FACTORS CAUSING DIFFERENCE IN FORCE OUTPUT AMONG MOTOR UNITS IN THE RAT MEDIAL GASTROCNEMIUS MUSCLE

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SUMMARY

1. The isometric contractile properties and morphological characteristics of the muscle unit portion of motor units were investigated in the medial gastrocnemius (MG) muscle of Fischer 344 rats. Individual motor units were functionally isolated by stimulating single MG axons in finely dissected ventral root filaments.

2. To study the mechanical properties of the motor units in the rat MG muscle, ninety-six motor units in five animals were classified into three categories (type FF, FR and S units) using two physiological criteria: presence or absence of the 'sag' property and fatigability. The relative distribution of the different motor unit types in the sample was 35.4% for type FF, 47.9% for type FR, and 16.7% for type S units.

3. There was little overlap in the distribution of twitch contraction time between type F (including types FF and FR) and type S units. The mean value was 17.1 ms for type FF, 15.7 ms for type FR, and 28.0 ms for type S units. Type FF units produced the largest tetanic tension (mean ± s.d.: 201±75 mN). Tension output of type S units was the smallest (15±6 mN), and that of type FR units was intermediate (100±45 mN). These values were significantly different.

4. A muscle unit portion of twenty-three motor units (8 FF, 6 FR, and 9 S units) was depleted of its glycogen through repetitive stimulation after characterization of its mechanical properties. Cross-sectional areas of unit fibres and innervation ratio were directly measured in sections stained for glycogen using a periodic and acid-Schiff (PAS) reaction. Specific tension of unit fibres was calculated by dividing the maximum tetanic tension of a unit by its total fibre area.

5. The number of unit fibres ranged from 44 to 77 for type S, 116 to 198 for type FR, and 221 to 356 for type FF units, and differences among their means (66, 154 and 271, respectively) were significant. Tetanic tension was correlated with innervation ratio for all of the twenty-three units, or units within a particular type.

6. Mean fibre area for type S units (1983 μm²) was significantly smaller than that for type FF units (3489 μm²). Fibres belonging to type FR units had an intermediate size (2648 μm²). Correlation between tetanic tension and fibre area was significant for either all units or units within a particular type.

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7. Total cross-sectional area was significantly different among the motor unit types, and was highly correlated to the maximum tetanic tension.

8. Specific tension was $16.7 \pm 2.9$ N/cm$^2$ for type S, $21.4 \pm 1.3$ N/cm$^2$ for type FR, and $25.1 \pm 2.9$ N/cm$^2$ for type FF units. These values were significantly different.

9. Stepwise multiple regression analysis revealed that variations in maximum tetanic tension among motor unit types could be explained principally by innervation ratio, whereas fibre size was the major factor to determine tetanic tension within a given motor unit type.

10. Unit fibre density for type S units was significantly smaller than that for either type FR or type FF units. There was no difference between type FR and type FF units.

11. Axonal conduction velocity was not correlated with innervation ratio for units within a particular type as well as for all twenty-three units of different types, suggesting that there was no or a weak, if any, correlation between axonal conduction velocity and the number of terminal branches for rat motoneurones.

**INTRODUCTION**

The maximum force outputs of individual motor units in a mixed muscle differ considerably (Wuerker, McPhedran & Henneman, 1965; Olson & Swett, 1966). There are also systematic differences related to motor unit type. Mean tetanic tension is greatest for type FF (fast twitch, fatigable) units, intermediate for type FR (fast twitch, fatigue resistant) units and smallest for type S (slow twitch) units (Burke, Levine, Tsairis & Zajac, 1973). These differences may be attributed to differences in (1) innervation ratio, i.e. the number of muscle fibres composing individual motor units, (2) mean cross-sectional area of the muscle fibres, and (3) specific tension, i.e. the force output per unit cross-sectional area of muscle fibres. The difference among individual motor units was usually explained in terms of the first factor until the mid 1970s (Henneman & Olson, 1965). The second and third factors appeared to have been ignored. Burke and his co-workers (Burke & Tsairis, 1973; Burke, Levine, Salcman & Tsairis, 1974) estimated relative innervation ratio by an indirect approach, and found that variation was very small among motor unit types. They proposed that difference in specific tension of unit fibres (i.e. muscle fibres composing a motor unit) was an important factor determining the difference in tetanic tension between type FF (or FR) and S units in cat muscles.

Recent development of histochemical methods for marking the fibres composing individual motor units, by depleting them of glycogen, has made it possible to study the relationships between these factors and the maximum tetanic tension directly (Edström & Kugelberg, 1968). Using this technique, Bodine, Roy, Eldred & Edgerton (1987) and Chamberlain & Lewis (1989) have studied the force output and morphological parameters of the muscle unit portion of individual motor units in the cat tibialis anterior and the rat soleus muscles, respectively. They showed that the principal factor determining the maximum tetanic tension was innervation ratio, although fibre cross-sectional area and specific tension did contribute to differences between unit types. The specific tension estimated from data of individual motor units was smaller for fibres of type S units than those of type F units (e.g. type FF and FR units), but the difference was much smaller than the values obtained by
indirect estimation. Number and types of motor units so far studied using the glycogen depletion method, however, are limited because of the inherent difficulties in this method.

It has been generally assumed that the number of terminal branches is proportional to motor axon diameter although there has been little direct evidence (Eccles & Sherrington, 1930). The notion seems to fit to empirical data showing that type S units produce less tetanic tension than type F units, because motoneurones belonging to slow motor units have slower conduction velocities (i.e. smaller axon diameters) than motoneurones of fast motor units (Burke, Rymer & Walsh, 1976; Dum & Kennedy, 1980; Fleshman, Munson, Sypert & Friedman, 1981; Dum, Burke O'Donovan, Toop & Hodgson, 1982). Further, tetanic tension correlates with conduction velocity for units of all types (McPhedran, Wuerker & Henneman, 1965; Wuerker et al. 1965; Appleberg & Emonet-Dénand, 1967; Bagust, Knott, Lewis, Luck & Westerman, 1973; Bagust, 1974; Proske & Waite, 1974; Jami & Petit, 1975). However, some reports have shown that this correlation does not hold especially for units within a particular motor unit type (Burke, 1967; Mosher, Gerlach & Stuart, 1972). The extent to which tetanic tension correlates to innervation ratio is also a subject of dispute as described above. Consequently, the relationship between the axonal conduction velocity of the motoneurone and number of branches is still an open question.

Using the glycogen depletion method, we measured innervation ratio, cross-sectional area of unit fibres, and axonal conduction velocity as well as various mechanical properties of individual motor units in the rat medial gastrocnemius (MG) muscle which contained three types (i.e. FF, FR and S) of motor unit. Total cross-sectional areas and specific tension were also calculated from these data. We analysed factors controlling the force production of individual motor units and differences among motor unit types. Preliminary accounts of these results have appeared elsewhere (Kanda & Hashizume, 1986; Kanda, 1988).

METHODS

Physiological analysis

Experimental procedures

Isometric contractile properties of the motor units were measured in the MG muscle of male Fischer 344 rats (11- to 14-month-old; body weight, 410–510 g). Each animal was anaesthetized with a mixture of urethane and chloralose (500 mg/kg and 50 mg/kg, respectively) or sodium pentobarbitone (35–40 mg/kg) administered intraperitoneally. In the leg, the MG muscle was dissected from the surrounding tissues, but was not separated from the lateral gastrocnemius muscle in order to maintain good blood circulation. The MG nerve was also freed of surrounding tissues to allow recording of the action potentials of MG axons. The hip and hindlimb muscles, except for the MG muscle, were widely denervated by cutting the nerves. The lumbosacral spinal cord was exposed by laminectomy. The leg and lumbosacral spine were immobilized in a metal frame by means of clamps. The distal tendon of the MG muscle was attached to an isometric strain gauge (BG-300, Kulite; compliance, 0.008 mm/N; natural frequency, 4-0 kHz) with a small steel hook. The exposed portions of the spinal cord and limb were covered by pools of mineral oil pools at 36–38 °C (for rectal temperature and spinal cord) or at 35–37 °C (for leg). Blood pressure and expired CO₂ level were monitored throughout the experiment. In some experiments, lactated Ringer solution (Otsuka Pharmaceuticals) or 4 % Ficoll (Pharmacia Fine Chemicals) solution was infused to maintain the blood pressure.

Motor units were isolated by stimulating single MG axons in fine dissected ventral root filaments. The ventral roots of the lower lumbar segments (usually L4 and L5) were sectioned at a point near
the entry to the cord. The peripheral cut end was dissected, and placed on a pair of bipolar stimulating electrodes (cathode was always distal). The criteria for single unit activity were all-or-none mechanical twitch, and all-or-none action potentials from EMG recordings. This was tested by gradual change in stimulus intensity, and repeated at least five times. Single, bi- or tri-phasic action potentials recorded from the MG nerve were also used to confirm single unit activity. The initial muscle tension was determined to allow production of the maximum whole-muscle twitch tension. Mechanical and electrical responses of muscle units were recorded and the motor unit types were determined at this optimum tension. The following was the sequence of stimulation protocols: (1) single pulse activation; (2) short (0.6 or 1.6 s) trains of pulses at various frequencies producing unfused or fused tetani; (3) alternation between single pulses and short (0.45 s) trains of pulses at 200 pulses/s, causing post-tetanic potentiation (or depression) of the twitch response; and (4) trains of pulses composed of thirteen pulses recurring at 40 pulses/s. Trains were repeated every 1 s for at least 2 min in order to assess sensitivity to fatigue.

The electrical activity of the active muscle units was recorded with fine flexible stainless-steel wire electrodes (100 µm in diameter) bared of insulation at the tip and hooked into the MG or adjacent muscles (usually the lateral gastrocnemius and the plantaris). Motor axon action potentials elicited by the stimulation of the ventral root filament at 3–5 times threshold for each motor unit tension were recorded from the MG nerve at the popliteal fossa with a pair of bipolar electrodes, and averaged 10–20 times. Conduction velocity was calculated by the latency for the averaged axonal action potential, using the conduction distance from the ventral root stimulation site. In some cases, the motor axon action potentials were recorded before and after registering mechanical properties. The latencies for these action potentials recorded at two different periods were consistent. The conduction distance was measured after the animal was killed.

Fatigue test

Motor units were classified into three categories (FF, FR and S) using criteria (i.e. the ‘sag’ property and fatigability) similar to those applied to cat and rat motor units (Burke et al. 1973; Sandercock, Faulkner, Albers & Abbrecht, 1985; Gardiner & Olha, 1987; Chamberlain & Lewis, 1989). The methods have been described elsewhere (Kanda & Hashizume, 1989). Briefly, the presence or absence of the sag property was tested by producing unfused tetani with short (0.6 or 1.6 s) trains of pulses at various frequencies (pulse interval 15–35 ms for fast-twitch units, and 25–65 ms for slow-twitch units), therefore, motor units were classified into two major groups: types F (fast twitch) and S (Fig. 1B–D and F–H). The maximum tension produced by each train of pulses did not decline constantly for the type F units if the fatigue test was performed after a few minutes of rest (Fig. 2A–H). The peak tension produced by the first train of pulses was usually the largest because of effective summation during the first few pulses. Tension declined during a couple of the following trials, then became larger because of potentiation until it reached a plateau, and finally declined gradually towards the end of this procedure. We termed the ratio of maximum tension produced during the 120th tetanus (i.e. 2 min of stimulation) to the tension output during the plateau phase the fatigue index for the type F units. In contrast to the complex pattern of tension change for the type F units, tension declined constantly or did not change apparently for the type S units (Fig. 2I–L). In these cases, the ratio of tension produced during the 120th tetanus to that produced during the first tetanus was taken as the fatigue index.

Muscle unit morphology and histochemistry

Tissue processing

Visualization of the fibres of a selected motor unit was attempted by the glycogen depletion method (Edström & Kugelberg, 1968) in twenty-eight rats (not including the rats used for the study of the general physiological properties of motor units in the MG). After registering mechanical properties, the unit was stimulated with train pulses of the same pattern as used for the fatigue test for 4 min under ischaemia produced by clamping the popliteal artery with a small clip. The stimulus frequency was thereafter reduced to 1 Hz, and the clamp was removed for 5 min. This sequence was repeated 5 times (Kugelberg & Lindegren, 1979). We paid special attention to ensuring that anaesthesia was deep enough to prevent any movement throughout the experiment and to maintain good blood circulation in the MG muscle in order to minimize the sporadic depletion of glycogen in unstimulated units. The muscle was quickly excised and was fixed approximately at a length at which tension had been recorded. It was then frozen in isopentane
cooled in liquid N\textsubscript{2}. After storage in a deep freezer (−75 °C), serial sections (10–15 \(\mu\)m) were cut in a cryostat. They were stained for glycogen by the periodic and acid–Schiff (PAS) method, for myosin ATPase (Khan, Papadimitriou & Kakulas, 1974), and for NADH diaphorase. The cross-sectional area of individual muscle fibres was measured using a microscope with a drawing apparatus, a digitizing tablet and a microcomputer. No correction was made for the pinnation of the muscle. The total cross-sectional area of the unit was calculated from the mean cross-sectional area of unit fibres and the innervation ratio.

Identification of unit fibres

Two major problems in obtaining an accurate number of unit fibres using the glycogen depletion method have been pointed out (Burke & Tsairis, 1973; Burke et al. 1974): one is the complex internal architecture (e.g. fibre pinnation) which limits the number of unit fibres that appear at any level of muscle cross section, and the other is the incomplete or non-uniform depletion of glycogen along the muscle fibres (Barker, Emonet-Dénand, Harker, Jami & Laporte, 1977; Toop, Burke, Dum, O’Donovan & Smith, 1982; Bodine et al. 1987; Nemeth, Norris, Lowry, Gordon, Enoka & Stuart, 1988). To overcome these problems and to count the number of unit fibres as accurately as possible, in the present experiments, we cut many sections from various levels of the muscle so that the entire motor unit territory could be covered for inspection in the pinnated MG muscle. The frozen whole muscle was first cut into cross-sectional blocks about 5 mm in thickness. Probe sections were cut from each block, and stained for glycogen. Several serial sections were cut at intervals of 100 \(\mu\)m to 1 mm from the blocks containing PAS-negative fibres (usually three to four out of five blocks). Along with major markers such as outlines of fasciculi and blood vessels, PAS-negative or very lightly stained fibres in each section were drawn on a sheet of paper using a drawing apparatus attached to a microscope. By comparing a drawing with that of the adjacent section, individual depleted fibres were identified, and an identification number was assigned to each. These procedures were performed consecutively until the entire territory of the unit was reconstructed.

Statistical analysis

Group comparisons were made initially with a one-way analysis of variance (ANOVA); post hoc comparisons were made with the Scheffe procedure (significance level \(P < 0.05\)). A stepwise multiple regression analysis was used to determine the relative importance of each variable measured in determining the maximum tetanic tension. Other statistical analyses are described in the text.

RESULTS

Physiological properties of motor units in rat MG muscle

To determine the general characteristics of motor units in the rat MG muscle, we measured ninety-six motor units in five animals. The number of motor unit samples from one animal ranged between seventeen and twenty-three. The total number of motor units in this muscle has been estimated to be about ninety-eight (Hashizume, Kanda & Burke, 1988).

Shape of unfused tetani and twitch contraction time

Eighty out of ninety-six units showed a clear ‘sag’ over a wide range of stimulus frequencies (Fig. 1B, C and D), while the rest (i.e. sixteen units) did not (Fig. 1F, G and H). The twitch contraction time (after maximum effect of tetanic stimulation) of the eighty motor units which showed ‘sag’ property was generally short, ranging from 12·0 to 21·3 ms (Fig. 1I). Only four had twitch contraction times greater than 20 ms (specifically, 20·8, 21·0, 21·2 and 21·3 ms). On the other hand, all of the units which did not show the ‘sag’ property had twitch contraction times greater than 20 ms (range: 20·2–36·2 ms). Thus, it seemed reasonable to classify rat MG motor units into fast twitch (type F) and slow twitch (type S) types by the presence or
Fig. 1. Mechanical response of fast twitch (A–D) and slow twitch (E–H) muscle units, and the histogram showing the frequency distributions of twitch contraction time (I). A, initial twitch response with electrical activity and a twitch contraction after maximum post-tetanic potentiation (PTP). B–D, intervals of train pulses for stimulation are 35, 25 and 15 ms, respectively. E, twitch response after maximum PTP with electrical activity. F–H, intervals of train pulses are 60, 40 and 20 ms, respectively. Note that the fast twitch unit shows the 'sag' property, while the slow twitch unit does not. Calibrations: 25 mN and 25 ms for A, 50 mN and 200 ms for B–D, 5 mN and 25 ms for E, and 20 mN and 200 ms for F–H. I, histogram showing the frequency distributions of twitch contraction time after maximum PTP. ☐, units with the 'sag' property; ☒, units without the 'sag'. Note that all the units without the 'sag' have a twitch contraction time longer than 20 ms.

absence of the 'sag' property like the motor units in the muscle in cats and rats (Burke et al. 1973; Gardiner & Olha, 1987; Chamberlain & Lewis, 1989).

Fatigue sensitivity and motor unit types

Distribution of fatigue indices was bimodal, and most of the units had a fatigue index of less than 0.5 or greater than 0.75 (Fig. 2M). All type S units had a fatigue index greater than 0.75. Type F units with a fatigue index equal to or greater than 0.75 were defined as type FR, and those with a fatigue index smaller than 0.75 as type FF in these experiments. Thus, in the present experiments, we adopted tripartite classification rather than tetrapartite including additional type FI (the unit with a fatigue index >0.5 but <0.75). In our recent series of experiments on rat MG motor units, the distribution pattern of the fatigue index showed no clear sign for
Fig. 2. Responses of two fast twitch (A–D, E–H) units and one slow twitch (I–L) unit to short duration (330 ms) 40 pulses/s tetani recurring every 1 s, and the frequency distributions of fatigue index (M). A, E and I, responses for the entire sequence of recurrent tetani (2 min). B, F and J, records of the first and the second trials with a faster time base. Note that, for the fast twitch units, the ‘sag’ phenomenon is observed during the first tetani, but is not seen clearly during the second tetani. C, G and K, responses gaining the maximum force during the sequence of recurrent tetani, shown by arrows. D, H, and L, responses at 2 min after the onset of the recurrent tetani. Calibrations: 50 mN and 100 ms for B–D, and F–H; 20 mN and 200 ms for J–L. M, the frequency distributions of fatigue index (see Methods) of the ninety-six motor units in these experiments. □, motor units with the ‘sag’; ⊙, units without the ‘sag’. Note that values are clustered below 0·5, or above 0·75, and that all of the units without the ‘sag’ have fatigue indices greater than 0·9.

separation between types FF and FI (authors’ unpublished data). Further, we have not confirmed yet that the muscle unit portion of units with a fatigue index between 0·5 and 0·75 have a unique histochemical profile (Burke, 1981). We therefore think that for the time being tripartite classification is suitable for the rat MG motor units. The relative distribution of the different motor unit types in the present sample was as follows: 35·4 % for type FF, 47·9 % for type FR and 16·7 % for type S motor units.
Fig. 3. Histograms showing the frequency distributions of tetanic tension produced by individual motor units classified into type FF (A), type FR (B), or type S (C). show the distribution of tetanic force output produced by the units which were depleted of their glycogen.

Fig. 4. Relationships between axonal conduction velocity and tetanic tension (A), and twitch contraction time (B). No correlation was found between axonal conduction velocity and tetanic tension in these experiments. Twitch contraction time was correlated to axonal conduction velocity for all units (r = 0.572, P < 0.001). , type S units; , type FR units; , type FF units.

**Tetanic tension**

Figure 3 show the distribution of maximum tetanic tension. As shown for the motor units in different muscles or animals, type FF units in the rat MG muscle produced the largest tetanic tension (mean ± s.d.; 201 ± 75 mN). Tension output of
the type S units was the smallest \((15\pm 6\text{ mN})\), and that of type FR was intermediate \((100\pm 45\text{ mN})\). All these values were significantly different.

**Axonal conduction velocity**

The conduction velocity of the type S units in this experiment \((\text{mean}\pm\text{s.d.}; 57.6\pm 5.4\text{ m/s}, n = 7)\) was significantly \((P < 0.001)\) slower than that of the type F units \((65.1\pm 3.8\text{ m/s}, n = 29)\). Within the type F units, units with the highest conduction velocity belonged to type FR, although the difference in mean values between types FF and FR was not statistically significant \((P > 0.1)\). Conduction velocity of the innervating motor axon did not correlate with tension output for all units \((r = 0.206, P > 0.02; \text{Fig. 4A})\) or for units within a subpopulation of a given motor unit type. Twitch contraction time inversely correlated with the axonal conduction velocity \((r = 0.572, P < 0.001; \text{Fig. 4B})\) although this relation did not hold true for units within a particular motor unit type. These relationships also held true for units in individual animals.

**Morphology of muscle unit portion of individual motor units**

We attempted to deplete glycogen in thirty motor units in twenty-eight muscles. We analysed twenty-three units (eight FF, six FR and nine S units), which had a single, simple-shaped territory with homogenous histochemical profiles. We excluded the following cases for further analysis: (1) only a few PAS-negative fibres were found (one case); (2) the territory was divided into two separate regions, and the mean sizes

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**Fig. 5.** Serial sections of a medial gastrocnemius muscle stained for glycogen (PAS) showing the distribution of fibres belonging to a single depleted motor unit (unit 6 in Table 1). Note that two PAS-negative fibres (shown by arrows in section A) are indistinguishable from fibres belonging to non-depleted motor units (i.e. PAS-positive) in section B. Calibration bar: 100 \(\mu m\).
of fibres in the two regions were significantly different (one case); or (3) most of the fibres were stained very lightly and discrimination between depleted and non-depleted fibres was difficult (five cases). Data for individual units are shown in Table 1. The distribution of tetanic force produced by these units is shown in Fig. 3. The mean value for each unit type (237 ± 68 mN for type FF units, 89 ± 34 mN for type

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MU, motor unit identification number; type, motor unit types; CV, axonal conduction velocity; \( P_0 \), maximum tetanic tension; IR, innervation ratio; mean CSA, mean cross-sectional area of the unit fibres; total CSA, total cross-sectional area calculated as IR \times mean CSA; specific tension, mean specific tension of the unit fibres calculated as \( P_0 / \text{total CSA} \).

FR units, and 22 ± 8 mN for type S units) was similar to that obtained from a larger sample of non-depleted units. Differences between unit types were statistically significant.

A non-uniform mode of depletion along the longitudinal axis of the muscle fibre belonging to the type S units was revealed during the reconstruction process. Some fibres (13.2–61.7% of total unit fibres) were depleted of glycogen at a certain level of the serial sections, but not in the sections at other levels. (Fig. 5). Such non-uniform depletion was less frequently found among type FR units (0–14.7% of total unit fibres), and not among type FF units. All fibres which showed the non-uniform depletion were considered as unit fibres because they were located within the territory of the unit and had a similar histochemical profile, which was judged from the sections stained for ATPase or NADH diaphorase.
Number of unit fibres (i.e. innervation ratio)

The number of fibres in the depleted units ranged considerably: 41–77 for the type S, 116–198 for type FR and 221–356 for type FF units (Table 1), and differences between their means (66, 154 and 271, respectively) were statistically significant.

Figure 6A shows the relationship between the maximum tetanic force and innervation ratio for the twenty-three units examined. Tetanic tensions were highly correlated to the innervation ratio for all units (correlation coefficient, $r = 0.953$, $P < 0.001$). However, the correlation coefficients for units within a particular type were lower: $r = 0.7$, $P > 0.5$ for the FF units; $r = 0.857$, $P < 0.05$ for the FR units; and $r = 0.191$, $P > 0.5$ for the S units.

Cross-sectional area of unit fibres

Cross-sectional areas of 60–100% of the unit fibres were measured in a single representative section for each depleted unit. Serial sections were cut perpendicularly to the long axis of the muscle. No correction was made for fibre pinnation and shrinkage during staining. The mean area for the type S units (mean ± s.d., $1983 ± 59 \mu m^2$, $n = 9$; range, 929–3070) was significantly smaller than that for the type FF units (3489 ± 701 $\mu m^2$, $n = 8$; range, 2669–4679). Fibres in the type FR
units had an intermediate size (2648±546 μm², n = 6, range: 1973–3256 μm²), which were not significantly different from either the type FF or S units. The relationships between tetanic tension and cross-sectional area are shown in Fig. 6B. Correlation coefficients were high for all units (r = 0.86, P < 0.001) or units within a particular type (r = 0.781, P < 0.05 for the FF units; r = 0.925, P < 0.01 for the FR units; and r = 0.76, P < 0.02 for the S units). The total cross-sectional area, which was the product of the mean cross-sectional area of the unit fibres multiplied by the innervation ratio, was significantly different among the motor-unit types: 0.1288±0.0363 mm² for type S units, 0.4151±0.1464 mm² for type FR units, and 0.9541±0.2996 mm² for type FF units. Tetanic tension was highly correlated to the total cross-sectional area (r = 0.86, P < 0.001 for all units; r = 0.781, P < 0.05 for the FF; r = 0.925, P < 0.01 for the FR; and r = 0.76, P < 0.02 for the S units; Fig. 6D).

Specific tension

Specific tension, calculated by dividing the maximum tetanic tension of a unit by its total cross-sectional area of fibres, was considerably different among twenty-three depleted units in the present experiments (range: 12.2–28.3 N/cm²). The mean values (±S.D.) for each type: 16.7±2.9 N/cm² for the type S units, 21.4±1.30 N/cm² for the type FR units, and 25.1±2.9 N/cm² for the type FF units differed significantly. The tetanic tension was also correlated with the specific tension for all units (r = 0.761, P < 0.001), whereas the correlation coefficients were low for units within a particular type (r = 0.013, P > 0.9 for the FF units; r = 0.556, P > 0.2 for the FR units; and r = 0.544, P > 0.1 for the S units; Fig. 6C).

Multiple regression analysis

A stepwise multiple regression analysis was performed to define the influence of each of three factors (i.e. innervation ratio, mean cross-sectional area, and specific tension) in the production of the maximum tetanic tension. The first step identified innervation ratio as the best single predictor of tetanic tension for all twenty-three units. The proportion of tetanic tension variance that can be predicted by this single variable regression equation was 0.908. The second step identified mean cross-sectional area as the best predictor to be used with innervation ratio to define a two-variable multiple regression equation. The proportion of tetanic tension variance that could be predicted by this two-variable multiple regression equation was 0.955.

On the other hand, the same analysis for units within a particular type revealed that the best single predictor of tetanic tension was mean cross-sectional area for all three types. The proportions of tetanic tension variance that could be predicted by this single variable regression equation were 0.61, 0.856 and 0.578 for FF, FR and S units, respectively. The best predictor to be used with mean cross-sectional area to define a two-variable multiple regression equation was innervation ratio. Additional innervation ratio raised the values to 0.875, 0.977 and 0.855 respectively. For the FF and S units, including the final variable, specific tension raised the values to 0.995 and 0.982, respectively.

Consequently, difference in innervation ratio appears to be the major factor in producing difference in tetanic tension among motor unit types, whereas fibre size is
A more important factor in determining tetanic tension within a given motor unit type. These two or three factors account for more than 95% of the variability in tetanic tension of individual units.

Unit fibre density and size of motor unit territory

Unit fibres were evenly distributed within a territory. The mean fibre density (±s.d.) for the type S units was 4·6±0·7 unit fibres/(100 fibres within the territory), and was significantly smaller than that for either the type FR (7·7±1·2) or FF (8·8±2·2) units. There was no significant difference in the unit fibre density between the type FF and FR units. The relative size of the motor unit territory, estimated from the values of unit fibre density, innervation ratio and total number of muscle fibres composing the muscle, was significantly larger for the type FF units (18·2±6·5% of total cross-sectional area) than that for either the type FR units (11·0±2·1%) or the type S units (8·2±2·3%). The difference between the type FR and type S units was not significant. Figure 7 shows the relationships between these two factors and the innervation ratio. Fibre density for units within a particular type did not correlate with the innervation ratio ($P > 0·005$). Positive correlation was found between territory size and innervation ratio for the FF ($r = 0·896, P < 0·005$) and S ($r = 0·874, P < 0·005$) units.
Innervation ratio and axonal conduction velocity

Figure 8 shows the relationship between axonal conduction velocity and innervation ratio for the depleted units in these experiments. Axonal conduction velocity was not correlated with the innervation ratio for units within a particular type as well as for all twenty-three units of different types ($r = 0.222$, $P > 0.2$). Conduction velocity was not correlated with the tetanic tension for the depleted units ($r = 0.373$, $P > 0.1$), either. The mean axonal conduction velocities for the S, FR and FF units were 59.1, 65.0 and 67.5 m/s, respectively. There were no differences among the motor unit types.

Motor unit organization

Bodine and co-workers (Bodine et al. 1987) reported that there was no difference in the innervation ratio between types FF and FR units. They found no significant difference in specific tension among any of the fast types (i.e. FF, FI and FR), either, although overall mean specific tension for the fast units was significantly different from the mean for the slow units in the cat tibialis anterior muscle. However, their sample number for each type (especially for type FR) seemed to be too small to draw any conclusions on this subject. The rat soleus muscle studied by Chamberlain & Lewis (1989) is composed only of type S and FR units. Consequently, it was not known if there were any systematic differences in these factors between the FF and FR subtypes and between S types in slow and mixed muscle in the rat. In the present experiments, we showed that three factors: the innervation ratio, the cross-sectional area, and the specific tension of the unit fibres in the rat MG muscle, all were graded in the order $S < FR < FF$. The mean values were significantly different among the motor unit types, except between the FR and S units for the mean cross-sectional area. These differences account for the systematic, large difference in tetanic tension related to the motor unit types. Further, a stepwise multiple regression analysis revealed that the most influential factor for producing differences in tetanic tension was innervation ratio for the units of all types. The same analysis for units within a given type showed that the best single predictor was the mean cross-sectional area of the unit fibres. This suggests that the rat MG motor unit population is comprised anatomically as well as physiologically of distinct unit classes (Fleshman et al. 1981).

The validity of the values of innervation ratio obtained in these experiments may be checked by comparing two values derived independently. That is, by knowing the number of motor-units and mean innervation ratio for each unit type, we can estimate the total number of fibres composing the MG muscle. This value is compared with the value obtained by a direct count ($18252 \pm 627$ (s.d.), $n = 3$ for the total; $1214 \pm 160$ for type I fibres; and $17038 \pm 471$ for type II fibres; Kanda & Hashizume, 1989). The number of motor units (i.e. the number of $\alpha$-motoneurones innervating the MG muscle: 98) was obtained in our previous experiments using horseradish peroxidase (HRP) retrograde labelling (Hashizume et al. 1988). Distribution of the motor unit types in the large, non-depleted unit sample was 16.7% for type S, 47.9% for type FR, and 35.4% for type FF units. Thus the number of motor units of each type were estimated to be 16, 47 and 35, respectively. The
mean innervation ratio for each unit type obtained in these experiments was 66 for type S, 154 for type FR, and 271 for the type FF units. Notice that the mean tetanic tensions of these units were similar to those obtained from a larger, non-depleted unit sample. From these values the total number of muscle fibres was estimated to be 17779, which is very close to the value obtained by direct count. Thus, we believe that the values for innervation ratio obtained in the present experiments are acceptable although glycogen depletion and PAS staining may not be a perfect method for identifying activated fibres (Toop et al. 1982; Nemeth et al. 1988).

Specific tension

Our estimates of specific tension: 16.7 N/cm² for the type S units, 21.4 N/cm² for the type FR units, and 25.1 N/cm² for the type FF units are similar to those obtained in former experiments performed by the glycogen depletion method and direct count of unit fibres (Burke & Tsairis, 1973; Bodine et al. 1987; Chamberlain & Lewis, 1989), and to those of whole muscle experiments (Sexton & Gersten, 1967, Bárány & Close, 1971; Murphy & Beardsley, 1974; Edjtehadi & Lewis, 1979; Faulkner, Niemeyer, Maxwell & White, 1980; Spector, Gardiner, Zernicke, Roy & Edgerton, 1980; Powell, Roy, Kanim, Bello & Edgerton, 1984; Côté & Faulkner, 1986; Brooks & Faulkner, 1988). However, Chamberlain & Lewis (1989) stated that their values should be lower than that at the optimal length of the muscle because of free shortening before freezing under their conditions. Their corrected values were much higher (38 N/cm² for the S units and 46 N/cm² for the FR units) than the values in these experiments in which the muscle was held at close to the optimal length during freezing. Specific tensions estimated indirectly for the S units in the cat soleus are about twice as large as those for the same types of units in the medial gastrocnemius muscle (Burke et al. 1974). This might also be true for rat motor units.

It has been reported that the specific tension for type I fibres or fibres of the type S motor units is somewhat less than that for type II fibres or fibres of the type FF and FR motor units (Burke & Tsairis, 1973; Dum et al. 1982; Bodine et al. 1987; Chamberlain & Lewis, 1989). The ratio, S:FR:FF = 1:1.3:1.5, in these experiments was similar to the results in the former experiments (Bodine et al. 1987; Chamberlain & Lewis, 1989). Experiments in whole muscles also showed a similar difference between slow and fast muscle (Kean, Lewis & McGarrick, 1974; Finol, Lewis & Owens, 1981). On the other hand, Lucas, Ruff & Binder (1987) examined the tension production of skinned fibres in vitro and reported that the specific tension for type I fibres was as large as that for type II fibres. Engel & Stonnington (1974) found that myofibrils formed approximately the same proportion of fibre area in both types. Hence, type I fibres should be less completely activated in situ than type II fibres. As has been shown (Barker et al. 1977), we also found in these experiments that some type S fibres were depleted of their glycogen unevenly along their length. More specifically, some fibres were depleted in cross-sections at certain levels, whereas the same fibres in sections at different levels were not depleted. We considered all such fibres as unit fibres of the depleted unit because most were located within the territory of the unit and had a similar histochemical profile. Bodine et al. (1987) stated that there was some degree of variation in PAS staining, but this was not enough to alter the classification of a fibre as either depleted or non-depleted in a
particular section. It is not known whether the variation is due to a combination of variation in glycogen level along the longitudinal axis of the fibre (Hintz, Chi & Lowry, 1984) and an incomplete depletion, or to a partial activation of contractile elements in situ. The relationship between this phenomenon and smaller specific tension for type S units is worthy of further study.

It is not known which factors account for the difference in specific tension between the FF and FR units. Engel & Stonnington (1974) reported that the proportions of myofibrils was the same in both slow and fast muscles, because the additional mitochondria of slow muscle almost exactly balance the abundance of sarcoplasmic reticulum of the fast fibres. Fibres of type FR motor units are probably rich in both mitochondria and sarcoplasmic reticulum of the fast fibres. Fibres of type FR motor units are probably rich in both mitochondria and sarcoplasmic reticulum. Consequently, a lower proportion of myofibrils and a lower specific tension for fibres of the type FR unit (i.e. type IIA muscle fibres) might be comparable to the fibres of the type FF units (i.e. type IIB muscle fibres).

**Axonal branching and conduction velocity**

It is generally assumed that larger axons send off more branches than smaller axons. The evidence for this has been provided by Eccles & Sherrington (1930) who dissected single axons and showed a higher incidence of branching in larger axons. This has been generally discussed in relation to maximum tetanic tension. A linear (or logarithmic) relationship between conduction velocity and maximum tetanic tension has been repeatedly reported in various muscles (McPhedran et al. 1965; Wuerker et al. 1965; Appelberg & Emonet-Dénand, 1967; Bagust et al. 1973; Bagust, 1974; Proske & Waite, 1974; Jami & Petit, 1975). However, these findings do not necessarily explain the relationship between axonal conduction velocity and the number of muscle fibres, because tetanic tension is governed by factors other than innervation ratio as shown in these experiments. We could find no correlation between innervation ratio and axonal conduction velocity for twenty-three depleted units in these experiments. There is a certain variability in correlation between axonal conduction velocity and cell size (Cullheim, 1978; Burke, Dum, Fleshman, Glenn, Lev-Tov, O'Donovan & Pinter, 1982). The number of terminal branches may be more closely correlated to cell size rather than to axonal conduction velocity. Alternatively, the number of axonal branches might be governed not only by motoneurone size (or axon size), but also by other factors, such as the motor unit type and/or muscle differences. There were no differences in axonal conduction velocity among the motor unit types whereas differences in the mean innervation ratio was highly significant. Type S units in the rat MG muscle appear to have a smaller innervation ratio (66±12·4 in the present experiments) than the same type of units in the soleus muscle (110±32·7, Chamberlain & Lewis, 1989). Similar values (80–100) for the type S units in the rat soleus muscle can be obtained indirectly from the total number of muscle fibres (i.e. about 2500; Gutmann & Hanzlíková, 1966; Caccia, Harris & Johnson, 1979; Ishihara, Naitoh & Katsuta, 1987; Ansved & Larsson, 1989) and the number of α-motoneurones innervating this muscle (i.e. 25–30; Gutmann & Hanzlíková, 1966; Caccia et al. 1979; Ishihara et al. 1987; Ishihara, Naitoh, Araki & Nishihira, 1988).
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REFERENCES


FORCE OUTPUT OF RAT MOTOR UNITS


