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Morphometric and Neurophysiological Analysis of Skeletal Muscle in Paraplegic Patients with Traumatic Cord Lesion

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Summary

Morphometric and electrophoretic properties of soleus and medialis gastrocnemius fibres from paraplegic patients were studied 1 to 10 months following complete traumatic cord transection (spinal cord level C5–T1).

In the short term of paraplegia (1–6 months) gastrocnemius medialis and soleus muscles showed predominant atrophy of IIA fibre types. In long term paraplegia (8–10 months) atrophy and reduction of type 1 fibres, with presence of high percentages of type IIB fibres, were seen in both studied muscles. The consistence in both muscles of IIB intermediate fibres in long term paraplegia, seems to indicate the initial stage of a mechanism of fibre transformation reflecting the adaptative capacity of the paretic muscle to spasticity. Electrophysiological studies of the H-reflex and the H/M ratio values reveal an increase of the H-reflex excitability in the soleus and gastrocnemius muscles during a 1 to 10 months follow-up.
Key words: *Skeletal muscle; Spinal cord injury; Morphometric analysis; Neurophysiological study.*

In the last decade the neurophysiological and morphometric properties of skeletal muscle from patients with different corticospinal lesions has been frequently studied. Longitudinal studies on patients with hemiplegia secondary to acute central pathology, in which the lesions impaired the lateral motor system, showed an ingravescent muscle fibre atrophy with patterns of denervation and signs of collateral reinnervation (Scelsi *et al.*, 1984; Messina *et al.*, 1984; Dattola *et al.*, 1988). While peripheral neuropathy or ventral root pathology were exluded in these patients (Troni *et al.*, 1988, Dattola *et al.*, 1988), some authors introduced for this neurogenic pattern the hypothesis of the transneuronal degeneration, as a

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consequence of interruption of input in the descending corticospinal tracts (Goldkamps, 1967, Kondo, 1987). Morphometric investigation of skeletal muscle from patients with paraplegia following traumatic complete cord lesion, revealed conflicting results.

In fact, whereas patterns of denervation were frequently reported by some authors (Scelsi *et al.*, 1982), the subsequent steps of fibre alteration or transformation in skeletal muscle are not unequivocal (Grimby *et al.*, 1976; Khoubesserian *et al.*, 1983). The purpose of the present study was to determine the morphometric modifications of soleus muscle, composed almost exclusively of slow-twitch fibres and medial gastrocnemius muscle, composed of a mixed fibre type population, in paraplegic patients with complete spinal cord transection. This longitudinal study on short and long term paraplegia was associated with an electrophysiological study of mono-synaptic reflexes (H-reflex and H/M ratio). An electrophoretic study of individual isoforms of myosin heavy chains was also performed on the same muscles.

Patients and methods

Ten paraplegic male patients aged between 16 to 54 years, without previously suspected neuromuscular disease were selected for this investigation. Two normal male subjects aged 18 and 35 years respectively, were used as controls.

The patients were divided into five groups on the basis of the interval between traumatic lesion and muscle biopsy, as seen in Table 1.

The patients had complete traumatic transection of the cervical and thoracic cord segments (C5–T1) and had a rehabilitative physiokinesiological therapy for the control of the muscular hypertonia and the avoidance of pressure sores. None of the examined patients showed ectopic calcification.

Electrophysiological studies

The present study was carried out for 10 months on 10 paraplegic patients. The patients were examined every 2 months for H-reflex excitability and the H/M ratio values. The H-reflex excitability was studied by simultaneous recording from soleus and medical gastrocnemius muscles using coaxial-needle electrodes. In order to obtain the H-reflex excitability curve, single rectangular pulses of increasing intensity were applied to the tibial nerve at the popliteal fossa. During the

Table I Mean muscle fibre diameter in soleus and gastrocnemius muscles of paraplegic patients

Group (time after lesion in months)	Soleus Fibre type			Gastrocnemius Fibre type		
	I	IIA	IIB	I	IIA	IIB
1 (1–2)	30	24	26	28	22	24
2 (3–4)	28	22	24	28	24	24
3 (5–6)	28	24	22	26	24	22
4 (7–8)	26	24	24	24	22	20
5 (9–10)	16	21	20	14	20	18
Control	50	48	44	48	46	46

electrophysiological procedures, the patients lay prone on the examination table turning their heads to the recording side and placing their feet off the examination table.

Morphometric studies

Open biopsies from the mid-portion of soleus and medialis gastrocnemius muscles were obtained under local anaesthesia. An incision of about one inch in length was made over the belly of the muscle in the direction of the fibres. After incision of the fascia, a cylindrical segment of muscle (about half inch in length) was removed. The specimens were frozen after removal in isopentane cooled in liquid nitrogen at −170°C.

Serial transverse cryostat sections were stained with Gomori's trichrome stain, PAS, Oil Red O, and the fibre typing was performed by myosin-ATPase pH 10·4 and 4·67. DPNH–diaphorase was used for the oxidative enzyme activities. The morphometric studies were performed on transverse cryostat sections treated for ATPase pH 4·67, in which the differentiation between type 1 (slow–ST) fibre, type IIA (Fast resistant FR) and type IIB (Fast fatigable FF) fibres is easily obtained.

Quantitative evaluation of fibre types, diameter and percentage was made with an automatic Interactive Image Analysis System–IBAS I–II (Kontron-Bildanalyse, Munich).

Each specimen was placed under a TV camera and its image was shown on a TV screen. The image was input and elaborated according to a program we devised and selected from a program menu of the IBAS system. Automatically the chosen structures were counted and measured. All measurements were calculated in microns.

Sodium lauryl sulphate polyacrylamide gel electrophoresis (SDS PAGE) of myosin heavy chains

Myosin, isolated and purified from muscle biopsies as previously described, was analyzed on 6% polyacrylamide gel slabs in the presence of SDS. Individuals isoforms of myosin heavy chains (MHC 1 type, IIA and IIB type), were distinguished according to their electrophoretic mobility. Relative amounts of MHC isoforms were determined by comparing the intensity of staining with Coomassie blue of the electrophoretic bands using a Shimadzu densitometer mod. CS 930 (Carraro *et al.*, 1983).

Results

Electrophysiology

The H-reflex excitability curves in the soleus and the medialis gastrocnemius muscles are summarised in Figure 1. An increase of H-reflex was seen in both examined muscles during the present follow-up. The same results were obtained when examining the H/M ratio values. In long term paraplegia (10 months after lesion), the H-reflex of the studied muscles showed some differences in the decrease of the excitability curve.

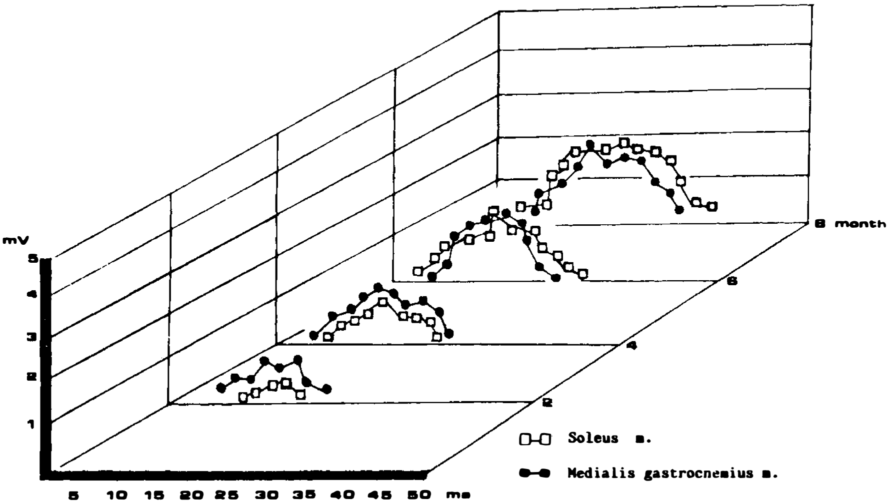

Figure 1 The H-reflex values at different months after a spinal cord lesion.

Table IIA Morphometric findings and myosin heavy chain isoforms (MHC) in the soleus muscle in paraplegia

Group	Time after lesion in months	Enzyme-histochemistry % fibre-type I	IIA	IIB	MHC% I	IIA	IIB
1	1–2	88	10	2	83	12	5
2	3–4	85	8	7	81	13	6
3	5–6	84	8	8	80	14	7
4	7–8	79	9	12°	68	28	14
5	9–10	65°	14	21°	70	11	19°
Control	—	90	8	2	94	4	2

°:P<0·001

Table IIB Morphometric findings and myosin heavy chain isoforms (MCH) in the medialis gastrocnemius muscle in paraplegia

Group	Time after lesion in months	Enzyme-histochemistry % fibre-type I	IIA	IIB	MCH% I	IIA	IIB
1	1–2	54	21	25	58	20	22
2	3–4	49	20	31	54	16	30
3	5–6	47	20	33	54	15	31
4	7–8	46	18	36	52	14	34
5	9–10	44	20	36	46	16	38°
Control	—	52	30	18	55	24	21

°:P<0·001

Morphometry and MHC isoforms

The diameter of the type I and type II fibres in soleus and medialis gastrocnemius vs months after the lesion are reported in Table 1.

At 1 to 4 months, the atrophy grade was moderate, with preferential type II atrophy. In the later stage of the cord lesion (7–10 months), a predominant type I fibre atrophy was seen. A summary of the fibre type percentage and of the myosin heavy chain isoforms in the soleus and medialis gastrocnemius muscles is reported in Table II A and II B.

While in short term paraplegia there were no significant changes in fibre type distribution, in long term paraplegia, mainly at 9 to 10 months after cord lesion, there was a progressive reduction of type I fibres, with increase of type IIB fibres mainly in the soleus muscle.

The type IIA fibres was numerically constant in all stages of the study. In all patients cytoarchitectural changes of muscle fibres was observed, groups of atrophic angular fibres and target-targetoid fibres, indicating denervation, were frequently seen.

Discussion

The present morphometric results, indicating a progressive skeletal muscle fibre atrophy in paraplegic patients with high spinal cord transection, which are similar to those described in previous studies (Scelsi *et al.*, 1982). In the above mentioned investigation, the fibre type differentiation was performed on alkaline ATPase that permits the characterisation of the two main fibre types. In the present study the characterisation of the fibre type was performed on soleus and medialis gastrocnemius by myosin ATPase pH 4·67 and with the electrophoretic differentiation of isoforms of MHC; by these methods, individual fibres were categorised as type I (ST), type IIA (SR) and type IIB (FF).

In short term spinal cord lesion, muscle fibre atrophy without reduction of type IIA fibres was seen. In long term spinal cord lesion (7–10 months) atrophy and numerical reduction of type I fibres was observed in both examined muscles.

In this stage a significant number of type IIB fibres, with intermediate enzyme activity was observed, these fibres have been interpreted as transitional form versus a different fibre type (Tellerman-Toppet *et al.*, 1971). Similar findings, characterised by rarefaction of type I fibres and by appearance of numerous type II intermediate fibres, were reported in spastic paraplegic patients with traumatic spinal cord lesions at various levels (Khoubesserian *et al.*, 1983). It is well known that the enzymatic profile of muscle fibres may be influenced and modified by electrical frequence of nerve stimulation (Kugelberg, 1968). It is suggested that spasticity, present in all the studied patients, may induce muscle fibre modifications, with possible fibre type conversion through the intermediate type IIB (FF) fibres, as observed in long term paraplegia. This hypothesis may be confirmed by experimental spinal cord transection, in that the rat and cat skeletal muscle properties would change, with almost complete type I to type II fibre transformation (Lieber *et al.*, 1986, West *et al.*, 1986).

Regarding the H- reflex studies, the present results are in agreement with those of Girlanda *et al.* (1987), which showed a progressive increase of the H-reflex excitability and of the H/M ratio values in soleus and gastrocnemius muscle of spastic paraplegic patients. These electrophysiological results are evidence of a time-dependent correlation with the clinical observations of increasing spasticity in the studied patients. On the other hand, our morphometric observations are not in agreement with the hypothesis of Burke (1983), in which the H-reflex is related to low threshold motor neurons, that cause the slow twitch motor unit activation. On the contrary our findings seem to indicate a possible modification of the innervating properties of the low-threshold motor neurons. On the other hand, some recent neurophysiological and morphometric studies of the contractile properties of soleus and EDL muscles, in the experimental cord transection, confirmed the morphometric findings of fibre type conversion from type I to type II fibres (Lieber *et al.*, 1986 I and II).

The project of the study has been vetted and accepted by the Ethical Committee of the Centro di Recupero e Rieducazione Funzionale G. Verdi, Villanova d Arda (Italy). Each patient gave his informed consent to the biopsy.

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