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Fine structural changes in electrostimulated human skeletal muscle

Evidence for predominant effects on fast muscle fibres

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Summary. Physical education students were subjected to electrical stimulation of relatively high frequency and current amplitude for 19 days. A quantitative study of several morphological parameters was performed on biopsy samples from gastrocnemius, using stereological methods at both light and electron microscopic levels. The main results were: muscle fibre size was increased; nuclear volume was also increased, suggesting that a proliferation of nuclei had occurred; this was paralleled by an increased content of nuclear DNA. The size of single myonuclei was increased, and their heterochromatin fraction was decreased, these changes being most pronounced in type II fibres. The increase in the mitochondrial fraction was also greatest in type II fibres. It is concluded that this type of electrical stimulation has predominant effects on type II fibres.

Key words: Electrical stimulation — Skeletal muscle — Fast muscle fibres — Training — Stereology

Introduction

Our knowledge of the effects of electrical stimulation of locomotor muscles is based mostly on animal experiments using long-term stimulation with relatively low frequencies (for references, see Nix and Vrbová 1986). This type of muscle activation is applied to humans for the restoration of denervated muscles (Lewis et al. 1986), in several muscle diseases (Scott et al. 1986), and also during the treatment of idiopathic scoliosis (Schmitt 1986). The cellular changes occurring during electrical stimulation, however, have so far mostly been described for animal models. After low-frequency long-term stimulation, a decrease in fibre size accompanied by a decline in muscle wet weight has been reported (Eisenberg et al. 1984). It has been assumed that this type of stimulation produced conditions of net protein catabolism (Salmons and Henriksson 1981).

Several authors found an increased nuclear fraction in trained muscles (James and Cabric 1982; Cabric and James 1983), others did not find such a change (Sieden 1976), and Atherton et al. (1981) reported a relative decrease in the amount of myonuclei in hypertrophied muscle, suggesting that no proliferation of nuclei had occurred. Whatever the experimental condition, morphological changes in nuclei might be accompanied by functional modifications in, for example, the heterochromatin/euchromatin fraction and the amount of DNA (Frenster et al. 1963; Littau et al. 1964).

With electrical muscular stimulation of relatively high frequency and high current amplitude, as used for strength training, changes in muscle morphology, especially in the nuclear fraction, might be expected, since nuclear proliferation and/or hypertrophy can be considered as a prerequisite for muscle fibre hypertrophy. Metabolic adaptations of muscle fibres to the increased activity caused by electrical stimulation can be most easily observed at the morphological level by changes in the mitochondrial fraction.

In this investigation the effects of electrical stimulation upon human muscles, which mimics a strength training exercise, have been studied morphologically with particular attention being paid to any differential effects on muscle fibre types.

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Materials and methods

Six male physical education students (age 19—22 years) participated in this study. They were moderately trained and did not participate in any competitive sport. After having given their informed consent to the experiment after explanation of the procedure and of possible risks, they were subjected to electrical stimulation for 10 min daily for 19 days. Surface stainless steel sheet-metal electrodes (45·55 mm) covered with water-soaked foam rubber were used for the stimulation of triceps surae. The upper electrode was placed below the knee over gastrocnemius, the lower electrode being at the point where gastrocnemius ends and the tendo calcaneus begins. During stimulation the leg was fixed in a special device to maintain a knee joint angle of 90°, the ankle joint being dorsiflexed at about 10°.

For stimulation, alternating currents of sinusoidal wave form with a frequency of 2500 Hz were used. The intensity was adjusted so that strong tetanic contractions occurred, this being achieved at 35—45 mA; the pulse width was 0.15 ms. No subject reported serious discomfort from this current. Each stimulus lasted for 10 s followed by a rest of 50 s, and 10 contractions were performed in each session.

One week before the beginning of the stimulations, and one day after the last stimulation, biopsies were taken from the lateral part of gastrocnemius under subcutaneous anaesthesia (Xylocain, 2% solution) using biopsy needles. Each specimen of muscle was immediately fixed with 3% glutaraldehyde in 0.1 M sodium cacodylate-HCl buffer (pH 7.4, 4°C) for 4 h, rinsed in the same buffer containing 0.12 M sucrose several times, postfixed with 1% aqueous osmium tetroxide, and was embedded in Epon 812 after dehydration. For light microscopic examination (LM) semithin longitudinal and transverse sections were made from the samples and were stained with toluidin blue.

Longitudinal and transverse ultrathin sections were contrasted with uranyl acetate and lead citrate for electron microscopic examination (EM). The morphometric investigation was performed at magnifications of ×215 (LM) and ×22,500 (EM).

For LM quantification of fibre size the cross-sectional area of the muscle fibres was measured using a semi automatic image analysing system MOP AM 03 (Kontron, Munich, FRG).

The density of nuclei as expressed as the number of nuclei per unit of muscle volume (N_V) was calculated from the number of nuclei per area of muscle cross section (N_A) and from the mean length of nuclei ($\bar{l}$) as measured in longitudinal sections at LM level using the relationship:

$$N_V = N_A \cdot \bar{l}^{-1} \quad \text{(Cruz-Orive 1980)}$$

The mean size of nuclei ($\bar{V}$) was estimated using the relationship:

$$\bar{V} = A_A \cdot N_V^{-1} \quad \text{(Underwood 1970)}$$

A_A being the fraction of the area of skeletal muscle occupied by myonuclei as estimated in transverse sections using a point counting technique (Unterwood 1970).

The DNA content per nucleus (DNA_N) was estimated using the value of the DNA content (DNA_c) previously found with biochemical methods (0.04 pg · μm^{-3}; David 1977) using the relationship

$$DNA_N = \bar{V} \cdot DNA_c$$

The morphometric analysis for the following parameters was performed at EM level, and the data were obtained separately for slow (Type I) and fast (Type II) fibres. Fibres were classified according to ultrastructural characteristics such as the amount and localization of mitochondria, and the thickness and pattern of the Z-line. Nuclear size was estimated by measuring its area on the MOP AM 03. From these nuclei the heterochromatin (HC) content was estimated by using specific stereological screens and a methodology described in detail in a previous paper (Cabric and James 1984). The mitochondrial fraction of the muscle fibres was estimated by using the point counting method (Underwood 1970; Weibel 1980).

The data from each subject were collected and mean values and standard deviations were calculated for the group. Significances of differences found after stimulation were tested using the Student t test.

Results

The electrical stimulation led to hypertrophy of muscle fibres (Table 1, Fig. 1). Their cross sectional area increased by 20% from 3321 μm^2 to 3979 μm^2 ($p < 0.05$). The number of nuclei per

Table 1. Morphometric parameters and calculations based on light microscopic evaluations

	Fiber size (μm^2)		N_A (mm^{-2})		$\bar{l}$ (μm)		N_V ($mm^{-3} \times 10^4$)		$\bar{V}$ (μm^3)		DNA_N ($pg \cdot N^{-1}$)	
	before	after	before	after	before	after	before	after	before	after	before	after
1	2359	2762	446	433	10.0	10.7	4.46	4.05	299	247	12.0	9.9
2	2993	3663	403	385	9.5	11.1	4.27	3.47	209	352	8.4	14.1
3	3517	4523	254	258	11.5	12.2	2.21	2.12	302	419	12.1	16.8
4	4171	5178	294	297	11.6	12.1	2.53	2.46	263	460	10.5	18.4
5	3405	3905	301	337	12.3	13.4	2.45	2.51	272	266	10.9	10.6
6	3484	3843	267	294	12.1	14.0	2.21	2.10	302	318	12.1	12.7
	3321	3979	327	334	11.2	12.2	3.02	2.78	274	344	11.0	13.7
SD	605	817	78	65	1.1	1.3	1.05	0.79	36	84	1.4	3.4
$P <$	0.05		n.s.		n.s.		n.s.		0.05		0.05	

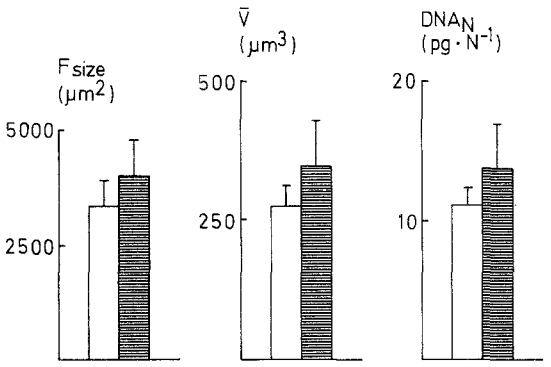

Fig. 1. Schematic representation of the data (cf. Table 1) for fibre size, nuclear volume, and DNA content per nucleus; open columns: before electrostimulation, dark columns: after electrostimulation

unit area (N_A) and per unit volume (N_V) did not change significantly, and the maximum diameter ($\bar{l}$) of the nuclei was unchanged. On the other hand the nuclear volume within a given tissue volume was increased by 25% (from 274 to 344 μm^2, $p < 0.05$). The increase in DNA content per nucleus was in a similar range (11.0 pg before, 13.7 pg after stimulation, $p < 0.05$).

With regard to the different fibre types (Table 2, Fig. 2), nuclear size expressed as its area in EM transverse sections showed the biggest increase in type II fibres, by about 50% from 219.4 to 328.5 μm^2 ($p < 0.001$). In type I fibres the nuclear size changed by 20% from 248.6 to 296.5 μm^2 ($p < 0.02$). The changes observed for the area fraction of heterochromatin showed a similar pattern; in type II fibres it decreased from 51.0% to 34.9% ($p < 0.001$), whereas in type I fibres the decrease was smaller (from 40.2% to 37.0%, $p < 0.05$). The mitochondrial fraction was considerably increased in type II fibres, from 14.1% to 21.9% ($p < 0.001$), whereas in type I fibres little change was found (from 36.9% to 39.4%, $p < 0.02$).

Discussion

Fibre size and volume are closely related to the volume of myonuclei. Casperson (1950) was one of the first authors to establish that increased activity of cells leading to cellular hypertrophy is paralleled by an increase in nuclear activity. Both the size and number of nuclei are augmented under similar conditions (Policard 1972). Clearly such changes can also be caused by electrical stimulation of a muscle. While N_A and N_V did not change significantly, these findings have to be considered in relation to the increased muscle fibre size: larger muscle fibres necessarily mean less fibres per unit volume and unit area. Thus the number of nuclei per single muscle fibre might have been increased. Moreover, the increase in mean size of the nuclei ($\bar{V}$) evident at the light microscopic level clearly indicates enlargement of individual nuclei during electrical stimulation. This finding was confirmed at the electron microscopic level, which showed that the increase in nuclear size was more pronounced in type II than in type I fibres.

The type of stimulation, especially the frequency, may be crucial to its effects on the myonuclei. The results of this study, which employed high frequencies, contrast with those which failed to describe changes in the nuclear fraction after long-term electrical stimulation at very low frequencies (Shah et al. 1985). Following this latter type of stimulation, fibre diameters were smaller

Table 2. Morphometric data as measured on electron micrographs

	Nuclear size (μm^2)				HC fraction of nuclei (%)				Mitochondrial fraction (%)			
	Type I fibres		Type II fibres		Type I fibres		Type II fibres		Type I fibres		Type II fibres	
	before	after	before	after	before	after	before	after	before	after	before	after
1	223.1	241.3	159.5	365.1	46.1	38.6	50.5	35.6	36.2	45.0	12.7	22.9
2	198.7	262.7	253.2	339.8	32.4	36.6	51.1	35.7	38.1	37.2	15.1	21.3
3	234.6	298.8	241.3	303.3	39.8	35.3	49.8	36.3	37.7	39.7	11.3	20.4
4	227.5	304.0	218.8	319.6	43.7	37.8	52.0	34.2	35.4	38.1	14.6	22.0
5	307.6	327.8	189.6	299.8	40.2	36.9	51.7	33.7	36.5	38.3	14.9	21.8
6	300.1	344.4	254.3	343.4	39.1	36.8	51.1	34.0	37.6	37.9	15.8	23.3
	248.6	296.5	219.4	328.5	40.2	37.0	51.0	34.9	36.9	39.4	14.1	21.9
SD	44.5	38.8	38.2	25.4	4.7	1.1	0.8	1.1	1.0	2.9	1.7	1.0
$p <$	0.02		0.001		0.05		0.001		0.02		0.001	

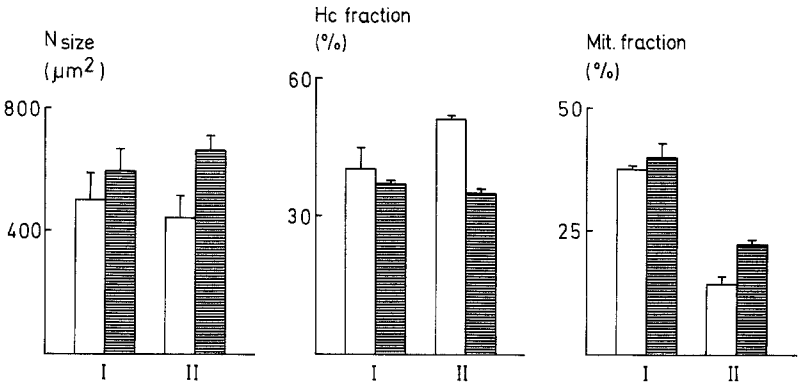

Fig. 2. Schematic representation of the data (cf. Table 2) for nuclear size, heterochromation fraction, and mitochondrial fraction in type I and type II fibres; open columns: before electrostimulation, dark columns: after electrostimulation

(Salmons and Henriksson 1981). However, in a recent study, results similar to those presented here have been described after electrical stimulation at such lower frequencies (50 Hz) but with a high current amplitude (Cabric et al. 1987).

Correlations probably exist between several physiological and morphological qualities and quantities of cells, such as cell and nuclear size and the amount of nuclear DNA (Olmo and Morescalchi 1975; 1978; Gupta 1976; Kuramoto 1981; Cabric and James 1985). The increased amount of DNA in single nuclei is indicative of increased cellular activity. A changed nuclear activity is evident from the observed decrease in the nuclear heterochromatin fraction, which indicates that the genetically active euchromatin fraction had been increased. This was found to a greater extent in type II fibres. The increases in the amounts of DNA in single nuclei, and the enlarged euchromatin fraction, most probably relate to an increased rate of RNA synthesis (Frenster et al. 1963; Littau et al. 1964). This is a prerequisite for the synthesis of cellular structures eventually leading to hypertrophy. Results similar to those described here have also been reported in trained animals as compared to sedentary animals (James et al. 1982; Cabric and James 1983) and in men (Cabric and Resic 1985).

The increase in the mitochondrial fraction was much larger in type II than in type I fibres, although the changes were significant in both types. A large general increase in mitochondrial volume has also been reported during the first three weeks of stimulation by Eisenberg and Salmons (1981), without putting any emphasis on different fibre types (Eisenberg et al. 1984). Similar findings have been reported after training in animals and humans (Holloszy 1967; Gollnick and King 1969; Howald, 1975; Hoppeler 1986). It is interesting that the type II fibres showed much larger increases in the mitochondrial fraction than the type I fibres: this may relate to the stimulation regime which, being of high frequency and high current amplitude, was more strength-oriented than endurance-oriented. By this type of stimulation the largest motoneurons innervating type II fibres are activated first and to the greatest extent. This is in contrast to the normal recruitment pattern, in which the smallest motoneurons (supplying type I fibres) are first activated, the larger ones being recruited with increasing voluntary strength. The selective activation of type II fibres is also reflected by the greater changes in nuclear size and heterochromatin fraction of myonuclei in type II fibres as compared with type I fibres.

In general it can be concluded that electrical stimulation of relatively high frequencies and current amplitude has major effects on type II fibres.

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