either of the synaptic transmission or of the subsarcolemmal system. To rule out a possible dysfunction of neuromuscular transmission produced by 2,4-D treatment, myotonic muscles were stimulated at high frequency (100 Hz) immediately after the fatigue test. As shown in Fig. 1, the output tension of myotonic muscles was normal at high frequency stimulation. This implies that synaptic transmission was still functional while the muscles were

TABLE 1

Fatigability and enzymatic activities of anterior tibialis

| EXPERIMENTAL CONDITION | | FATIGUE INDEX | ENZYME ACTIVITY (U/mg protein) Lactate Dehydrogenase | Glycogen Phosphorylase | Malate Dehydrogenase |
|---|---|---|---|---|---|
| CONTROL | | 0.90 ±0.07 (n = 7) | 2.38 ± 0.16 (n = 14) | 0.41 ± 0.03 (n = 13) | 1.41 ± 0.16 (n = 6) |
| 2,4-D | 4 h | 0.35 ± 0.04 (n = 7) | 2.23 ± 0.09 (n = 4) | 0.44 ± 0.03 (n = 4) | --- |
| 2,4-D | 24 h | 0.33 ± 0.08 (n = 5) | 2.19 ± 0.26 (n = 10) | 0.49 ± 0.02 (n = 9) | 1.17 ± 0.07 (n = 7) |
| Overall ANOVA | | $p < 0.01$ | $p > 0.05$ | $p > 0.05$ | $p > 0.02$ Mann-Whitney |

fatigued. Recovery of the tension output at high frequency stimulation was not due to the small rest period before 100 Hz stimulation, because the muscle remained fatigued when stimulated at 30 Hz after 100 Hz stimulation.

The AT muscle capacity to produce energy for contraction appeared normal after treatment with 2,4-D, although drug-treated muscles were less resistant to fatigue than control muscles. As shown in Table 1, the activity of both enzymes involved in anaerobic glycogen metabolism, glycogen phosphorylase and lactate dehydrogenase, remained at normal levels for at least 24 h after drug treatment. Malate dehydrogenase activity was slightly reduced 24 h after 2,4-D treatment. However, the difference between drug-treated and control muscles was not significant (Table 1). Histochemical studies revealed no change in the glycogen content of AT muscle, even at 24 h of 2,4-D treatment.

## DISCUSSION

Muscles from myotonic rats were less resistant to fatigue than those from normal control rats. Since drug treatment did not impair neuromuscular transmission, the dysfunction underlying muscle fatigability should be located in the muscle fiber itself.

Contractile activity needs an adequate supply of energy. This depends on the availability of substrates and the activity of the enzymes involved in energetic metabolism. Glycogen catabolism is initiated by Ph b, while LDH converts pyruvate to lactate, the final step in glycolysis. Since both enzymes retained their normal activity after drug treatment, the glycolytic capacity of this muscle was apparently not modified by 2,4-D treatment. Substrate availability appeared also normal, since no change was detected in the glycogen content of drug-treated muscles. This is in agreement with results of Iyer *et al.*, (1977), who reported a normal glycogen content in rat muscles after 4 h of treatment with 2,4-D.

Resistance to fatigue has been associated with the activity level of the enzymes involved in oxidative metabolism. Fast fatigable muscles transformed into fatigue-resistant ones by chronic stimulation show a concomitant increase in their oxidative capacity (Kwong and Vrbova, 1981; Pette and Vrbova, 1985). Furthermore, the activity of MDH is roughly proportional to muscle fiber endurance (Nemeth *et al.*, 1980). In 2,4-D-treated muscles, however, no significant change in MDH activity was detected, although myotonic muscles were less resistant to fatigue than control ones. Intramuscular acidosis has been also mentioned as a cause of muscle fatigue (Hermansen, 1981; Sahlin, 1978). However, when comparing the pH of normal and 2,4-D-treated muscles after a fatigue test no difference was found, although only the drug-treated muscles were fatigued (Ramírez and Soza, 1988). Therefore, muscle fatigue in 2,4-D-

treated rats seems to be due to a factor other than a dysfunction of the enzymes involved in energy production, a decreased pH or substrate availability.

The specific site of action of 2,4-D on skeletal muscle has not yet been determined. However, it probably interferes with the sarcolemmal membrane since myotonia has been attributed to altered ionic-channel properties of the muscle fiber membrane (Bryant and Morales-Aguilera, 1971; Rüdel and Senges, 1972; Rüdel and Lehmann-Horn, 1985). The increased muscle fatigability could be a consequence of the changes induced in the muscly by the agent that triggers the myotonia.

The present results indicate that the tension output recovered after high-frequency nerve stimulation of the fatigued muscles, showing that the neuromuscular transmission and the contractile mechanism were functional after drug treatment. Since 2,4-D-treated muscles developed low-frequency fatigue despite the presence of functional neuromuscular transmission and contractile capacity, one may suppose the existence of a failure between excitation and contraction, that is, a dysfunction in the excitation-contraction coupling.

In summary, no correlation was found between resistance to fatigue and the energetic metabolism in muscles from 2,4-D-treated rats. The present results suggest that a failure of the excitation-contraction coupling might be involved in muscle fatigability in this experimental model of myotonia.

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