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Correlation between shortening velocity, force–velocity relation and histochemical fibre-type composition in rat muscles

K. W. RANATUNGA* and P. E. THOMAS

Department of Physiology, The Medical School, University of Bristol, Bristol BS8 1TD, UK

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Summary

Isometric and isotonic contractions of three muscles in the rat hind leg (soleus, extensor digitorum longus (EDL) and peroneus longus (PL)) were recorded *in situ* at 35° C and with nerve stimulation. Additionally, the histochemical muscle fibre-type composition of the three muscles was determined by the method of Guth and Samaha (1970). The data obtained from soleus and EDL muscles were similar to those reported in previous studies. On the basis of twitch contraction time, rate of rise of tetanic tension and maximum shortening velocity, the contraction speed of EDL was 2–3 times higher than in soleus. In the PL muscle, the twitch contraction time, rate of tension rise and shortening velocity were 17 ms, 30 *Po*/s and 12 muscle fibre lengths/s, respectively; the data showed that the contraction speed of PL muscle was intermediate between that of the soleus and EDL muscles. In the case of soleus, more than 75% of the cross-sectional area was occupied by type 1 (slow) fibres; in both EDL and PL muscles more than 90% of the area was occupied by type 2 (fast fibres). However, the two fast muscles (EDL and PL) had different proportions of type 2B fibres; the area occupied by the type 2B fibre complement was less than 5% in PL, whereas it was around 70% in EDL muscle. The differences in shortening velocity and force–velocity relation among the three muscles could be explained on the basis of their respective muscle fibre-type compositions.

Introduction

The shortening velocity of a single muscle fibre is a characteristic property (Julian *et al.*, 1986) and is correlated with the rate of ATP hydrolysis by myosin contained within it (Bárány, 1967). Experiments on rat soleus muscle have shown that the shortening velocity of a whole muscle as determined from the force–velocity relation is some average function of the individual velocities of different muscle fibre types (Claflin & Faulkner, 1985, 1989). Histochemical staining of mammalian limb muscles reveal three main muscle fibre types (see Close, 1972), type 1, type 2A and type 2B, each containing a specific isoform of myosin heavy chain (Dalla Libera *et al.*, 1980). Additionally, experiments on skinned muscle fibres have shown differences in shortening velocity between these fibre types (Sweeney, *et al.*, 1986, 1988). These findings indicate that the contraction characteristics, or more specifically the shortening velocity and the force–velocity relation, of different muscles would be closely correlated with their histochemically-determined muscle fibre-type compositions.

Contraction characteristics of the soleus and extensor digitorum longus (EDL) in the rat hind limb have been examined in considerable detail in a number of previous studies (Close, 1964, 1969; Ranatunga, 1977, 1982). These studies have shown on the basis of measurements such as twitch contraction time, rate of rise of tetanic tension and shortening velocity, that the contraction speed of EDL is 2–3 times higher than in soleus muscle. Such a difference is expected on the basis that soleus contains a predominant (about 80%) type 1 fibre (slow) complement, whereas EDL contains a predominant type 2 fibre (fast) component (see Close, 1972). We report in this paper the contraction characteristics and the histochemical composition of soleus, EDL and a third hind limb muscle (peroneus longus) in the rat. It is found that the three muscles have significantly different contraction speeds (including shortening velocity) as well as different histochemical muscle fibre type compositions. A simulation model developed in an attempt to correlate the histochemical fibre-type composition of a muscle with its shortening velocity and force–velocity relation is presented. Some preliminary results from this study have been briefly reported (Ranatunga & Thomas, 1988, 1989).

Materials and methods

Contraction characteristics and histochemical muscle fibre type composition were determined for soleus (slow-twitch), peroneus longus (PL) and extensor digitorum longus (EDL) (fast-twitch) muscles in the hind leg of ten-week-old male Wistar rats.

* Author to whom correspondence should be sent.

Rats were anaesthetized by intraperitoneal injection (60 mg kg^{-1} body-weight) of sodium pentobarbitone (Sagatal® May & Baker Ltd.) and were maintained under deep anaesthesia throughout the experiments.

Contraction recording

The three muscles and their nerves were prepared for *in situ* contraction recording as previously described for cat muscles by Buller and Lewis (1965). The muscles and other exposed tissues were immersed in a pool of paraffin maintained at 35 ± 0.5° C by a thermostatically-controlled heating lamp. The distal muscle tendon was connected to an aluminium lever designed to record both muscle tension and shortening in either isometric or isotonic mode (see Ranatunga & Thomas, 1988). The overall design of the lever system was basically similar to the one used for *in vitro* experiments (see Ranatunga, 1982), and consisted of two vertical levers. The movement of the primary (aluminium) lever (72 mm long, equivalent mass 0.3 g) was monitored photoelectrically (Ranatunga, 1984) and recorded as muscle shortening. Isometric contractions were recorded with the primary lever locked in vertical position by means of a screw; the compliance associated with the system allowed less than 2% shortening of muscle fibre length during an isometric tetanus. For isotonic contractions, the primary lever was afterloaded via a secondary (titanium) lever (combined lever ratio of 16:1) using a series of metal springs (Ranatunga, 1982). Tension developed by muscles during either isometric or isotonic contractions was monitored by two strain gauge elements (foil type, RS components Ltd.) bonded to the primary lever near its base.

Experimental procedure

Isometric twitch and tetanic contractions were first recorded at optimal muscle length and with supramaximal nerve stimuli (stimulus duration was 0.05 ms). In the case of tetanic contractions, the duration of the tetanic train was 200 ms and the frequency was adjusted to give the maximal rate of tension rise as monitored by the differentiated tension record. This stimulus frequency (usually 300–400 Hz) was then used for recording isotonic shortening although the train duration was reduced to 100 ms to minimize muscle fatigue. Two series of isotonic contractions under different afterloads (total 15–20) were recorded in order to determine the force–velocity relation of a muscle. The interval between two tetanic contractions was longer than 3 min. At the end of each series of such contractions, the isometric tetanic tension (*Po*) was checked and if *Po* was found to be reduced to less than 90% of its original value the preceding data were rejected. The mean ± standard error of the mean (SEM), percentage tetanic tension recorded last in an experiment was 97.5 ± 3.1 (n = 8) for soleus, 96.9 ± 4.4 (n = 4) for EDL and 98.9 ± 3.7 (n = 5) for PL muscle. The measured velocities and isotonic tensions, within the force range of 5–50% of maximal isometric force (*Po*), were fitted with the A. V. Hill (1938) equation by a non-linear least squares technique (see Ranatunga, 1984). The force range of 5–50% *Po* was used in curve fitting so as to be comparable with previous studies on mammalian muscles (Ranatunga, 1982, 1984; Claflin & Faulkner, 1985, 1989).

The muscle length used for contraction recording was measured *in situ* before its removal from the animal. The isolated muscle, pinned at that muscle length, was examined under a dissecting microscope to estimate the muscle fibre length; the average of three measurements was taken. The muscle was then blotted on filter paper and weighed. The fibre length and the wet muscle weight were used in determining muscle cross-sectional area for specific tension calculations.

Histochemistry

At the end of an experiment, each muscle was pinned at optimal length to a cork board, frozen in isopentane cooled by liquid nitrogen and stored at −70° C. Frozen sections (10 μm) were stained for myosin ATPase (Guth & Samaha, 1970). In alkaline (pH 10.4) preincubated sections, fibres were classified as type 1 (pale staining) or type 2 (dark staining); in acid (pH 4.4) preincubated sections, fibres were classified as type 1 (dark staining), type 2A (pale staining) or type 2B (intermediate staining) (Brooke & Kaiser, 1970).

The fibres identified above as type 2B may include a subset, named type 2X (see Schiaffino *et al.*, 1986) and more recently it has been shown that type 2X has a shortening velocity considerably lower than type 2B (Schiaffino *et al.*, 1988). Therefore, three EDL and three PL muscles were additionally processed (pH 10.5) to identify the type 2X component (dark staining) from type 2B proper (pale staining); it is known that soleus contains very few or no type 2B fibres (Brooke & Kaiser, 1974; Results).

Fibre-type composition of a muscle was determined from counts of all the muscle fibres falling on the major diameter and its orthogonal in a full section. In three muscles, a cross-section through the mid region contained between 2400 and 4000 fibres, and the above method involved counting of 100–200 fibres. In two soleus, two PL and one EDL sections, and in sections of a number of other muscles not reported here, the fibre composition was determined by counting all the fibres in a full section as well as by the above method; the comparison from a total of 15 sections showed that the percentage of a fibre type estimated by the two methods was within ±10% of each other. In three sections, the cross-sectional area of different fibre types was estimated by measuring the major diameter (d_1) and its orthogonal (d_2) in 40–50 fibres and calculating the area as $\pi \cdot d_1 \cdot d_2/4$. These average area values for fibre types in different muscles were used in converting fibre-type number to the area occupied by a fibre type in a cross-section.

Model

A simulation model was developed to examine whether the differences in the force–velocity relation among the three muscles could be accounted for on the basis of the differences in their histochemical fibre-type composition. The main features of the model are:

1. The cross-sectional area occupied by a fibre type was taken to be directly proportional to its contribution to muscle force. The underlying assumption was that different fibre types have similar specific tensions; indeed, there is no strong evidence to the contrary (see Close, 1972; Ranatunga, 1984; Sweeney *et al.*, 1986, 1988; Lucas *et al.*, 1987; Greaser *et al.*, 1988).
2. Characteristic relative maximum shortening velocities (*Vmax*) of 1, 2.25 and 4.5 were assigned for type 1, type 2A and type 2B muscle fibre types, respectively. Such data are not available for rat muscle fibres; the particular *Vmax* values are based on unloaded shortening velocities (*Vo*) of skinned rabbit muscle fibres (Sweeney *et al.*, 1988) after normalization to

type 1 *Vmax*. *Vmax* of type 2X fibre was taken to be the same as for type 2A on the basis of the findings of Schiaffino and co-workers (1988).

3. The force–velocity relation for each muscle fibre type was assumed to be a hyperbola as described by the Hill (1938) equation:

$$(P + a)(V + b) = (Po + a)b,$$

where P represents force, Po maximum isometric force, V velocity of shortening and a and b constants with dimensions of force and velocity, respectively. The a/*Po* ratios used in the model were 0.27 for type 1 and 0.44 for type 2 fibres. A lower ratio indicates a greater curvature and this curvature is higher in slow twitch muscles (containing type 1 fibres) than in fast twitch muscles (containing type 2 fibres) (Wells, 1965; Close, 1969; Ranatunga, 1982, 1984; Elmubarak & Ranatunga, 1988). A greater curvature can arise from fibre heterogeneity of a muscle (see Josephson & Edman, 1988; present model), but a greater curvature was observed even in cat soleus muscle (Buller & Pope, 1977; Buller *et al.*, 1987) which is known to be a homogenous slow muscle (see Close, 1972). A lower a/*Po* ratio was assigned, therefore, for type 1 fibres than for type 2 fibres: the particular values were chosen so as to satisfy certain specific aspects of the force–velocity relation of rat soleus muscle (see Results).

4. The method of determining the composite force–velocity relation of a muscle from its individual components was similar to that described recently by Claflin and Faulkner (1989) and Josephson and Edman (1988). Using Hill's equation, with specifically assigned *Vmax* and a/*Po* ratio, the force contributions of different muscle fibre types were calculated and summed at each of a series of shortening velocities; if *Vmax* of a fibre type was lower than the shortening velocity under consideration, its contribution to muscle force was taken to be zero. The resultant force and velocity data (force range 5–50% *Po*, n = 15–22) were fitted to Hill's equation as described for experimentally determined data of a muscle.

It is clear from the above description that the model is based on several other assumptions. They include:

(1) All three muscles have a parallel fibre arrangement. In two animals we determined the muscle fibre alignment in the three muscles by microscopical examination. In agreement with Close (1964), the angle of fibre alignment to the axis of muscle action was small ($< 10°$) in both soleus and EDL muscles; the estimates made for PL muscle were not much different ($< 15°$). Such differences would only account for $< 5\%$ difference in shortening velocity between the muscles (Sacks & Roy, 1982);

(2) All the muscle fibres in a given muscle are equal in length. The variability in fibre length has been found to be small in a given muscle (see Close, 1964; Elmubarak & Ranatunga, 1984);

(3) Muscle fibres of a particular histochemical type are uniform with respect to shortening velocity in the three muscles. This is not fully supported by experimental evidence from other muscles (Sweeney *et al.*, 1986; see Greaser *et al.*, 1988). Basically a dearth of direct results from rat single muscle fibres made this simple assumption necessary; and (4) In the present study, the shortening velocities were estimated from the initial phase of after-loaded isotonic contractions; this is the common method used for *in situ* experiments on relatively large mammalian muscles (Close, 1964; 1969; Buller & Pope, 1977; Buller *et al.*, 1987). The major criticism of the method is that the shortening velocity may be limited by the degree of muscle fibre activation, as found in single frog muscle fibres (Cecchi *et al.*, 1978) and isotonic release from tetanic tension plateau (Jewell & Wilkie, 1958) would be more appropriate. However, at least for rat muscles, the two methods yield similar velocity data when using relatively high tetanic stimulation frequencies, as used here (Ranatunga, 1982; Claflin & Faulkner, 1989).

All computations were made on a BBC B computer using software written in Basic.

Results

Isometric contractions

Figure 1 shows isometric contractions of the three muscles from one rat, where twitch contractions are shown on the left and the tetanic contractions on the right. It is seen that the duration of the twitch contraction is longest in soleus and shortest in EDL; that of PL has an intermediate duration. The pooled data for time to peak and time to half-relaxation measured from twitch contractions in a number of animals are given in Table 1; the difference in the twitch duration seen among the three muscles is clearly illustrated by these data.

Examination of the tetanic contractions in Fig. 1 indicates that the rising phase of the tetanus is also different in the three muscles. The positive peak of the differentiated tension record was measured from such

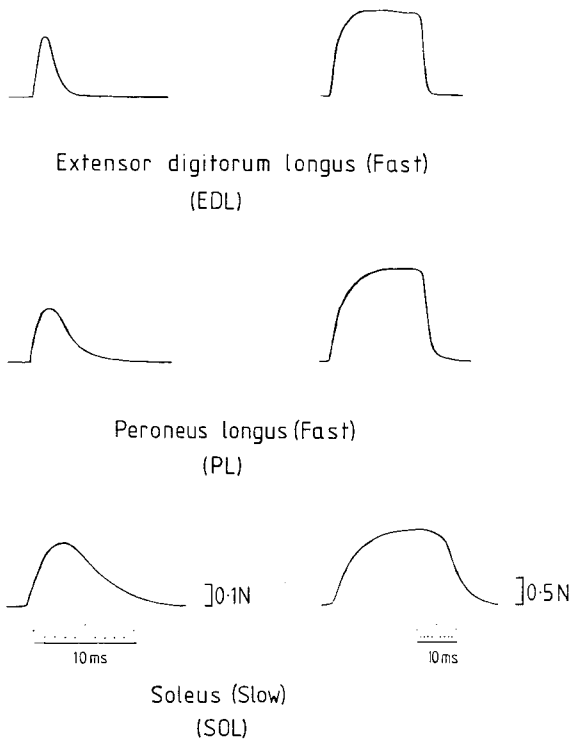

Fig. 1. Isometric contractions of the three muscles from one rat. Isometric twitch contractions are shown on the left and tetanic contractions on the right. Note that on the basis of twitch duration and rate of tetanic tension rise, the contraction speed of PL is intermediate between soleus and EDL.

Table 1. Physiological contraction parameters measured at 35 °C: isometric contraction characteristics.

	Twitch time to peak (ms)	*Twitch time to half relaxation (ms)*	*Rate of tension rise (Po/s)**	*Twitch/tetanus tension ratio*	*Specific tension ($kN\ m^{-2}$)*
Soleus: mean	34.1	40.3	19.1	0.21	129
±SEM	1.0	1.0	1.7	0.01	5.2
n	9	9	8	8	9
PL: mean	17.5	16.4	31.2	0.2	144
±SEM	0.4	0.9	2.1	0.02	23
n	8	8	6	8	8
EDL: mean	14.5	14.3	50.7	0.23	132
±SEM	1.3	1.9	1.5	0.02	9.5
n	4	4	4	4	4

* *Po* = maximum tetanic tension.

contractions (Buller & Lewis, 1965), and normalized with respect to tetanic tension (*Po*). Taking this as in index of the rate of tetanic tension rise, the data shows (see Table 1) that the rate is approximately 20 *Po*/s in soleus and 50 *Po*/s in EDL; the rate is intermediate (30 *Po/s*) in PL muscle. Data in Table 1 also show that the three muscles have similar twitch: tetanus tension ratios and specific tensions.

Force–velocity relation

Figure 2a shows a pair of shortening and tension records from each muscle at a tension level of 9–11% *Po*; the records are from the same animal. It was consistently found that the shortening velocity of PL was intermediate between those of soleus and EDL muscles. Figure 2b shows force–velocity plots of the three muscles from another animal, where the curves represent the Hill (1938) equation fitted to each set of points. The three muscles clearly have different shortening velocities over a wide range of relative tensions. Figure 2c illustrates the complete range of force–velocity curves obtained in the study from a total of eight soleus, four EDL and five PL muscles; the data show that whereas there is some variability in each muscle type, the distinction among the three muscles remains clear even in this presentation.

The pooled data for the force–velocity curves are given in Table 2. When represented in muscle fibre length per second, the maximum shortening velocities were approximately 7, 12 and 20 for soleus, PL and EDL muscles, respectively. Similar differences were seen when the velocities actually measured at the same relative isotonic tension (9–11% *Po*) were compared. The *a*/*Po* ratio was lowest in soleus and highest in PL muscle.

In a given animal, the average muscle fibre length was longer and the muscle fibre length/muscle length ratio higher in soleus than in EDL (Ranatunga, 1982; Elmubarak & Ranatunga, 1988). With respect to both measurements, the PL was similar to EDL muscle (see Table 2).

Histochemical composition

As determined by alkaline preincubation, the type 2/type 1 percentage ratio was 30/70 for soleus and 94/6 for EDL muscle. These data are in good agreement with other studies on Wistar rats and especially those in which all the fibres in a section were counted (see review by Eddinger *et al.*, 1985). PL contained predominantly type 2 fibres; the average type 2/type 1 percentage ratio was 87/13. The percentage of different muscle fibre types determined after acidic preincubation showed that most of the type 2 fibres were type 2A in soleus, whereas they were type 2B in EDL. Thus soleus contained around 25% type 2A and 70% type 1 fibres and EDL contained 70% type 2B, 20% type 2A and 10% type 1 fibres. The composition of PL muscle was clearly different from either soleus or EDL muscle; it had about 45% type 2B, 40% type 2A and 15% type 1 fibres. Figure 3 shows representative areas from EDL and PL muscles illustrating the different proportions of type 2B/type 2A in them.

In determining the fibre-type composition in a given muscle, some consideration should be given to possible variation along muscle length. Since different studies have given very similar fibre-type compositions for soleus and EDL muscles, it may be assumed that their fibre-type composition does not vary significantly along the longitudinal axis. In PL muscle, we investigated this aspect by taking four sections along its length. The full range of percentage fibre number obtained from all the sections (n = 12, three muscles) was 36–54% for type 2B, 28–51% for type 2A and 6–21% for type 1; a high percentage of type 2A fibres was evident in all the sections. The data given in Tables 3 and 4 were based on average estimates of fibre numbers from two sections taken from the proximal and distal halves of a muscle.

Table 3 gives pooled data for the fibre-type compositions of the three muscles after correction for the differences in fibre cross-sectional area (see Materials and

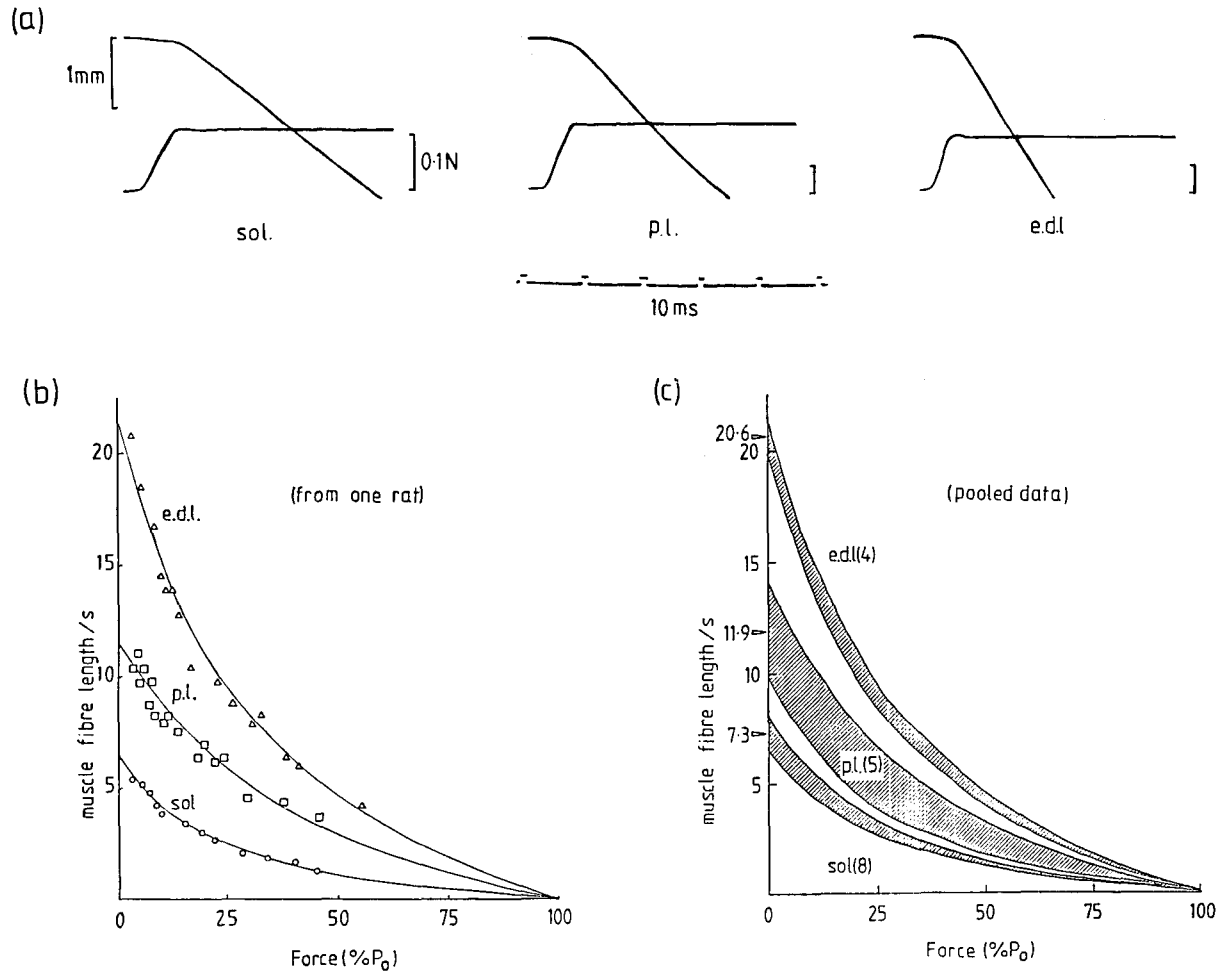

Fig. 2. (a) Oscilloscope records of isotonic contractions recorded at 9–11 % *Po* for soleus (sol), (PL) and (EDL) muscles from one animal. In each case the upper trace represents lever movement (downward displacement indicating muscle shortening) and the lower trace is the tension record. (b) Force–velocity plots for each of the three muscles from another animal. Shortening velocity in muscle fibre lengths per second is plotted in the ordinate, against the isotonic tension as a percentage of *Po* in the abscissa. The curves represent Hill's (1938) equation fitted to data points. The three muscles have clearly separate force–velocity relations. (c) Full range of force–velocity curves for each of the three muscles. Even presented in this manner a clear distinction remains between the muscles.

Table 2. Physiological contraction parameters measured at 35 °C: force–velocity characteristics.

	Vmax (*m.f.l./s*)	*V*(10% *Po*) (*m.f.l./s*)	*a/Po*	*Muscle fibre length* (*mm*)	*Muscle fibre length/ muscle length ratio*
Soleus: mean	7.3	4.5	0.26	14.9	0.52
±SEM	0.3	0.2	0.03	0.4	0.02
n	8	8	8	8	8
PL: mean	11.9	8.2	0.35	10.6	0.42
±SEM	0.7	0.7	0.03	0.4	0.02
n	5	5	5	5	5
EDL: mean	20.6	13.3	0.31	11.5	0.41
±SEM	0.3	0.5	0.02	0.3	0.01
n	4	4	4	4	4

Po = maximum tetanic tension; *Vmax* = maximum shortening velocity obtained by extrapolation of Hill's curve to zero force; *V*(10% *Po*) = measured shortening velocity at 9–11% *Po*.

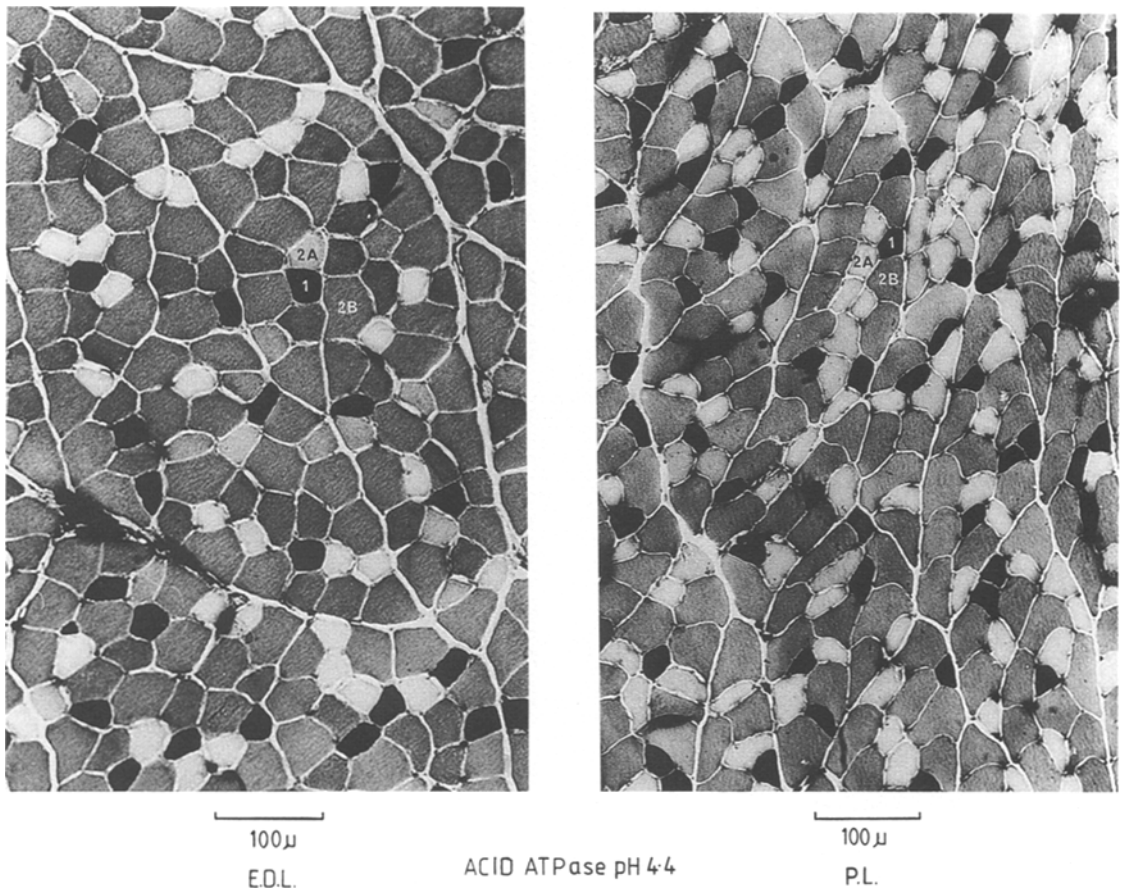

Fig. 3. Muscle fibre type composition in EDL and PL muscles. Representative area of each muscle is shown. The sections were preincubated in acid (pH 4.4) and stained for myosin ATPase. The dark fibres were identified as type 1, the unstained as type 2A and others as type 2B; note that PL muscle has more type 2A fibres.

methods); thus, the mean values given in Table 3 represent the areas occupied by different muscle fibre types in cross-sections of the three muscles. Our data show that, in a soleus cross-section, 77% area is occupied by type 1 fibres, 20% by type 2A fibres and a small area of 3% by another subset of type 2 fibres. Type 2B fibres are evidently not found in rat soleus (Brooke & Kaiser, 1974) and this small population has been classified as type 2C (Brooke *et al.*, 1971). In the model below this small component of fibres in soleus was included in type 2A category; indeed the motor unit studies also have recognized only two groups of fibres in soleus (Close, 1967). Results also show that in both EDL and PL the area

Table 3. Histochemical composition: fibre-type composition determined after preincubation at pH 4.4.

	Type 1	*Type 2A*	*Type 2B*
Soleus: mean	77.2	19.5	3.3*
±SEM	4.2	2.1	0.8
n	6	6	6
PL: mean	9.0	29.7	61.3
±SEM	0.9	2.2	3.7
n	5	5	5
EDL: mean	5.9	9.3	84.8
±SEM	0.4	0.4	2.0
n	3	3	3

* Note that in soleus these fibres are identified as type 2C. Values represent the percentage cross-sectional area occupied by a fibre type.

Table 4. Histochemical composition: fibre-type composition of fast muscles after subdividing the type 2B component into type 2X and type 2B proper.

	Type 1	*Type 2A*	*Type 2X*	*Type 2B*
PL: mean	9.0	29.7	57.2	4.1
±SEM	0.9	2.2	3.4	0.3
n	5	5	5	5
EDL: mean	5.9	9.3	14.7	70.1
±SEM	0.4	0.4	0.4	1.6
n	3	3	3	3

Values represent the percentage cross-sectional area occupied by a fibre type. Note that the predominant fibre type is type 2B in EDL and type 2A + 2X in PL muscle.

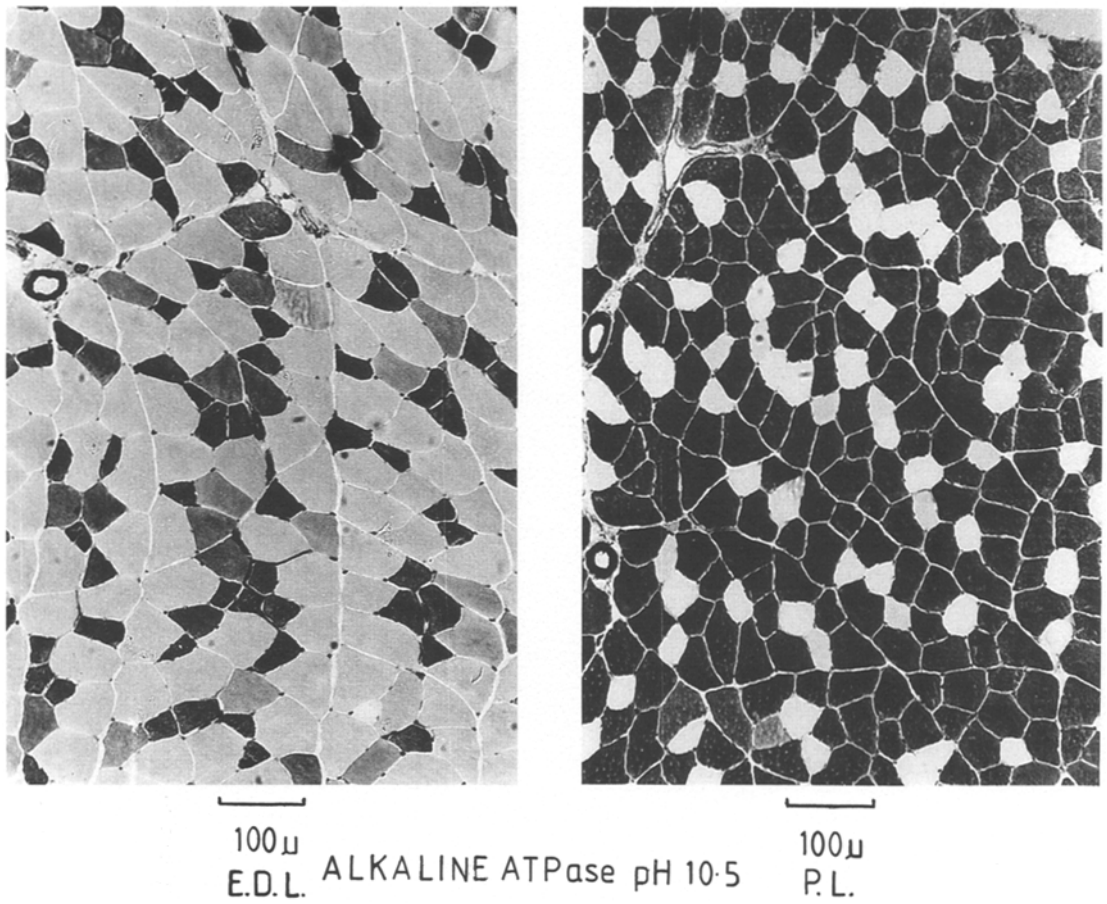

Fig. 4. Muscle fibre-type composition in EDL and PL muscles after high alkaline preincubation (pH 10.5). Pale or unstained fibres represent type 2B (proper) and type 1, whereas the dark fibres are other subsets of type 2 (type 2A and type 2X). The PL muscle contains a predominant type 2A + 2X component.

occupied by the type 1 fibres is less than 10%. Additionally, although there is a clear difference in the actual proportions, the predominant fibre type in both muscles is type 2B. The large difference in shortening velocity observed between the two fast muscles could not be readily explained on this basis.

After preincubation at pH 10.5, PL sections contained around 80% dark staining fibres whereas EDL sections contained only about 30% of such fibres, the majority of fibres in EDL being pale staining type 2B (and type 1) fibres. The clear difference seen between the two muscles under these conditions is illustrated by the sections shown in Fig. 4. On comparison with the proportion of type 2A fibres (determined after acid preincubation), it became clear that most of the fibres identified above as type 2B in PL muscle, in fact, were type 2X fibres. Table 4 gives fibre-type compositions of the two fast muscles after the type 2B category in Table 3 was subdivided into type 2X and type 2B (proper).

Simulated force–velocity relations

Details of the model are given in Materials and Methods. Soleus was taken to consist of two fibre types (1 and 2A), whereas both EDL and PL were taken to contain three muscle fibre types (1, 2A + 2X and 2B). The force–velocity curves assigned to different fibre types are shown in Fig. 5a; in addition to different shortening velocities, type 1 and type 2 fibres were given different a/*Po* ratios of 0.27 and 0.44, respectively. The particular curves in Fig. 5a were considered appropriate because using them the model could satisfactorily simulate the experimental force–velocity relation of the soleus muscle. Figure 5b shows the two component curves for the fibre types in soleus, drawn appropriately scaled with respect to the mean cross-sectional areas (from Table 3). The symbols represent the computed values for the composite force–velocity relation and the curve drawn through the symbols represent Hill's equation (1938) fitted to velocities within the range of 5–50% cross-sectional area (= *Po*), as done for experimental data. The *Vmax* (extrapolated) of the composite curve is 1.4 and its a/*Po* ratio is 0.23. These predicted data were considered satisfactory on the following grounds. First, *Vo* (*Vmax* of fastest fibres) in the model is 2.25, so that the ratio *Vo*/*Vmax* for model soleus is 1.6; this is the same as that found experimentally by Claflin and Faulkner (1989) for soleus muscle. Second, the a/*Po*

ratio of the model (0.23) is within the range (0.2–0.26) obtained for rat soleus muscle at 35° C (Ranatunga, 1982, 1984; Elmubarak & Ranatunga, 1988; present study).

The force–velocity curves for EDL and PL muscles were computed using the same fibre type force–velocity curves as used in the soleus model and taking the fibre-type compositions given in Table 4. The *Vmax* and a/*Po* ratios derived from these computations are plotted against the experimentally determined data in Fig. 6a (a/*Po* ratio) and Fig. 6 (*Vmax*). The horizontal bars represent ± SEM associated with experimental values and the vertical bars indicate the range predicted when ± SEM associated with fibre-type composition, were used in the calculations. The relative *Vmax* values were first converted into muscle fibre length per second using the experimental and the model data of soleus and the individual values for muscle fibre types are indicated by arrows on the ordinates. The plots show the general agreement for parameters of the force velocity relation obtained with these simulations.

Discussion

Our results from physiological contraction measurements showed distinct differences between the soleus, PL and EDL muscles in the rat. The data from soleus and EDL muscles were similar to those reported in many previous studies (see Elmubarak & Ranatunga, 1988). In contrast, the contraction speed of PL muscle, including its shortening speed, was intermediate between soleus and EDL muscles. Since both EDL and PL muscles contain predominantly type 2 (fast) fibres, differences between their contraction speeds are of particular interest. In whole-muscle recording, differences in twitch duration and rate of tension rise can result from differences in series elasticity (Hill, 1949). However, both EDL and PL muscles had similar muscle fibre lengths and fibre length/muscle length ratios indicating similar series elasticity. Moreover, their differences in shortening velocity cannot be accounted for on that basis. Additionally, the fibre arrangement with respect to the longitudinal axis was not sufficiently different to explain the different shortening velocities. Therefore the differences in the contraction speed observed among the muscles must reflect their different muscle fibre type composition.

Determination of the histochemical muscle fibre-type composition by conventional techniques clearly showed differences among soleus, EDL and PL muscles. Although each muscle was mixed in fibre type, the predominant

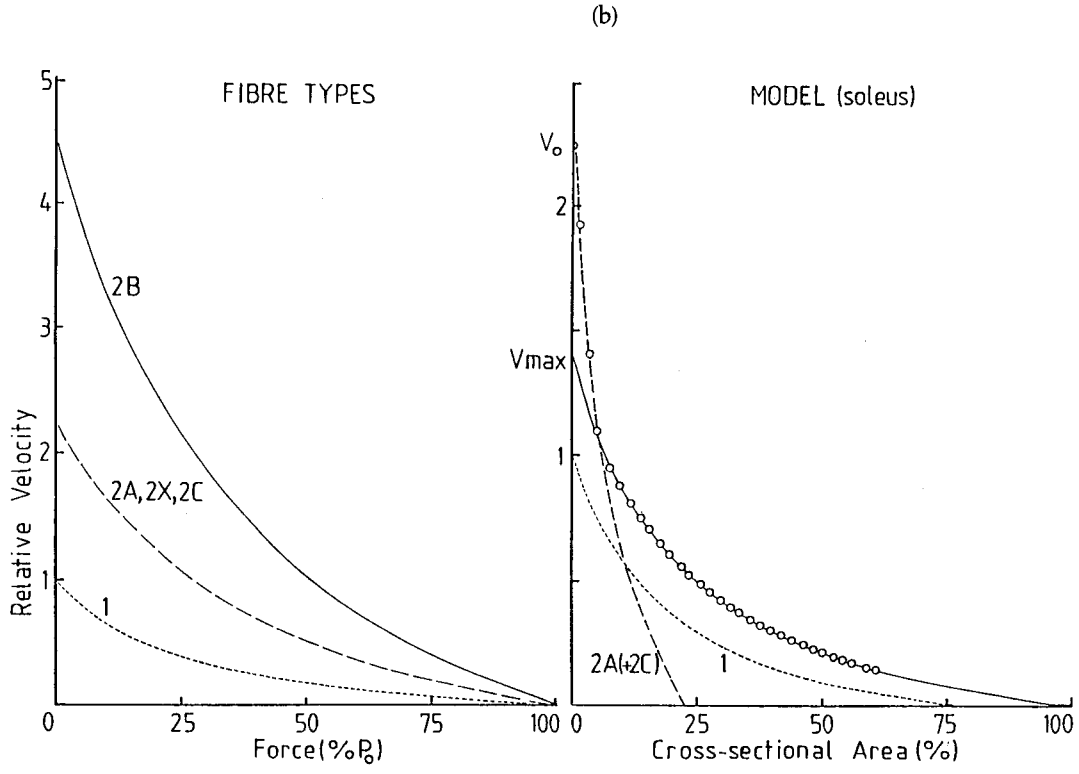

Fig. 5. (a) Component force–velocity curves used in the model for different fibre types. Relative *Vmax* values of 1, 4.5 and 2.25 were assigned to type 1, type 2B and other subsets of type 2, respectively; type 1 fibres were given an a/*Po* ratio of 0.27 and type 2 fibres 0.44. The curves were drawn fitting these values to Hill's equation (1938). (b) Component and the composite force–velocity curves of the model soleus muscle. The component curves are scaled according to the mean cross-sectional area occupied by the fibre types (from Table 3). ○—○, computed data for the composite curve of the muscle. The curve drawn through the symbols represents Hill's equation (1938) fitted to the velocities (n = 15–25) between 5–50 % *Po* (cross-sectional area) using a non-linear least squares technique. The curve for model soleus is characterized by a/*Po* ratio of 0.23 and *Vo*/*Vmax* ratio of 1.6; the values are similar to those experimentally determined for rat soleus muscle.

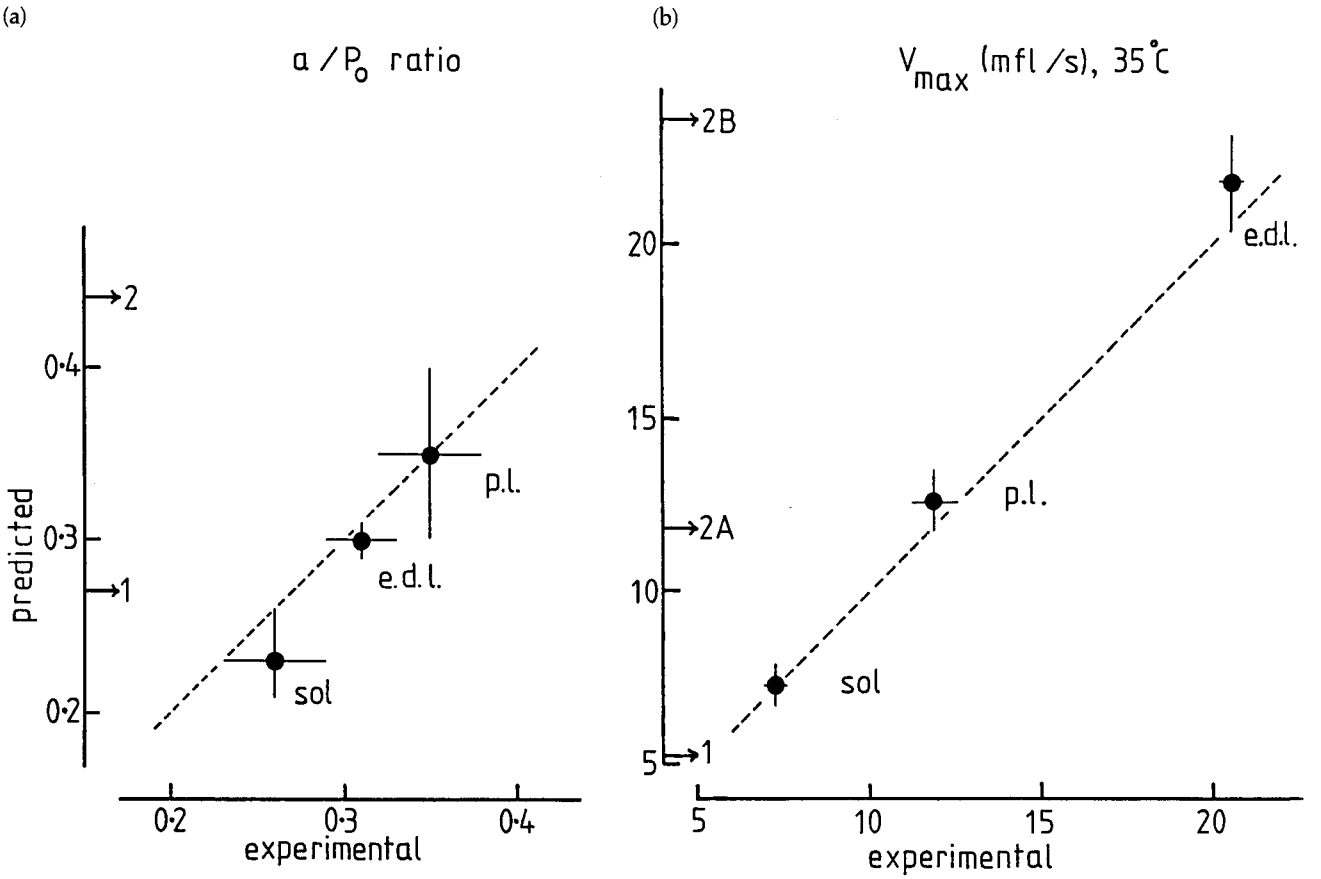

Fig. 6. Comparison of the model predictions with experimental data; dotted lines represent exact correlation. (a) Values of a/*Po* ratio (Hill's 1938 equation) predicted by the model (ordinate) are plotted against those experimentally determined in the present study. Note that a/*Po* ratios for type 1 and type 2 fibres (arrows on the ordinate) were fixed so as to obtain soleus muscle a/*Po* ratio and *Vmax* (Fig. 5b), and those for PL and EDL were computed using these values. (b) Predicted *Vmax* values plotted against the experimental values; each *Vmax* value was obtained by extrapolation to zero load of a curve fitted to data between similar (5–50 % *Po*) force range. The velocities assigned to each fibre type are indicated by the arrows on the ordinate. Note that predicted *Vmax* values were converted to muscle fibre lengths/s using soleus data.

fibre type (> 70%) was type 1 in soleus, type 2B in EDL and type 2A + 2X in PL muscle. The fibre-type compositions of soleus and EDL were generally comparable with those reported in previous studies. The PL muscle is one of four muscles which are often grouped together as peroneal muscles, and the study by Armstrong and Phelps (1984) showed a high proportion (around 40%) of type 2A fibres in the rat peroneal muscles. A similar proportion of type 2A fibres was found in cat PL muscle (see Donselaar *et al.*, 1987). Identification of type 2X was not made in either study but their values for type 2A component compare well with that obtained in the present study. Our results also show that rat PL muscle contains a large component of type 2X fibres, occupying > 50% of its cross-section (see Tables 3 and 4).

The fact that there is a general correlation between histochemical fibre type and other physiological properties (e.g. twitch contraction time, fatiguability, etc.) has been known from motor unit studies (see Burke *et al.*, 1973). Indeed, our data from isometric contraction measurements also show differences (see Tables 1 and 2) which may be related to different fibre-type compositions. It may be argued that a more direct physiological correlate of a histochemical fibre type is the shortening velocity, since shortening velocity is determined by myosin ATPase activity (Bárány, 1967). Therefore, a model was used to test whether the observed differences in average histochemical composition of the muscles could predict the major differences in their force–velocity relations. The model predictions were satisfactory (see Fig. 6). For example, taking the maximum shortening velocity extrapolated from the force–velocity relation and the velocity measured at 10% *Po* (Tables 1 and 2), the soleus : PL : EDL shortening velocity ratio was 1 : 1.6–1.8 : 2.8–3.0; the ratio predicted by the model was similar (1:1.7:3.1). Also, a/*Po* ratio increased (or the curvature of the force–velocity relation decreased), in sequence, from soleus–EDL–PL muscles in both the experimental data and the model.

There are a number of associated implications which may be used to check the validity of the model. Firstly, the maximum shortening velocities of fibre types in rat

muscles should have differences comparable with those in rabbit muscles; calculations showed that when corrected for differences in temperature (from Ranatunga, 1984), the velocities of rat fibres would be 50–60% higher than those measured from rabbit fibres (Sweeney *et al.*, 1988). Second, the force–velocity relation of type 1 fibres would be more curved (because of their smaller a/*Po* ratio) than that of type 2 fibres. Third, the ratio of unloaded shortening velocity/maximum shortening velocity extrapolated from the force–velocity relation would be high (1.9) for PL (4.5/2.4) and low for EDL muscle (1.1); this ratio for soleus has been experimentally determined to be 1.6 (Claflin & Faulkner, 1989). Additionally, whether a model of this type would be of value in comparing data from other muscles remains to be investigated.

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