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EXPERIMENTAL CHANGES TO LIMB MUSCLES ELICIT CONTRALATERAL REACTIONS: THE PROBLEM OF CONTROLS

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Summary

The extensor digitorum longus muscle (EDL) or soleus muscle (SOL) in rats was mechanically overloaded on one side. The muscles were (i) untreated (normal) or (ii) self- or foreign-reinnervated (leading to persisting muscle fibres) or transplanted (leading to regenerating muscle fibres). The effects of the different procedures were studied in the treated and untreated muscles on the operated side and in the untreated muscles on the contralateral side. Overloading led to an absolute increase in mass (*versus* control values) in the normal muscles and to a relative increase in mass (*versus* the lower mass after reinnervation) in the treated muscles. The mechanism underlying this gain in mass was usually a compensatory hypertrophy. Overloading was followed by transformation of fibres from fast to slow in normal muscles. In the reinnervated muscles, the fibre distribution changed in response to the new nervous input and then remained constant. The majority of the experimental procedures elicited significant muscular changes in the contralateral muscles, including hyperplasia, fibre transformation and fibre hypertrophy or atrophy. The changes are interpreted as the consequence of a general compensatory neuromuscular activity designed to maintain a symmetrical posture during walking and running. These frequent and substantial muscular changes in the unoperated muscles clearly show that the muscles of the contralateral side cannot be used as normal controls.

Key words: contralaterality, extensor digitorum longus, soleus, rat, surgical overloading, muscle, controls.

Introduction

Skeletal muscles are composed of motor units with different properties giving rise to the well-known morphological, physiological and metabolic heterogeneity of muscular tissue. Once the adult fibre pattern has been established in a given muscle, the proportions of the different fibre types remain stable throughout most of life. This preservation of specific tissue characteristics exists in parallel with a potential to respond in a plastic manner to a variety of systemic or local stimuli, such as training, hormones (mainly thyroid hormones), immobilization, weightlessness, synergistic tenotomy, artificial chronic stimulation or foreign-reinnervation (for further references, see Booth and Thomason, 1991; Florini, 1987; Gordon, 1995; Howald, 1982; Jolesz and Sreter, 1981; Müntener et al., 1987b; Pearson and Sickles, 1987; Pette and Staron, 1997; Pette and Vrbova, 1985; Riley et al., 1987; Salmons and Henriksson, 1981; Schantz, 1986).

While these muscular reactions have been known for a long time, contralateral changes after local disturbances were noticed much later. Rotshenker (1979) showed that unilateral denervation of the cutaneous-pectoris muscle in the frog induced nerve sprouting and the formation of synapses in both the denervated and the intact innervated muscle of the opposite side. Similarly, contralateral fibre transformation following cross-reinnervation of rabbit soleus (SOL) or rat extensor digitorum longus (EDL) muscle has been observed (Müntener et al., 1987a; Müntener and Srihari, 1984; Reichmann et al., 1983; Srihari et al., 1981). Changes in the muscle geometry of the contralateral gastrocnemius muscle have been reported after unilateral hindlimb immobilization in rats (Heslinga et al., 1992).

Compensatory hypertrophy of skeletal muscle induced by functional elimination of synergistic muscles (tenotomy or ablation) has been widely investigated, mainly in SOL, EDL or plantaris muscle (Donovan and Faulkner, 1986; Finkelstein et al., 1991; Frischknecht and Vrbova, 1991; Gutmann et al., 1971; Ianuzzo et al., 1976; Oakley and Gollnick, 1985; Pearson and Sickles, 1987; Phelan and Gonyea, 1997; Roy and Edgerton, 1995; Schiaffino and Pierobon Bormioli, 1973; Sugiura et al., 1993; Williams and Goldspink, 1981; Zhou et al., 1998). However, in these investigations, contralateral changes were not studied. Instead, the muscles on the experimental side were usually compared with the corresponding ones in the unoperated contralateral limb to serve as controls (Bishop and Milton, 1997; Finkelstein et al., 1991; Frischknecht and Vrbova, 1991; Gutmann et al., 1971; Ianuzzo et al., 1976; Oakley and Gollnick, 1985; Phelan and Gonyea, 1997; Schiaffino and Pierobon Bormioli, 1973; Skorjanc et al., 1998; Sugiura et al., 1993; Williams and Goldspink, 1981; Zhou et al., 1998).

Unilateral functional overloading of a given limb muscle causes two different effects. On the operated side, the muscular changes (hypertrophy and altered fibre composition) result from an increase in performance. On the contralateral side, the changes are probably induced by altered afferent activity (resulting from the locomotory disturbance), which elicits alterations in the efferent activity. This process may be regulated by higher centres within the brain (Reichmann et al., 1983).

In this study, either the fast EDL or the slow SOL was functionally overloaded on one side in the adult rat. The overloading procedures were performed on both normal and transformed muscles. Muscular transformation was induced by self- or cross-reinnervation. However, in non-overloaded muscles, Donovan and Faulkner (1987) have shown that regenerating fibres adapt more rapidly to foreign-reinnervation than do surviving fibres. Overloading was, therefore, performed not only on transformed muscles with persisting fibres (self-, cross-reinnervation) but also on transformed muscles with regenerating fibres (transplantation). In all experiments, the reactions after overloading were analysed in the muscles on both the operated (ipsilateral) and the non-operated (contralateral) side. In addition, the effects of unilateral excision of either the EDL or SOL were analyzed. A major point of interest was a comparison between the response of a fast muscle (EDL) and that of a slow muscle (SOL).

Materials and methods

Animals

Male rats (strain Zur:SIV) supplied by the Institute of Laboratory Animal Science, University of Zürich, were used. All animals were singly caged, provided with food and water *ad libitum* and maintained at a constant temperature and on a 12 h:12 h light:dark cycle. For surgical procedures, the animals were anaesthetized with 0.25 ml kg^{-1} body mass Innovar-Vet (Pitman-Moore) intramuscularly combined with 2.5 mg of Valium (Roche) and 7.5 mg of Nembutal (Abbott) intraperitoneally. The rats were not given antibiotics and there was no incidence of infection.

Surgical procedures

The experiments were always performed on the left side, either on the extensor digitorum longus (EDL) or the soleus (SOL) muscle. The time course of the different procedures with the corresponding survival times is summarized in Fig. 1.

Overload of normal muscles

In 5-month-old animals (*N*=18), muscular enlargement of the EDL (O-N-EDL) was produced by ablation of the tibialis anterior muscle. The tendon of the tibialis anterior muscle was isolated and severed, and the muscle was lifted and severed from its origin. Enlargement of the SOL (O-N-SOL) was induced by ablation of the gastrocnemius muscle. The tendon of the gastrocnemius muscle was cut and the muscle severed. Care was taken to avoid damaging neural or vascular supplies to surrounding muscles. Using this procedure, approximately three-quarters of the medial head and two-thirds of the lateral head of the gastrocnemius muscle were removed.

Overload of transformed muscles

Persisting fibres. Self-reinnervation of either the EDL (S-EDL) or SOL (S-SOL) was performed on 4-week-old animals (*N*=39). The motor nerves to both the EDL and SOL were transected and loosely adapted (without suture).

Cross-reinnervation. Preliminary experiments have shown that cross-reinnervation of the EDL cannot be performed satisfactorily. Spontaneous self-reinnervation of the EDL could not be prevented without damaging the nerve supply to the tibialis anterior muscle. This would have led to an untimely overload of the freshly cross-reinnervated EDL muscle. For this reason, only the SOL was cross-reinnervated (X-SOL) in 36 animals by suturing the EDL nerve at the point of entry of the SOL nerve. The proximal SOL nerve stump was cut near where it branched from the tibial nerve. Care was taken not to damage the branches to the gastrocnemius muscle. The EDL muscle was excised.

Thus, in a total of 75 rats, the EDL or SOL was self- or cross-reinnervated (S-EDL, S-SOL or X-SOL). After a recovery period of 4 months in 35 animals, these muscles were surgically overloaded (O-S-EDL, O-S-SOL, O-X-SOL) as described above. In the remaining 40 animals, the muscles were not overloaded and were used for comparison.

Regenerating fibres. In 4-week-old animals (*N*=54), transplantation of the EDL (T-EDL) and SOL (T-SOL) was accomplished as described by Carlson and Gutmann (1974). The muscle in the recipient site was isolated, removed and discarded. The EDL muscles were autografted into the site of the SOL muscles with reinnervation by the SOL nerve, or the SOL muscles were grafted into the site of the EDL muscles with reinnervation by the EDL nerve. The proximal and distal tendons of the muscles were sutured to the corresponding tendon stumps. The length was adjusted to maintain the original resting length of the muscle. When suturing the tendons, care was taken to ensure that the tendon stumps of the recipient site were free of any remnants of the original muscle fibres. To obtain successful reinnervation, the nerve stumps were carefully placed near the site of entry of the original nerve and loosely adapted.

After a recovery period of 5 months in 30 animals, the T-EDL and T-SOL were surgically overloaded (O-T-EDL, O-T-SOL) as described above. Again, the remaining animals (*N*=24), whose T-EDL or T-SOL muscles were not overloaded, were used for comparison.

Controls

Age-matched untreated animals (*N*=18) served as controls (N-EDL, N-SOL). After cross-reinnervation and transplantation on the experimental side, either the EDL or SOL was lacking. So, in an additional control group (*N*=18) of 4-week-old animals, either the EDL or SOL was excised without further treatment (Exc EDL, Exc SOL).

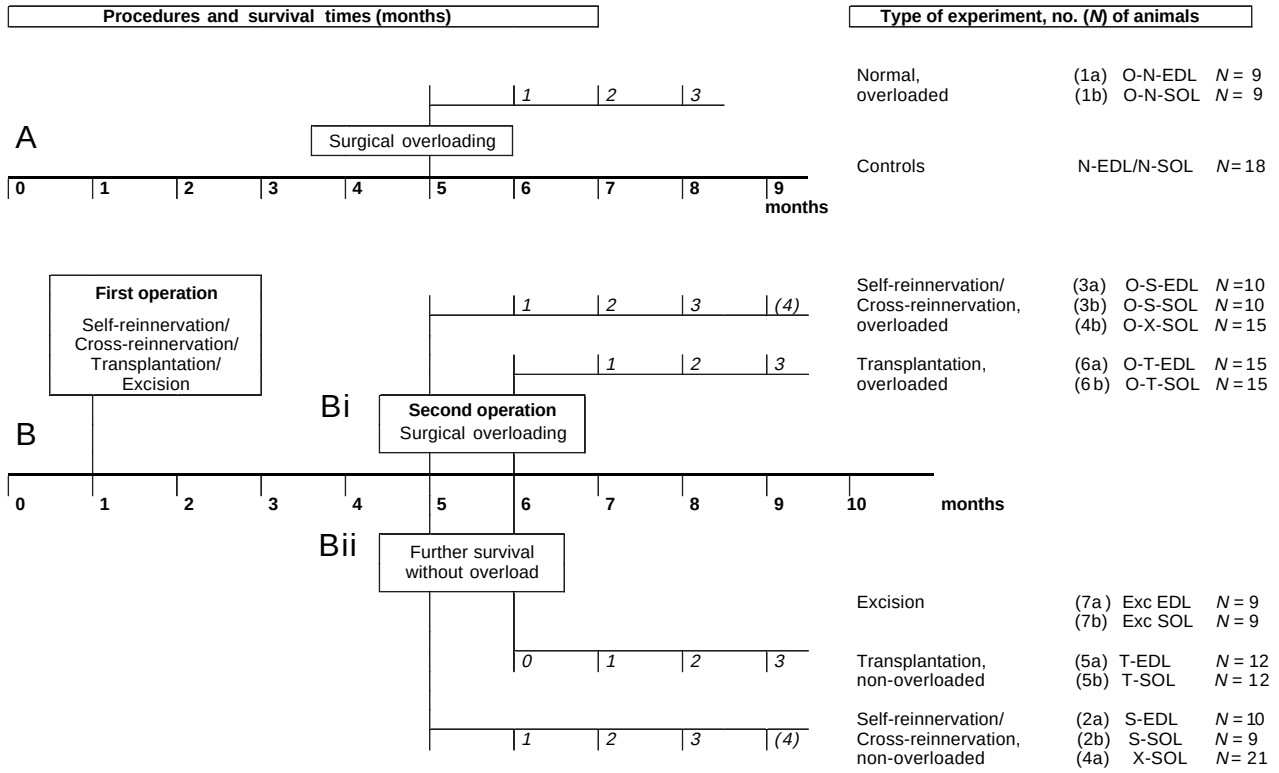

Fig. 1. Synopsis of the different procedures and survival times. (A) Normal animals that were either left untreated to serve as controls or that had either the extensor digitorum longus (EDL) or soleus (SOL) muscle mechanically overloaded at the age of 5 months. (B) Animals (*N*=147) in which either the EDL or the SOL was either experimentally altered (self-reinnervated, cross-reinnervated or transplanted) or excised at the age of 1 month. In 65 animals, the experimentally altered muscles were mechanically overloaded at the age of 5 or 6 months (Bi). The overloaded muscles were investigated from then on at monthly intervals. In the remaining 82 animals, the non-overloaded muscles were investigated in a similar manner (Bii). The survival stages are indicated by numbers in italics. In rats with transplanted, non-overloaded muscles (T-EDL/T-SOL), stage '0 month' was also investigated. Animals with overloaded and non-overloaded cross-reinnervated SOL (X-SOL and O-X-SOL) muscles were additionally examined after 4 months of survival (numbers in parentheses). At every stage, at least three animals were examined. N-EDL, normal EDL; N-SOL, normal SOL; O-N-EDL, overloaded normal EDL; O-N-SOL, overloaded normal SOL; S-EDL, self-reinnervated EDL; S-SOL, self-reinnervated SOL; O-S-EDL, overloaded self-reinnervated EDL; O-S-SOL, overloaded self-reinnervated SOL; X-SOL, cross-reinnervated SOL; O-X-SOL, overloaded cross-reinnervated SOL; T-EDL, transplanted EDL; T-SOL, transplanted SOL; O-T-EDL, overloaded transplanted EDL; O-T-SOL, overloaded transplanted SOL; Exc EDL, excised EDL; Exc SOL, excised SOL.

Histochemistry

Overload

Rats with normal and transformed muscles (O-N-EDL/SOL, O-S-EDL/SOL, O-X-SOL, O-T-EDL/SOL) were deeply anaesthetized with Nembutal 1, 2 and 3 months after the overloading operation (Fig. 1).

Non-overload

These animals (S-EDL/SOL, X-SOL, T-EDL/SOL, Exc EDL/SOL) were killed at corresponding stages. Rats with transplanted muscles (T-EDL/SOL) were also examined at the age corresponding to the overloading operation (stage '0 month', Fig. 1). In the cross-reinnervation experiments (with and without overload), animals were also examined after a survival time of 4 months (Fig. 1). Control animals (N-EDL/SOL) were investigated at 5, 6, 7, 8 and 9 months, corresponding to the survival stages '0', 1, 2, 3 and 4 months, respectively (Fig. 1).

The muscles on the experimental side (EDL and/or SOL) and the contralateral EDL and SOL muscles were removed, weighed and frozen in melting isopentane (−160 °C). Control muscles were processed in the same fashion. Cryocut cross sections (12 μm thick) of the whole muscle were reacted for myofibrillar ATPase (E.C. 3.6.1.3.) according to Guth and Samaha (1970) with the modifications described by Müntener (1979, 1982). To demonstrate the presence of alkali-stable ATPase, preincubation at pH 10.4 and 10.5 was combined with incubation at pH 9.5 or 9.6. For the demonstration of acid-stable ATPase, preincubation at pH 4.25 or 4.3 was followed by incubation at pH 9.5.

Consecutive sections (12 μm thick) were fixed with 2 % paraformaldehyde in 0.2 mol l^{-1} phosphate buffer (pH 7.4) for 10 min and incubated for cytochrome *c* oxidase (E.C. 1.9.3.1.) with added catalase according to the method of Wong-Riley (1979). Consecutive sections (12 μm thick) were incubated for succinate dehydrogenase (SDH; E.C. 1.3.99.1.) according to Nachlas et al. (1957).

Fibres were classified into the following types: IA (equivalent to the classical 'slow-twitch oxidative', i.e. type I fibre); IB and IIC (both transitional fibres; Brooke and Kaiser, 1970; Jansson et al., 1978; Karpati et al., 1975); and IIA ('fast-twitch oxidative glycolytic') and IIB ('fast-twitch glycolytic'). Fibre typing and counting of the numbers of type IA, IB and IIC fibres were performed as described elsewhere (Müntener, 1982). To determine the total numbers of type I, IIA and IIB fibres, sections stained for myofibrillar ATPase were directly enlarged (by projection) onto photographic paper, and all fibres were counted.

Morphometric procedures

Photographs of five areas each displaying between 100 and 150 fibres were taken from every muscle. The areas were regularly spaced along the deep to superficial axis in the EDL and along the medial to lateral axis in the SOL muscle. The photographs were projected at a final magnification of 1:600 on a graphics tablet. The projection of a frame containing 16 regularly arranged dots was superimposed. The cross-sectional areas of those fibres coincident with (or nearest to) a dot were assessed by means of planimetry using an HP-86B microcomputer connected to the graphics tablet. Fibre diameters were calculated (for better comparability) by assuming that the muscle fibres were cylindrical.

Statistical analyses

The overloaded transplanted, self- and cross-reinnervated muscles were compared with the corresponding non-overloaded muscles while these, as well as the other experimental and all contralateral muscles, were compared with the corresponding normal ones.

All the values reported are means ± standard deviation (S.D.), which were calculated from individual values using standard procedures. The two-tailed unpaired *t*-test was used to compare the mean values for each fibre type. A significance level of $P<0.01$ was chosen for all tests.

Results

The fibre distribution and fibre size of normal EDL and SOL muscles at the different ages are summarized in Table 1. In all seven groups of experiments in each animal, the operated and the non-operated (ipsi- and contralateral) muscles were investigated in the same way, i.e. total fibre number, fibre distribution and fibre diameter were determined. For simplicity, however, the majority of these extensive data is presented semi-quantitatively. Table 2 shows all significant changes and the time of the strongest reaction in the EDL or SOL of the operated side (operated muscles only) and in the EDL and SOL of the contralateral (non-operated) side. Table 3 shows the total number of fibres of the muscles investigated at the survival times with the strongest reactions. Fig. 2 displays schematically the occurrence of significant muscular changes in all non-operated (ipsi- and contralateral) muscles.

Operated side

Non-overloaded muscles (experiments 2a,b, 4a, 5a,b; Tables 2, 3)

These muscles were compared with the corresponding normal ones.

The most conspicuous finding was the loss of muscle mass. In self- and cross-reinnervated SOL muscles, the loss of mass varied between 15 % and 24 %. The reduction in mass of the transplanted muscles (EDL and SOL) was even greater at 50–70 %. Fibre diameters were generally reduced by 15–20 % in the self- and cross-reinnervated muscle and by 20–50 % in the transplanted muscle.

The total numbers of muscle fibres remained unchanged, with the exception of the self-reinnervated EDL and the transplanted SOL (Tables 2, 3). The fibre number of the S-EDL was increased at all stages, by an average of 25 % (14–32 %), while that of the T-SOL was reduced by 12 % (0–21 %).

Changes in fibre type distribution were seen in all muscles.

Table 1. *Morphometrical data for normal muscles (N-EDL/N-SOL)*

Muscle	Age (months)	Muscle mass (mg)	Fibre number	Distribution of fibre types (%)			Diameter of fibres (μm)		
				I	IIA	IIB	I	IIA	IIB
N-EDL	5	214±20	2701±444	2.0±0.9	43.1±4.6	54.9±4.8	33.4±2.3	44.8±1.1	66.8±1.5
	6	260±30	3036±111	3.0±0.9	46.0±4.8	51.0±5.2	35.9±2.1	49.6±1.5	73.3±1.0
	7	244±12	3125±445	3.2±1.1	50.0±4.4	46.8±5.2	32.8±1.6	45.3±1.1	65.4±1.8
	8	240±18	3002±428	3.4±1.6	45.3±4.9	51.3±5.1	33.4±2.5	45.5±2.6	66.2±1.9
	9	235±24	2888±265	3.0±0.6	48.1±2.2	48.9±2.6	32.9±2.4	44.6±2.7	66.8±1.7
N-SOL	5	204±22	2816±143	95.9±3.2	3.3±2.6	0.8±1.2	63.8±2.9	49.8±2.0	38.7±1.0
	6	221±39	2648±297	93.2±3.9	6.4±4.0	0.4±0.4	68.2±4.8	64.7±6.4	45.7±5.4
	7	220±29	2708±328	93.6±5.9	6.2±5.4	0.2±0.1	70.1±5.3	62.8±3.6	40.2±4.3
	8	273±13	3011±123	95.6±6.4	4.2±6.3	0.2±0.1	70.2±2.9	57.5±3.0	42.6±5.6
	9	228±31	2746±362	97.4±1.5	2.4±1.3	0.2±0.2	71.5±2.7	57.1±4.9	44.8±5.5

Values are means ± S.D.; $N=18$.
EDL, extensor digitorum longus; SOL, soleus.

Table 2. *Summary of significant changes occurring in the operated and contralateral muscles in the different experimental procedures*

	Operated muscle					Contralateral muscle					
Experiment	Muscle mass	Fibre no.	Fibre type (%)	Fibre diameter	Time of strongest reaction (months)	Muscle	Muscle mass	Fibre no.	Fibre type (%)	Fibre diameter	Time of strongest reaction (months)
1a O-N-EDL	⇑	nc	IIA⇑, IIB⇓	IIA⇑	2, 3	EDL	nc	nc	IIA⇑, IIB⇓	nc	1–3
						SOL	nc	nc	nc	nc	–
1b O-N-SOL	⇑	nc	IIA⇓, IIC⇑	I⇑	1–3	EDL	⇑	⇑	nc	nc	1–3
						SOL	nc	nc	nc	I↑, IIA⇓	1–3
2a S-EDL	nc	⇑	I⇑, IIA⇑, IIB⇓	I↓, IIB⇓	1–3	EDL	nc	nc	nc	IIB⇓	1
						SOL	nc	nc	nc	nc	–
2b S-SOL	⇓	nc	IIC↑	nc	1, 3	EDL	nc	↑	IIA↑, IIB↓	IIB⇓	1
						SOL	↑	nc	nc	I↑	1
3a O-S-EDL	↑	nc	nc	IIB↑	1–3	EDL	nc	⇑	nc	IIA↓, IIB↓	1
						SOL	⇑	↑	nc	IIA⇑, IIC⇑	1, 3
3b O-S-SOL	↑	nc	nc	nc	1–3	EDL	nc	nc	IIA↑, IIB↓	IIB⇓	1, 3
						SOL	⇑	nc	I⇓, IIA⇑	I⇑, IIA⇑	3
4a X-SOL	↓	nc	I⇓, IIA⇑	I⇓, IIA⇓	0–4	EDL	nc	⇑	IIA⇑, IIB⇓	IIA⇓, IIB⇓	0–3
						SOL	nc	nc	nc	I↓, IIA↑	0, 4
4b O-X-SOL	↑	nc	nc	nc	1, 2	EDL	nc	nc	nc	IIA⇓, IIB⇓	1, 3
						SOL	nc	nc	nc	I↓	4
5a T-EDL	⇓	nc	I⇑, IIA⇓, IIB⇓	I⇑, IIA⇓	0–3	EDL	nc	⇑	IIA⇑, IIB↓	I↓, IIB⇓	0–3
						SOL	nc	nc	nc	I↓	2, 3
5b T-SOL	⇓	↓	I⇓, IIA⇑, IIB⇑	I⇓, IIA⇓	0–3	EDL	nc	nc	nc	I⇑, IIA⇑	0, 1
						SOL	nc	nc	nc	nc	–
6a O-T-EDL	↑	↓	nc	I↑	1–3	EDL	nc	nc	I⇓, IIA↓, IIB↑	IIB⇓	3
						SOL	nc	nc	nc	IIA⇓	1
6b O-T-SOL	↑	nc	nc	IIA↑	1–3	EDL	nc	nc	nc	IIB⇓	1–3
						SOL	nc	nc	IIC↑	IIC⇑	1
7a Exc EDL						EDL	nc	nc	nc	IIA⇑	1–3
						SOL	nc	nc	IIC⇑	I↓, IIA↓	1, 2
7b Exc SOL						EDL	nc	nc	IIA↑, IIB↓	IIA⇑	2, 3
						SOL	nc	nc	IIC↑	nc	1

The time of strongest reaction (months) indicated corresponds to the survival times shown in Fig. 1 (↑ or ↓ = $P<0.01$; ⇓ or ⇑ = $P<0.001$; nc, no change).

EDL, extensor digitorum longus; SOL, soleus; O-N-EDL, overloaded normal EDL; O-N-SOL, overloaded normal SOL; S-EDL, self-reinnervated EDL; S-SOL, self-reinnervated SOL; O-S-EDL, overloaded self-reinnervated EDL; O-S-SOL, overloaded self-reinnervated SOL; X-SOL, cross-reinnervated SOL; O-X-SOL, overloaded cross-reinnervated SOL; T-EDL, transplanted EDL; T-SOL, transplanted SOL; O-T-EDL, overloaded transplanted EDL; O-T-SOL, overloaded transplanted SOL; Exc EDL, excised EDL; Exc SOL, excised SOL.

The S-EDL displayed a considerable increase (30 %) in the number of oxidative fibres (types I and IIA) at the expense of glycolytic fibres (type IIB), while in the S-SOL the number of IIC fibres was increased at the 1 month stage. Changes in fibre composition (according to the new innervation) were more pronounced in the T-SOL than in the X-SOL. Type IIB fibres were present in small amounts (1–2 %) in the T-SOL, but were lacking in the X-SOL.

Overloaded muscles (experiments 1a,b, 3a,b, 4b, 6a,b; Tables 2, 3)

The overloaded muscles were compared with the corresponding non-overloaded ones.

Muscle mass was increased throughout. In both the overloaded normal EDL and SOL, the increase in mass varied considerably from 18 % to 103 % for the EDL and from 43 % to 81 % for the SOL. In the overloaded self- and cross-reinnervated muscles, the

Table 3. *Total number of muscle fibres in the operated and contralateral EDL and SOL at the survival times showing the strongest muscular reactions*

Experiment	Operated muscle Fibre no.	Contralateral muscle		Survival time (months) No. of animals
		Muscle	Fibre no.	
1a O-N-EDL	3025±429	EDL	3014±452	2
		SOL	2901±300	*N*=3
1b O-N-SOL	2813±222	EDL	3772±125**	1
		SOL	2955±149	*N*=3
2a S-EDL	3897±481**	EDL	3028±109	1
		SOL	2999±167	*N*=3
2b S-SOL	3080±235	EDL	3623±340*	1
		SOL	3183±138	*N*=3
3a O-S-EDL	4349±679	EDL	3790±295**	1
		SOL	3475±188*	*N*=3
3b O-S-SOL	2954±168	EDL	3494±143	3
		SOL	3024±87	*N*=3
4a X-SOL	3061±156	EDL	3544±172**	1
		SOL	2852±223	*N*=4
4b O-X-SOL	302±378	EDL	3906±657	3
		SOL	3126±282	*N*=4
5a T-EDL	2523±963	EDL	4072±312**	2
		SOL	2775±107	*N*=3
5b T-SOL	2273±134*	EDL	2495±331	3
		SOL	2752±746	*N*=3
6a O-T-EDL	1770±461*	EDL	335±701	3
		SOL	3106±329	*N*=5
6b O-T-SOL	225±963	EDL	3428±534	3
		SOL	3084±337	*N*=5
7a Exc EDL		EDL	2336±208	2
		SOL	2791±90	*N*=3
7b Exc SOL		EDL	3130±461	3
		SOL	2820±264	*N*=3

Survival times correspond to those given in Fig. 1; see also Table 2. *P<0.01; **P<0.001. Values are means ± S.D.

EDL, extensor digitorum longus; SOL, soleus; O-N-EDL, overloaded normal EDL; O-N-SOL, overloaded normal SOL; S-EDL, self-reinnervated EDL; S-SOL, self-reinnervated SOL; O-S-EDL, overloaded self-reinnervated EDL; O-S-SOL, overloaded self-reinnervated SOL; X-SOL, cross-reinnervated SOL; O-X-SOL, overloaded cross-reinnervated SOL; T-EDL, transplanted EDL; T-SOL, transplanted SOL; O-T-EDL, overloaded transplanted EDL; O-T-SOL, overloaded transplanted SOL; Exc EDL, excised EDL; Exc SOL, excised SOL.

increase in mass was more uniform, varying between 10 % and 39 %. In the transplanted muscles, overloading again led to a variable increase in muscle mass (34–96 %).

The total number of muscle fibres showed a slight decrease in the O-T-EDL but remained unchanged in the other overloaded muscles.

Changes in fibre distribution were restricted to the O-N-EDL and O-N-SOL. In the former, the percentage of IIA fibres increased by between 18 % and 38 %, while the relative proportion of IIB fibres decreased correspondingly. The O-N-SOL exhibited a reduction in type IIA fibres of up to 80 %; the numbers of IIC fibres were again increased at the 1 month stage.

The untreated muscles on the operated side (experimental groups 1, 2, 3 and 7) were compared throughout with the corresponding normal muscles. The majority showed significant changes (Fig. 2), such as an increase in the muscle mass, an increase in the fibre number, an alteration in the fibre distribution and fibre atrophy.

Contralateral side

All contralateral muscles were compared with the corresponding normal ones.

Non-overloading experiments (experiments 2, 4a, 5, 7; Tables 2, 3)

The muscle mass of the contralateral muscles was largely unaffected. Changes in the total fibre number were only seen in the contralateral EDL. In three experiments (S-SOL, X-SOL and T-EDL), this muscle displayed significant increases in the total fibre number at some stage varying between 16 % and 28 % (Table 3).

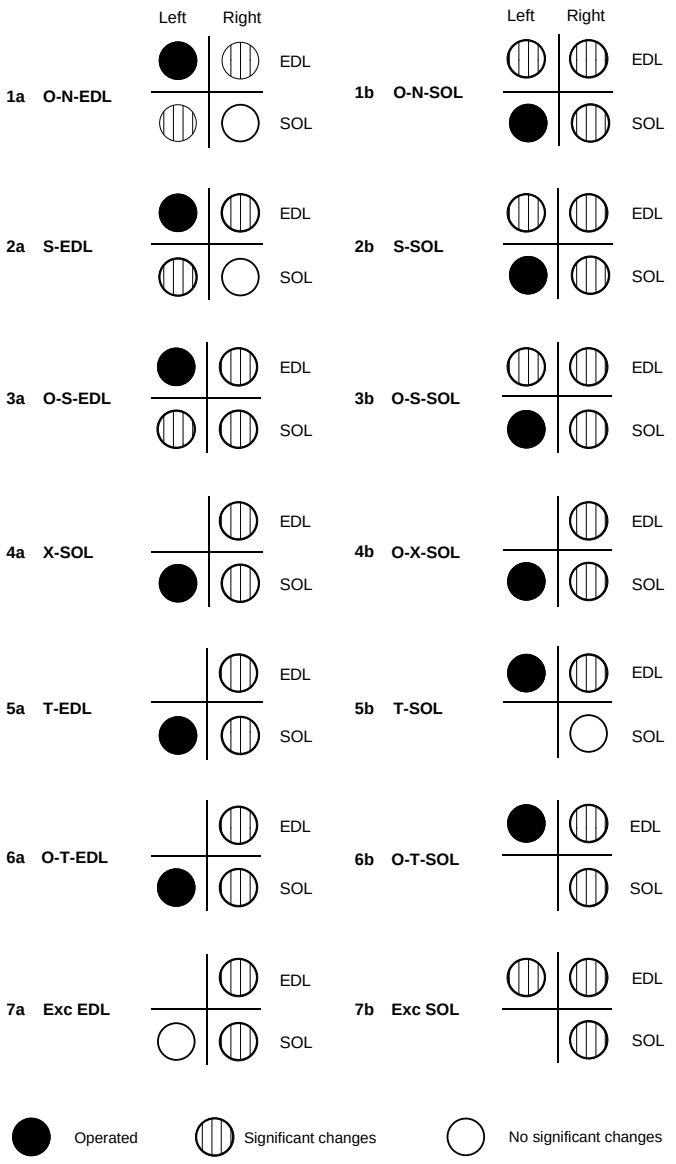

Fig. 2. Pictographic representation of all operated and non-operated muscles in the seven different experimental procedures (numbering as in Fig. 1). The muscles are shown in their definitive position at the time of examination; e.g. in experiment 5a, the transplanted extensor digitorum longus (T-EDL) is at the site of the soleus (SOL). Correspondingly, the transplanted SOL (T-SOL) in experiment 5b is at the site of the EDL. In the non-operated (ipsi- and contralateral) muscles, the occurrence of significant changes in at least one survival stage (according to Table 2) is indicated (hatched circle). Note that the contralateral EDL shows significant alterations in all experiments. O-N-EDL, overloaded normal EDL; O-N-SOL, overloaded normal SOL; S-EDL, self-reinnervated EDL; S-SOL, self-reinnervated SOL; O-S-EDL, overloaded self-reinnervated EDL; O-S-SOL, overloaded self-reinnervated SOL; X-SOL, cross-reinnervated SOL; O-X-SOL, overloaded cross-reinnervated SOL; T-EDL, transplanted EDL; T-SOL, transplanted SOL; O-T-EDL, overloaded transplanted EDL; O-T-SOL, overloaded transplanted SOL; Exc EDL, excised EDL; Exc SOL, excised SOL.

In addition, significant changes in fibre distribution were observed in these experiments. An increase in the percentage of type IIA fibres of between 10 % and 43 % was paralleled by a corresponding decrease in the relative amount of type IIB fibres. This type of shift of fibre distribution was also observed in the contralateral EDL when the SOL was excised. The majority of the contralateral muscles showed, at least at one survival stage, changes in fibre diameter. All fibre types displayed both increases and decreases in fibre diameter.

Overloading experiments (experiments 1, 3, 4b, 6; Tables 2, 3)

In three experiments (O-N-SOL, O-S-EDL/SOL) in the contralateral EDL and/or SOL, increases in muscle mass (27–57 %) and/or total fibre number (21–30 %) largely involved type II fibres; the relative amounts of type IIA and IIB fibres were correspondingly increased or decreased by 25–40 %. As in the non-overloading experiments, increases and decreases in the diameter of the oxidative fibres (type I and IIA) occurred in equal proportion while IIB fibres exhibited only decreases in fibre diameter.

Discussion

Unilateral surgical overloading of normal and experimentally altered muscles led, on the operated side, to absolute or relative increases in mass of the muscles caused by overloading and these were paralleled by fibre transformations resulting from changes in nervous input. On the contralateral side, the most conspicuous findings were a hyperplasia in the fast EDL.

Operated side

Muscle mass and fibre diameter

An increase in muscle mass was seen in all overloaded muscles. Overloaded normal muscles showed the well-documented absolute gain in mass. In overloaded reinnervated muscles (self-, cross-reinnervated and transplanted), however, there was only a relative increase over the generally lower mass found after reinnervation. Compensatory hypertrophy could generally be confirmed as the underlying mechanism (Gutmann et al., 1971; Ianuzzo et al., 1976; Oakley and Gollnick, 1985; Pearson and Sickles, 1987; Roy and Edgerton, 1995; Schiaffino and Pierobon Bormioli, 1973; Sugiura et al., 1993). The fibre types most commonly involved were the oxidative fibres.

Fibre number

Interestingly, the self-reinnervated, non-overloaded EDL (S-EDL) displayed a significant increase (25 %) in fibre numbers at all stages paralleled by a decrease in the size of type I and IIB fibres. Hyperplasia in animals and humans is generally considered to be a reaction to mechanical overload, stress and exercise (for further details, see Antonio and Gonyea, 1993b; Kelley, 1996). To our knowledge, hyperplasia as a consequence of self-reinnervation has not been reported in the literature. In the present investigation, the underlying mechanism (fibre splitting or satellite cell activation) of the hyperplasia could not be determined. On the operated side, hyperplasia occurred only in the S-EDL. In the contralateral

muscles, hyperplasia was found in five experiments and these usually involved the fast EDL (see below).

Fibre distribution

Fibre transformations have been observed in both overloaded normal and self-reinnervated non-overloaded muscles (Jansson et al., 1978, 1990; Jaschinski et al., 1998; Müntener, 1982; Müntener et al., 1987a; Pette and Staron, 1997; Schantz et al., 1982). In the rat, they occur according to the following (simplified) scheme:

$$\text{I} \leftrightharpoons \text{IIC} \leftrightharpoons \text{IIA} \leftrightharpoons \text{IIB}.$$

In both the EDL and SOL, the direction of the transformations was from right to left in the scheme illustrated above; for example, from the fast-twitch glycolytic type IIB or the fast-twitch oxidative glycolytic type IIA to the slow-twitch oxidative type I. In the SOL muscle, evidence for the fibre transformation was provided by an increase in the relative amount of the intermediate IIC fibres (Jansson et al., 1978; Müntener, 1982; Müntener et al., 1987b; Schantz et al., 1982). The transformation of fibres towards a higher oxidative capacity is accompanied in the overloaded EDL by capillary sprouting (Zhou et al., 1998).

Foreign-reinnervated muscles with persisting or regenerating fibres showed a transformation that corresponded to the new nervous input. Regenerating fibres (transplanted muscles) adapted more rapidly and to a larger degree than persisting fibres to the foreign nervous input (cross-reinnervated muscles). In the transplanted T-EDL, transformation was nearly complete. This result is in accordance with the study of Donovan and Faulkner (1987). The reason for this difference between regenerating and persisting fibres is not understood. However, myogenesis is recapitulated to a large extent in regenerating muscle fibres. A number of molecules present at myogenesis are re-expressed, including myogenic regulators (myogenin, MyoD), cell adhesion molecules (tenascin, M-cadherin, N-CAM) and the end-plate-associated molecule agrin (Billington, 1997; Irintchev et al., 1994; Moore and Walsh, 1993).

Overloading of both types of foreign-reinnervated muscle (persisting or regenerating fibres) did not lead to further changes in the fibre distribution.

The muscular changes in the untreated muscles on the operated side (e.g. EDL left in S-SOL) were largely the same as in the corresponding contralateral muscles (see below).

Contralateral side

In many experimental and clinical studies, the muscles contralateral to the manipulated side are used as controls because they are assumed to remain normal. It is well documented from earlier studies (Müntener and Srihari, 1984; Srihari et al., 1981) that even minimal experimental interventions or pathological conditions on one side provoke significant changes on the contralateral side. These studies did not, however, analyze the contralateral changes.

In 11 of the 14 experimental procedures (Fig. 2), both contralateral SOL and EDL exhibited a polymorphic picture of muscular changes, such as an increase in fibre numbers and/or alterations in fibre distribution and/or an increase or decrease in fibre diameter. In the remaining three experiments (O-N-EDL, S-EDL and T-SOL), the contralateral alterations were restricted to the EDL while the SOL remained unchanged.

Fibre number

In five experiments on the contralateral side, a significant increase (12–37 %) in the number of muscle fibres was observed. This was almost exclusively confined to the EDL. Again the cause of this hyperplasia could not be determined with any certainty. Mechanical compensation in the right leg during walking and running, as an effect of stretching or a general exercise, could have activated satellite cells (Antonio and Gonyea, 1993a; Rosenblatt and Parry, 1993; Russell et al., 1992). There is already strong evidence that daily low levels of asymmetric activity can induce muscle fibre hyperplasia in humans (Sjöström et al., 1991).

Fibre distribution

As with hyperplasia, fibre transformations were more frequent and more pronounced in the contralateral EDL than in the contralateral SOL. In the EDL, they led to a shift in the direction of fibre distribution from fast type IIB to slow type I (see scheme above), while in the SOL they appeared as an increase in the relative amount of type IIC fibres. A shift in the proportion of myosin heavy chains towards slower isoforms has also been observed in the contralateral EDL after chronic low-frequency stimulation of the left EDL (Skorjanc et al., 1998).

Fibre diameter

In both the EDL and SOL, increases and decreases in the diameter of oxidative type I and IIA fibres were equally frequent. Hypertrophy and atrophy, respectively, were independent of overloading operations. Changes in the diameter of the glycolytic type IIB fibres (lacking in SOL) were observed only in the direction of atrophy; increases in size were never observed.

This broad spectrum of muscular change occurring on the non-operated (contralateral) side after unilateral experiments clearly demonstrates that the contralateral side does not remain normal and, therefore, that side cannot be used as a control. Only untreated animals should be used as normal controls.

In the case of foreign-reinnervation (cross-reinnervation, transplantation), two types of contralaterality must be distinguished (Müntener and Srihari, 1984). For example, the cross-reinnervated (or transplanted) SOL of the left side receives its motor input from the EDL motoneurone pool and its afferent fibres project to the central nervous system *via* the EDL pathway. Thus, it becomes ‘neuronally’ linked to the EDL of the right side, while it remains ‘mechanically’ contralateral to the right SOL. In the present study, there was no difference in the reaction pattern of ‘neuronally’ and ‘mechanically’ contralateral muscles. Irrespective of the type of experiment, the different muscular changes on the contralateral side were always more pronounced in the EDL than in the SOL.

Neuronal mechanisms for these contralateral effects have been discussed since Rotshenker (1979) observed nerve sprouting and the formation of synapses in the contralateral muscles after unilateral denervation of the cutaneous-pectoris muscle in the frog. Experiments producing cross-reinnervation of the SOL (Reichmann et al., 1983; Srihari et al., 1981) and EDL (Müntener and Srihari, 1984) in rats confirmed these changes. Altered afferent activity derived mainly from the reinnervated muscle was thought to be transmitted, either by direct spinal interneurones or by superior integrating units, to the contralateral leg, resulting in modified efferent activity. The symmetrical reaction of 'neuronally' contralateral muscles was interpreted as support for this hypothesis (Müntener and Srihari, 1984).

Alternatively, the contralateral changes could be the consequence of a general compensatory mechanical activity aimed at maintaining a symmetrical posture during walking and running. The lack of any signs of reinnervation (such as fibre type grouping) as well as the heterogeneity of muscular changes (fibre hypertrophy, atrophy, muscle hyperplasia, muscle fibre transformations) provide strong evidence that supraspinal mechanisms are involved in the phenomena associated with contralaterality. The supraspinal mechanisms could also be responsible for the similarity between the muscular changes in the untreated muscles on the operated side and those in the corresponding ('mechanically') contralateral ones. In the experiment in which the SOL is self-reinnervated (S-SOL), for example, both the left and the right EDL show hyperplasia, with the percentage of IIA fibres increasing at the expense of atrophying IIB fibres.

While investigations of the mechanisms involved in contralaterality are lacking in animals, the problem has been intensively studied in humans with amputation of a lower or upper limb. Different methods and parameters have been used, such as alpha motoneurone excitability (Fuhr et al., 1992), cortical motor stimulation threshold and cortical topography after transcranial magnetic brain stimulation (Hall et al., 1990), sensory testing (Knecht et al., 1995) or regional blood flow measurements using electromyographic and cortical stimulation methods (Kew et al., 1994). The absence of changes in the excitability of the alpha motoneurone pool suggests reorganisation processes proximal to the alpha motoneurone level, while muscular responses evoked at lower thresholds and from a larger cortical area demonstrate a substantial reorganisation of the corticospinal system, suggesting that bilateral pathways are much more involved in neuronal plasticity than previously thought.

In conclusion, after unilateral manipulations involving a single muscle, there appears to be a complex rearrangement in the central nervous system that is paralleled by conversions in the corresponding contralateral motor units. The motor reprogramming in the central nervous system that follows a peripheral unilateral perturbation can be expected to influence more than just 'neuronally' or 'mechanically' contralateral muscles. Future studies should focus on evidence and principles of muscular plasticity in other distant ipsi- and contralateral muscles. It is clear from the present findings, however, that the contralateral muscles cannot be used as normal controls in such experiments.

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