Motor unit heterogeneity with respect to speed and fatiguability in cat muscles after chronic stimulation or paralysis

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Chronic stimulation of fast-twitch muscles in rabbit (Salmons & Vrbova, 1969; reviewed by Pette & Vrbova, 1985) and cat (Eerbeek et al., 1984; reviewed by Kernell & Eerbeek,1989) has been shown to change contractile properties toward those of slow muscles. Activation with total daily amounts of activity of > 30%, 5% and <0.5% of the day, corresponding roughly with the activity of slow (S), fast-fatigue resistant (FR) and fast fatiguable (FF) units, appeared to convert the muscle contraction characteristics toward those of the predicted motor unit (MU) type (Eerbeek et al., 1984; Kernell et al., 1987a,b; Westgaard & Lomo, 1988). If activity causes complete conversion, the prediction would be that all MUs in the stimulated muscles would become homogeneous in their properties. We therefore asked 2 questions: 1) what are the effects and the time course of increasing or decreasing activity on muscle speed and endurance? and 2) do MUs become homogeneous with respect to force, speed and endurance as predicted if activity converts muscles rather than modulates their properties within an adaptive range (Ausoni et al., 1990; Westgaard & Lomo, 1988).

Experimental methods

Silastic cuffs containing 3 stainless steel electrodes were aseptically implanted unilaterally around the nerve to medial gastrocnemius (MG) muscle in 13 adult cats (3-4 Kg), under pentobarbitone (60mg/Kg; i.p.) anaesthetic. A bipolar pad electrode was sutured to the fascia of MG muscle and the wires of all electrodes led out through the skin for 1) connection to a portable stimulator mounted on a basket carried on the cat's back for chronic stimulation and 2) for connection to external stimulators and amplifiers for regular recording of evoked EMG and isometric force.

The cats were divided into 2 groups. In group A (n=7), MG muscle was stimulated supramaximally at 20Hz with a 50% duty cycle (2.5 sec on, 2.5 sec off) for 6 to 34 weeks. In group B (n=6), the spinal cord was hemisected at L1 and the right hindlimb deafferented extradurally (L1-S2). Evoked EMG and isometric muscle force were measured at weekly intervals in both groups under halothane anaesthesia (for details see Gordon & Stein, 1982a) to monitor changes in force, contractile speed and fatiguability. The fatigue index (FI) for the muscle was calculated as the ratio of force generated at the end and beginning of a 2 minute period of tetanic trains of 13 pulses at 40Hz/1 sec. In a final acute experiment, 6-34 weeks later, all hindlimb muscles other than the MG were denervated and a laminectomy performed under pentobarbitone anesthesia, for isolation and characterization of muscle and single motor unit properties, using criteria described in detail by Gordon & Stein (1982b). Muscles were frozen and 10μm cross-sections were later cut for histochemical classification of muscle fibres (see Gordon et al., 1988). Muscle and motor unit properties were also studied in a third group C (n=6) which did not undergo surgery and provided the control data for comparison.

Results

There was little change in either contractile force or speed in MG paralysed by hemisection and deafferentation but muscle endurance decreased significantly. The mean (± S.E) tetanic force of

6 muscles which were paralysed for an average of 236 ± 45 days was 62 ± 5 N, as compared to 69 ± 6 N in contralateral control muscles. The twitch contraction time (CT) of 46 ± 2 ms was similar to that of the normal muscles, 50 ± 1.7 ms. FI was significantly less in paralysed muscles (cf. 0.25 ± 0.07 and 0.02 ± 0.01). There was a corresponding increase in the proportion of FF units (51% as compared with 31%) and fast glycolytic (FG) muscle fibres (58% as compared with 45%) which accounted for the decline in FI of the whole muscle. The relative number of S units and slow oxidative fibres (SO) of 28% did not change. Examination of a large population of MUs (n=180) showed that paralysed muscles contain the normal heterogeneous population with a wide range of unit CT and FI (Fig. 1).

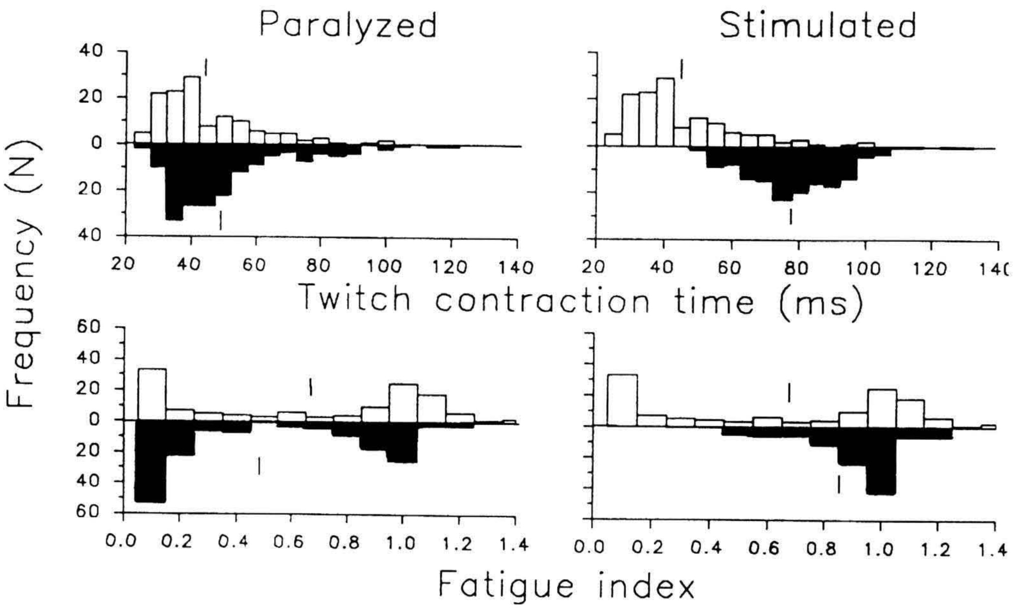

Figure 1. Comparisons of frequency distributions of twitch contraction times and fatigue indices of motor units sampled from paralysed (Group A) and chronically stimulated (Group B) cat MG muscles (solid histograms) with normal (Group C) muscles (open histograms). Mean values are shown as vertical bars. Further details are provided in the text.

In contrast, the contractile force and speed declined in the stimulated muscles and the endurance increased, as previously shown (Eerbeek et al., 1984; Kernell et al., 1987a,b). At six weeks, muscle force decreased (47 ± 5 N as compared to 63 ± 8 N in 3 cats) with relatively little change in CT or FI (cf 48 ± 2 ms and 51 ± 3 ms, CT; 0.32 ± 0.01 and 0.25 ± 0.05, FI). Over longer periods (158 ± 30 days, n=4), muscle twitch CT and FI increased significantly from 46 ± 2 ms to 96 ± 4 and 0.25 ± 0.07 to 0.7 ± 0.16, respectively.

There was a corresponding increase in mean CT and FI in isolated MUs: 78 ± 1 and 0.85 ± 0.02 in 168 MUs in 4 stimulated muscles, as compared with 43 ± 1 and 0.64 ± 0.04 in 134 units in 6 normal muscles (vertical bars in Fig. 1). However, the range of CT and FI was NOT reduced to the range of normal S units, as predicted for complete transformation of the MG from fast-twitch to slow-twitch. Comparison of the frequency histograms for MUs in normal and stimulated muscles shows that twitch CT is increased after stimulation but covers as large a range as normal. The normal bimodal distribution of FI becomes unimodal but the range is not confined to values above 0.75 as expected for complete conversion of MUs to non-fatiguable S MUs (see Kernell & Eerbeek, 1989).

Discussion

Although it has generally been accepted since the classical work of Sarah Tower (1937) that paralysed muscles undergo atrophic changes, we find surprisingly little change in properties of the paralysed cat MG muscle, other than a significant decrease in muscle endurance (see also Mayer et al., 1984; Pierotti et al., 1992, Munson et al., 1986). Similar findings have been observed in ankle flexor muscles (Kernell et al., 1987a,b). For the many different models of disuse which have been studied, effects of reduced muscle activity vary widely but there is general agreement that disused muscles become more fatiguable (Roy et al., 1991).

Results of slowing of fast-twitch muscles, increase in contractile speed of slow- twitch muscles, and conversion of muscle fibre types after cross-reinnervation or chronic stimulation have been interpreted as a "switch" from one phenotype to another, consistent with the appropriate expression of slow or fast isoforms of regulatory and contractile proteins and metabolic enzymes in the converted muscles (Vrbova et al., 1978; Pette & Vrbova, 1985). However, recent findings which show a significant increase in the number of muscle fibres expressing more than one isoform of these proteins, despite a change in muscle properties and muscle fibre types, suggest that muscle properties may be adapted rather than converted (reviewed by Pette & Staron, 1990). From studies that recognise differences in myotube phenotype prior to innervation, which are subsequently modulated by innervation during development (Miller & Stockdale, 1987), the possibility arises that intrinsic properties of muscle fibres are modulated by neural activity rather than converted: a plasticity which is limited within an adaptive range preset by intrinsic properties (see Ausoni et al., 1990). Analysis of motor unit properties after synchronous activation of all MUs in chronically stimulated muscles shows that the MU population is NOT homogeneous with respect to force, contractile speed and fatiguability which is the result predicted if activity CONVERTED muscle properties to that of slow MUs. Rather stimulation led to slowing of ALL MUs and increased their endurance, consistent with a modulation of properties. Thus, apparent conversion of fast-twitch to slow-twitch muscles by chronic stimulation is not simply a "switch" from a fast-phenotype to slow but rather a modulation of the MU population within an adaptive range, possibly preset by intrinsic mechanisms which are independent of activity.

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