Contractile properties of isolated muscle spindles of the frog

Edman, Paul; Radzyukevich, T; Kronborg, Britta

Published in:
Journal of Physiology

DOI:
10.1113/jphysiol.2001.016220

2002

Journal of Physiology (2002), **541.3**, *pp.* 905–916
DOI: 10.1113/jphysiol.2001.016220

www.jphysiol.org

Contractile properties of isolated muscle spindles of the frog

K. A. P. Edman, T. Radzyukevich and B. Kronborg

Department of Physiological Sciences, Biomedical Centre, F11, University of Lund, S-221 84 Lund, Sweden

Force and isotonic shortening velocities were studied (0.6–4.0 °C) in isolated single muscle spindles from the anterior tibialis muscle of *Rana temporaria* using techniques that enabled measurements both from the spindle as a whole and from marked segments of the preparation. The force–velocity relationship during tetanic stimulation exhibited the same biphasic shape as previously described for extrafusal muscle fibres. However, the maximum speed of shortening of the spindle fibres was merely 0.95 ± 0.006 lengths s^{-1} (mean ± S.E.M., $n = 11$), which is approximately half the value recorded in extrafusal fibres of the same muscle. The maximum tetanic force, 91 ± 10 kN m^{-2}, $n = 14$, was likewise only approximately half that produced by extrafusal fibres. The force generated by the capsule segment was lower than that produced by the whole spindle resulting in elongation of the capsule region during a fixed-end tetanus. The intracellular calcium ion concentration reached during the plateau of the tetanus, 1.7 ± 0.1 μM ($n = 8$), was substantially lower than the value attained in extrafusal fibres under equivalent conditions. In accordance, the spindle fibres did not become fully activated during supramaximal electrical stimulation as indicated by the finding that the tetanic force could be further increased by 16.6 ± 0.04 % ($n = 5$) on addition of 0.5 mM caffeine. Inadequate activation may thus, to a certain extent, account for the relatively low force per cross-sectional area of the spindle fibres. The contractile properties of the intrafusal fibres should make the spindle organ suited to provide feedback control during eccentric (forced lengthening) and static (isometric) contractions and, with reduced effectiveness, during slow muscle shortening.

(Received 23 December 2001; accepted after revision 30 March 2002)
Corresponding author K. A. P. Edman: Department of Physiological Sciences, Biomedical Centre, F11, University of Lund, S-221 84 Lund, Sweden. Email: paul.edman@farm.lu.se

Muscle spindles exist in relatively large numbers in vertebrate skeletal muscles and are thought to play an essential part in controlling the mechanical activity of the muscles *in vivo* (e.g. Stein, 1974; Hunt, 1990; Walro & Kucera, 1999). The spindle has a neural portion, the stretch receptor, which is functionally integrated with a bundle of very thin intrafusal muscle fibres. Activation of the intrafusal fibres (via γ-motoneurons) leads to strain of the sensory region and, by reflex action mediated by the spindle afferents, to increased α-motoneuron activity and stimulation of the regular (extrafusal) muscle fibres. As the muscle shortens and the spindle becomes unloaded, the feedback output from the stretch receptor ceases.

The contractile performance of the intrafusal fibres can be presumed to be of vital importance for the function of the muscle spindle, as the speed of shortening of the spindle fibres, at the various loads encountered, will determine how effectively the spindle is able to uphold its control of the muscle activity. Although measurements of force and shortening velocity have been performed on isolated muscle spindles in the past (e.g. Smith, 1964; Diete-Spiff, 1967; Jahn, 1968; Boyd, 1976; Fukami, 1984), it is unclear whether the contractile properties of the spindle fibres are significantly different from those of the regular muscle fibres. Since knowledge on this point is essential to evaluate the physiological role of the muscle spindle, a detailed study of the contractile properties has now been carried out on isolated muscle spindles of the frog. With the techniques used it has been possible to measure active force and shortening velocity both from the muscle spindle as a whole and from short marked segments along the intact spindle, also including measurements from the capsule region. The results demonstrate that the force–velocity relationship of the intrafusal muscle fibres has the same general features as previously described for extrafusal fibres (Edman, 1988) exhibiting a characteristic biphasic shape with an inflexion point near 80 % of maximum tetanic force. There are, however, marked quantitative differences in function between the intra- and extrafusal fibres in that the spindle fibres are found to shorten at a considerably lower velocity and to produce a much lower force per unit cross-sectional area than the regular muscle fibres. Some of the results have been presented in a preliminary form (Edman *et al.* 2000).

METHODS

Preparation and mounting

The experiments were performed according to procedures approved by the Animals Ethics Committee of the University of Lund. Single muscle spindles were isolated from the anterior tibialis muscle of cold-adapted *Rana temporaria*. The frogs were killed by decapitation and subsequent destruction of the spinal cord. Care was taken to free the muscle spindle from adherent connective tissue along its entire length and to avoid undue stretching of the very thin fibre bundle during dissection. Only spindles forming one distinct bundle and having one capsule region were selected for these experiments (see Fig. 1). Using these criteria it was possible to isolate fully intact muscle spindles with no obvious damage to individual fibres. The spindles, dissected on the day of the experiment, were mounted horizontally between a force transducer and an electromagnetic puller in a temperature controlled Perspex chamber. Both tendons were provided with clips of aluminium foil for firm attachment to the hooks of the force transducer and the puller arm as previously described (Edman & Reggiani, 1984). The experiments were carried out just above slack length (sarcomere length ~2.3 μm, see later).

Solutions. The bathing solution had the following composition (mM): NaCl, 115.5; KCl, 2.0; $CaCl_2$, 1.8; Na_2HPO_4 + NaH_2PO_4, 2.0; pH 7.0. In one series of experiments 0.5 mM caffeine was added to the bathing fluid. The solution was pre-cooled and perfused through the muscle chamber (volume *ca* 2.5 ml) at a speed of approximately 2 ml min^{-1}. The bath temperature was constant to within 0.2 °C during any given experiment but varied between 0.6 and 4.0 °C (mean 1.9 ± 0.2 °C) among the different experiments. Fibre length and sarcomere length were determined as described by Edman & Reggiani (1984).

Cross-sectional area. After completion of the mechanical measurements the muscle spindles were transferred to an inverted microscope where the spindle, submerged in Ringer solution, was examined at × 500 magnification. The individual fibres could generally be identified in this way and their diameter measured at two or more places along their length by means of an ocular micrometer scale. A mean value of the diameter was determined for each fibre and the cross-sectional area was calculated assuming a circular shape. In nine spindles the 'effective' cross-sectional area of the spindle was estimated by summating the cross-sectional areas that were derived from the individual fibres. In five additional preparations the borders of the individual fibres could not be clearly identified. In these cases the smallest and largest diameters of the spindle outside the capsule region were measured after the spindle had been twisted one turn at slack length. The cross-sectional area of the spindle was calculated from the two diameters assuming that this area had an elliptical shape.

Stimulation

Rectangular pulses of 0.2 ms duration were passed between a pair of platinum plate electrodes that were placed symmetrically on either side of the muscle spindle approximately 2 mm from it. The stimulus strength was set approximately 15 % above the value required to produce maximal tetanic force. A train of pulses of appropriate frequency (15–20 Hz) was used to produce a fused tetanus of 1–2 s duration.

Force transducer

The principal unit of the force transducer was a semiconductor strain-gauge element (AE 801, Aksjeselskapet Mikroelektronikk, Horten, Norway). A thin extension (approximately 5 mm in length) made of wood was fixed by epoxy resin to the silicon bar outside the domain of the strain-gauge elements. The tip of the extended arm was provided with a hook of stainless steel wire (0.1 mm diameter) to be used for attachment of the muscle spindle (see above). The resonant frequency was approximately 2.0 kHz when the transducer was submerged in the bathing fluid.

Recording of intracellular Ca^{2+} transients

The Ca^{2+}-sensitive fluorescent dye fluo-3 (Minta *et al.* 1989) was used to monitor the intracellular Ca^{2+} transients during isometric twitch and tetanus of isolated muscle spindles. A description of the loading procedure and of the approach used for recording the fluo-3 signal has been described previously (Caputo *et al.* 1994). Briefly, the muscle spindle was immersed in Ringer solution containing about 20 μM fluo-3 AM (Molecular Probes Europe BV, Leiden, The Netherlands) for about 45 min at room temperature. After the preparation had been loaded with fluo-3, the dye was removed from the bath by perfusing the muscle chamber with ordinary Ringer solution for at least 20 min before experimentation. The muscle chamber was mounted on the stage of a Zeiss inverted microscope (Axiovert 35) equipped with an epi-fluorescence attachment. The light source was a 100 W Hg lamp driven by a stabilized power supply. The filter combination used for fluo-3 was (excitation/dichroic/barrier) 450–490 nm/510 nm/520 nm. A programmable shutter was used to illuminate the fibre only during recording of the light signal. The signal was collected from an area of about 0.9 mm which was kept constant during the experiment.

The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was calculated from the fluo-3 signal by taking account of the on- (k_+) and off- (k_-) rate constants for the Ca^{2+}–fluo-3 complex following the procedure described by Caputo *et al.* (1994). The numerical values of k_- and k_+ for fluo-3 in the myoplasm were chosen as described by Sun *et al.* (1996).

Measurements of length changes

An electromagnetic puller of the type described previously (Edman & Reggiani, 1984) was used to record changes in the overall length of the muscle spindle. The technique used for recording changes in length of discrete segments of the preparation was the same as that described by Edman & Lou (1990), which was an updated version of the technique described in detail by Edman & Reggiani (1984). Opaque markers that were cut from letter-press (size approximately 75 μm × 75 μm; mass approximately 0.1 μg) were attached to the upper surface of the spindle with the non-glue side of the letter-press material facing downwards, the distance between two adjacent markers (one segment) being 0.4–0.7 mm. Laser light was shone through the spindle and a magnified image of the spindle was projected onto a photodiode array (Fairchild CCD 133). The distance between two selected adjacent markers was read throughout a contraction with a time resolution of 40 μs. An analog circuit converted the output from the photodiode array to a signal proportional to the percentage change of the segment length. The accuracy of the measurement was better than 0.2 % of the segment's length.

In some experiments a selected segment was held at constant length throughout the tetanus period. This was achieved by adjusting the overall length of the preparation appropriately using the segment-length signal for feedback control of the electromagnetic puller.

Force–velocity relation

Measurements of the force–velocity relationship were performed as previously described for single extrafusal muscle fibres

(e.g. Edman, 1979, 1988). The muscle spindle was stimulated to produce a fused isometric tetanus at 3 min intervals, and the data collection was preceded by at least 10 such trials. In tetani with shortening, the spindle was released during the tetanus plateau to shorten against a preset load. This was achieved by rapidly changing the mode of operation of the electromagnetic puller from fibre-length control to force control. The preparation remained under force control for the remainder of the stimulation period.

Recording and measurement of data

The signals from the force transducer, the electromagnetic puller and the segment length recording were fed into an acquisition and analysis program (Labview, National Instruments, Austin, TX, USA) and stored on disks. For the analysis of the force–velocity relationship the slope of the digital length records was measured within the relatively straight portion of the record that occurred after the initial transient had been passed during the force-clamp manoeuvre. The slope was measured over a time interval of 40–100 ms, depending on the speed of shortening, using a computer program based on the least-squares method.

The force–velocity relation had two distinct curvatures with a breakpoint near $0.8P_0$, similar to the situation in regular (extrafusal) muscle fibres, and the biphasic equation previously described by Edman (1988) was used to fit the experimental data:

$$V = \frac{(P_0^* - P)b}{P + a}\left(1 - \frac{1}{1 + e^{-k_1(P - k_2P_0)}}\right). \quad (1)$$

V denotes the velocity of shortening, P the load on the muscle spindle and P_0 is the measured isometric force. The first term of the equation expresses the force–velocity relation below $0.8P_0$ and represents a rectangular hyperbola as originally described by Hill (1938). P_0^* is the isometric force predicted from this hyperbola and the constants a and b are constants with dimensions of force and velocity, respectively. The second term within parentheses (referred to as the 'correction term'; Edman, 1988) reduces V at high loads to fit the distinct, upward–concave curvature at loads greater than approximately $0.8P_0$. The constant k_1 in the correction term has the dimension of 1/force, whereas k_2 is dimensionless.

Statistics

Student's t test was used for the determination of statistical significance. All statistics are given as means ± S.E.M.

RESULTS

Arrangement of the muscle fibres in the spindle organ

The contractile portion of the frog spindle organ is made up of a bundle of striated muscle fibres that run the entire length between the two tendons of the muscle. The number of fibres in the spindle was generally found to be 4–6 with diameters varying from 4 to 25 μm in 10 preparations in which detailed measurements of the fibre diameters were performed (see Methods). The spindle fibres are thus considerably thinner than the regular, extrafusal muscle fibres. In fact, the entire width of the spindle is comparable to that of a thin extrafusal frog muscle fibre.

Figure 1 illustrates a typical spindle of the kind used in the present study, photographed while mounted in the test chamber. The capsule, containing the sensory organ, was found to be located in the mid-region of the spindle,

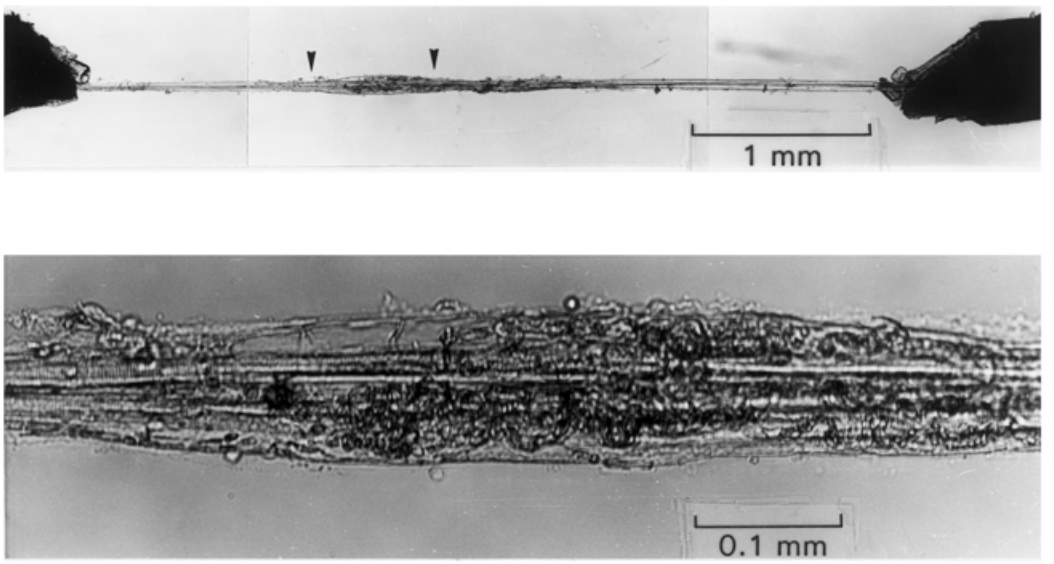

Figure 1. Representative example of muscle spindle isolated from the anterior tibialis muscle of *Rana temporaria*

The upper panel shows the spindle at rest length as mounted in the experimental chamber. The capsule is seen in the middle region of the spindle. The tendons have been trimmed and are provided with clips of aluminium foil (visible as a sharp contour on the right tendon approximately 0.5 mm from the fibre–tendon junction) for firm attachment to force transducer and puller arm. The lower panel is a magnified view of the capsule region (between arrows in upper panel) illustrating individual fibres of various thickness that pass through the capsule structure.

usually closer to one of the tendons, and the individual muscle fibres were found to pass through the capsule without any apparent change in width when inspected in a light microscope at × 500 magnification. In line with the view that each fibre spans the entire distance between the tendons, the same number of fibres could be identified on either side of the capsule. The fibres exhibited a regular sarcomere pattern outside the capsule region producing a distinct laser diffraction pattern like that derived from a single extrafusal muscle fibre (Cleworth & Edman, 1972). The striation pattern was less distinct in the capsule region and was obscured in several places by the abundant fibrous material that surrounded the fibres within the capsule. No systematic study of the sarcomere pattern in the various regions within the capsule was therefore feasible. However, measurements of the sarcomere spacing in rows of visible striations performed by direct microscopy, using an ocular micrometer scale at × 500 magnification (cf. Cleworth & Edman, 1972), did not reveal any obvious differences in sarcomere length between fibres inside and outside the capsule.

The sarcomere length measured outside the capsule region was close to 2.3 μm when the spindle was kept just taught. The 'slack' sarcomere length was thus slightly larger than that recorded in extrafusal fibres of the same muscle, which is generally 2.05–2.1 μm (Edman & Reggiani, 1984; also see Gordon *et al.* 1966).

With the supramaximal field stimulation used in the present study all muscle fibres within the spindle can be presumed to have contributed to the mechanical responses reported below. No bending of individual fibres that would indicate failure of activation was observed when the spindles were released to shorten below slack length.

Contractile properties

Isometric force. Figure 2 shows representative records of a fused isometric tetanus and a single isometric twitch from an isolated muscle spindle at 2.3 μm sarcomere length. The rising phase of the tetanus (Fig. 2*A*) can be seen to be relatively slow compared to that recorded in extrafusal fibres of the same muscle species at low temperature. The time required to reach 90 % of maximum force was 473 ± 34 ms (n = 20) in muscle spindles (1–3 °C) as compared to 134 ± 4 ms (n = 11) recorded in ordinary tibialis anterior muscle fibres (1–3 °C, Edman & Lou, 1990). After the rising phase the tension generally remained quite stable, with no appreciable 'creep', during the remainder of the stimulation period (1–2 s). In accordance with the slow rise of force during the tetanus a relatively low tension was attained during a single twitch (Fig. 2*B*). The twitch/tetanus ratio of the muscle spindles, typically 0.1–0.2, was thus considerably lower than that normally recorded in extrafusal fibres (see Edman & Lou, 1990).

The relatively slow onset of tension rise of the muscle spindle during tetanic stimulation was not attributable to a large series compliance that would prolong the rising phase. This is demonstrated in Fig. 3 in which a fixed-end tetanus is compared with a tetanus recorded from the same preparation while a marked segment was held at constant length during the stimulation period. The segment, 0.7 mm in length, was located midway between one of the tendons and the capsule of the spindle (cf. Fig. 1). The total amplitude of the force produced by the length-clamped segment can be seen to be greater than that of the fixed-end tetanus. However, the rate of rise of force was not substantially different in the two recordings suggesting that the slow onset of force is an inherent property of the contractile system of the muscle spindle.

The contractile strength, measured per unit cross-sectional area, was markedly lower in the muscle spindles than in the regular muscle fibres. The mean tetanic force recorded in 14 isolated spindles (for measurement of the cross-sectional area, see Methods) was 91 ± 10 kN m^{-2}. This is to be compared with a mean tetanic force of

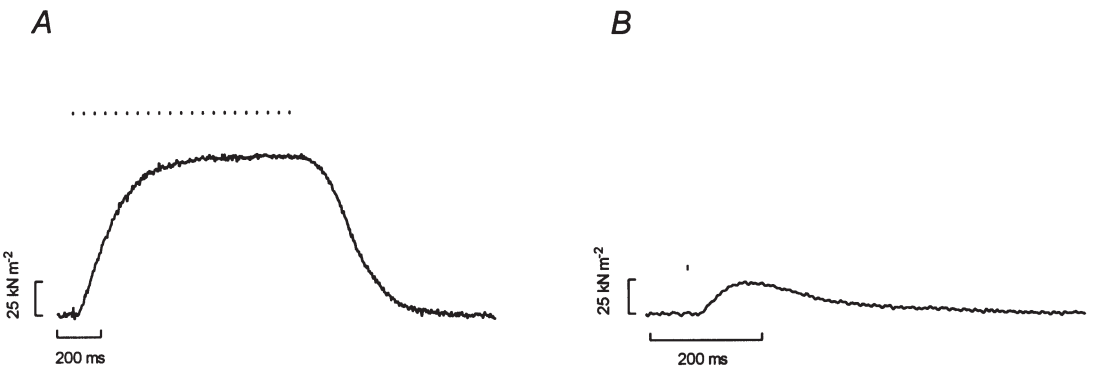

Figure 2. Example records of fused isometric tetanus (*A*) and single isometric twitch (*B*) of isolated muscle spindle

Note the relatively slow rise of tension during the tetanus and the low twitch/tetanus ratio. Stimulation pulses displayed above force records. Temperature: 3.5 °C.

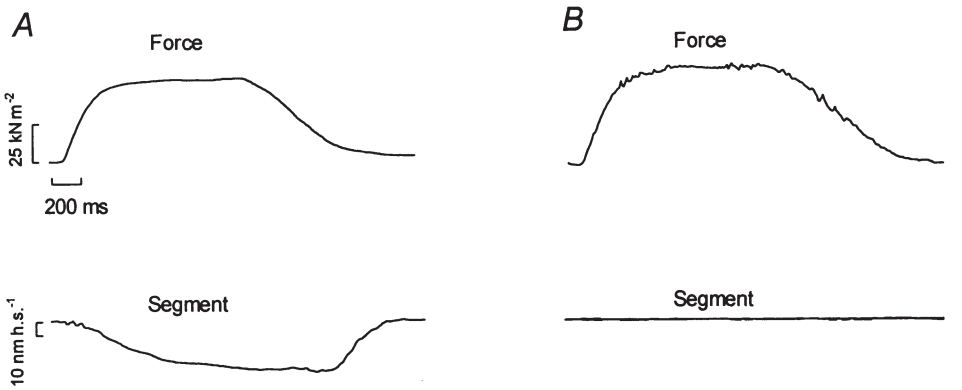

Figure 3. Time course of tetanic force recorded with the overall length of the spindle constant (standard 'isometric' recording, *A*) and with a marked segment held at constant length (segment-length clamp, *B*)

The segment length records in *A* and *B* refer to the same marked segment, shortening downwards. Note that there is a slow onset of force development also during segment-length recording. Temperature: 2.0 °C.

280 ± 10 kN m^{-2} (n = 20) produced by ordinary muscle fibres of the same muscle species (frog *anterior tibialis* muscle) under equivalent test conditions, i.e. 0.8–3.3 °C and 2.1–2.2 μm sarcomere length (K. A. P. Edman, unpublished data).

Similar to the situation in ordinary muscle fibres the 'isometric' relaxation of the muscle spindle had an initial linear phase and a second pseudo-exponential phase. The transition between the two phases occurred at 70–80 % of the plateau force and resulted in a 'shoulder' of the force myogram (Fig. 2) similar to that seen in ordinary muscle fibres. The speed of relaxation of the muscle spindle was not significantly different from that of ordinary fibres. The time measured from the last stimulus to the point at which tension had been reduced to 10 % of its maximal value was 673 ± 61 ms in 13 fibres, a value in good accord with measurements in ordinary muscle fibres at the same (1–4 °C) temperature (cf. Fig. 4*A* in Edman & Flitney, 1982).

There is ample evidence that the contractile system of regular (extrafusal) muscle fibres becomes maximally activated during a fused isometric tetanus (e.g. Sandow, 1965; Edman & Lou, 1990). The lower force output per unit cross-sectional area of the spindle fibres could mean that the intrafusal fibres, contrary to extrafusal fibres, do not reach mechanical saturation during tetanic stimulation. In order to test this point a series of five experiments was performed in which twitch and tetanus responses of isolated muscle spindles were compared in ordinary Ringer solution and after exposure to 0.5 mM caffeine. The spindle was stimulated to alternately produce a single isometric twitch and a fused isometric tetanus at regular 2 min intervals, care being taken that the stimulus strength was supramaximal. Measurements were first carried out in ordinary Ringer solution followed by a series of recordings in the presence of caffeine. The preparation was thereafter re-immersed in control Ringer solution followed by a second period of exposure to 0.5 mM caffeine. Example records from one experiment are illustrated in Fig. 4. The results show that caffeine produced a marked potentiation of the isometric twitch with increased time to peak tension and prolongation of the relaxation time. These effects of caffeine on the twitch force accord well with previous

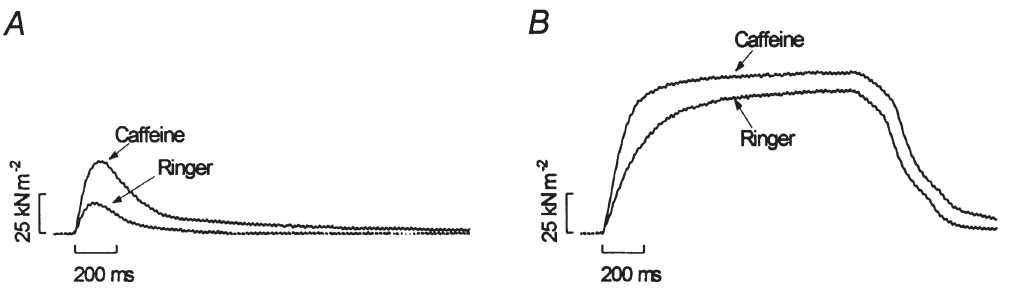

Figure 4. Effects of caffeine on twitch (*A*) and tetanus (*B*) of muscle spindle

Superimposed records illustrating potentiating effects of 0.5 mM caffeine on twitch (*A*) and tetanus (*B*) of isolated muscle spindle. Note that caffeine, in addition to potentiating the isometric twitch, also markedly increased the maximum tetanic force. For further information, see text. Temperature: 3.3 °C.

observations in extrafusal frog muscle fibres (e.g. Edman & Lou, 1990) and are in line with the observation that caffeine enhances the release of activator calcium from the sarcoplasmic reticulum (Sandow, 1965; Weber & Herz, 1968; Herrmann-Frank *et al.* 1999). Figure 4 furthermore demonstrates that caffeine increased the rate of rise of force during tetanic stimulation and also enhanced the maximum tetanic force of the muscle spindle, these effects being rapidly reversed after removal of caffeine from the muscle chamber. The increase in maximum tetanic force by caffeine provides evidence that spindle fibres, unlike extrafusal muscle fibres, do not reach mechanical saturation during tetanic stimulation. The mean increase in tetanic force produced by caffeine in the five preparations was 16.6 ± 0.04 % (± S.E.M.) of the control value in Ringer solution ($P < 0.02$). This mean value is based on one calculated value per fibre including two pairs of measurements (tests and controls) from each of the two series of recordings performed in a given preparation.

Force–velocity relation. The force–velocity relationship of muscle spindles exhibits the same characteristic features as previously demonstrated in ordinary striated muscle fibres (Edman, 1988). Thus, as is illustrated in Fig. 5, the force–velocity relation of the spindle has two curvatures, each one exhibiting an upward–concave shape, located on either side of a breakpoint near 80 % of the isometric force, P_0. The biphasic shape of the force–velocity relation and the distinct transition between the two curvatures is most clearly demonstrated in the semilogarithmic plot in Fig. 5*B*. The force–velocity data shown in the standard diagram in Fig. 5*A* could be fitted well with the biphasic equation (eqn (1), Methods) that has previously been applied to ordinary muscle fibres (Edman, 1988). The constants k_1 and k_2 in this equation determine the high-force curvature and the transition between the two portions of the force–velocity relation, respectively. The numerical values of k_1 and k_2 used for the curve fitting in Fig. 5*A* (see caption) are similar to those employed in studies of extrafusal muscle fibres (Edman, 1988). The high-force curvature did not come out clearly in all spindle preparations. Non-uniform sarcomere behaviour among the spindle fibres is likely to have smoothed out the two phases of the force–velocity relationship in such cases, since the shape of the force–velocity relationship varies with sarcomere length (see further Edman, 1988).

The curvature of the force–velocity relation at low and intermediate loads (extrapolated by dashed line in Fig. 5*A*) is given by the ratio $a/P_0{}^\star$ in eqn (1), an increased value indicating a decrease in curvature. Force–velocity measurements performed on 11 isolated muscle spindles gave an $a/P_0{}^\star$ value of 0.34 ± 0.03, which is in good agreement with measurements on ordinary fibres of frog anterior tibialis muscles (Edman, 1988). By contrast, the maximum speed of shortening (V_{max}), calculated from the fitted hyperbolic function, was markedly lower in the spindles than in the ordinary muscle fibres. The mean V_{max} based on measurements from the entire length of the spindle was thus merely 0.95 ± 0.06 lengths (L) s^{-1} ($n = 11$). Simultaneous force–velocity measurements performed on a marked segment outside the capsule area provided a very similar value of V_{max}, 0.96 ± 0.07 L s^{-1} ($n = 6$). The maximum speed of shortening of the spindle fibres was thus approximately half the velocity recorded in extrafusal fibres at the same temperature (Edman, 1988).

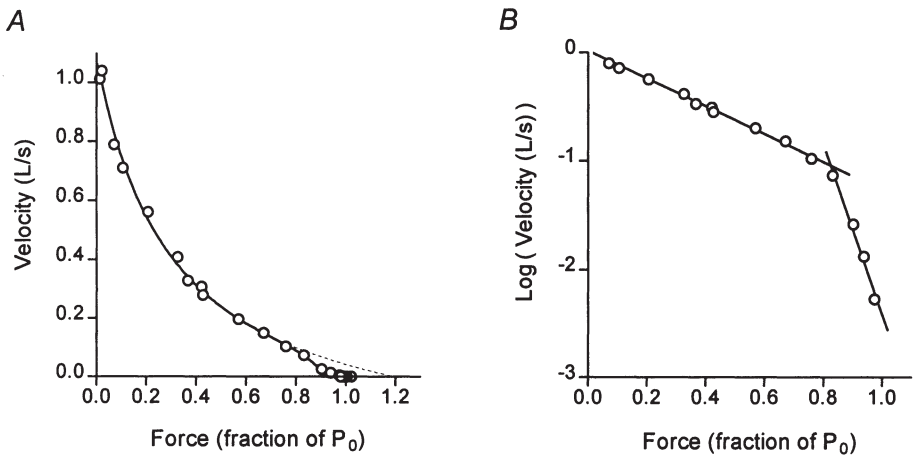

Figure 5. Biphasic force–velocity relationship in muscle spindle

Force velocity data derived from isolated muscle spindle are illustrated in a conventional diagram in *A* and as a semilogarithmic plot in *B*. Data in *A* fitted by eqn (1) (see Methods) using the following numerical values for constants: $k_1 = 26P_0{}^{-1}$, $k_2 = 0.84$, $a/P_0{}^\star = 0.30$. The straight lines in *B* are linear regressions of velocity upon force on either side of the breakpoint of the force–velocity relationship. Dashed line in *A*, continuation of hyperbola derived at low and intermediate loads. Note distinct breakpoint of the force–velocity relationship near $0.8P_0$. Temperature: 0.8 °C.

Segmental differences in contractile strength. In this series of experiments three to five markers were placed on the upper surface of isolated muscle spindles and the change in length between adjacent markers (one segment) was recorded in repeated tetani as described in Methods. The markers (placed 0.4–0.7 mm apart) were generally positioned so as to provide one segment over the capsule and 1–2 segments in areas next to the capsule. Figure 6 shows example records from such an experiment illustrating differences in contractile behaviour of segments located outside and inside the capsule region during tetanic stimulation. One extracapsular segment (segment 1, Fig. 6) can be seen to shorten considerably when the spindle was stimulated to contract while the ends of the spindle were fixed. The capsule segment, on the other hand (segment 3, Fig. 6), was markedly stretched indicating a lower force-producing capability within this region of the spindle. This is further illustrated during segment length clamp of segment 3 in Fig. 6, in which the length of the capsule segment was held constant by feedback control during tetanic stimulation. The force produced by the length-clamped segment can thus be seen to be substantially lower than the force recorded from the spindle as a whole. Marked segments within the capsule region were consistently found to elongate during fixed-end tetani in all spindles investigated.

The degree of shortening of the extracapsular regions was not uniform along the preparation, and the pattern of length changes, furthermore, varied from one spindle to another. Thus, whereas one region could be seen to shorten extensively, another region shortened only moderately or remained stationary. An occasional extracapsular segment, like segment 2 in Fig. 6, was even found to elongate during the fixed-end tetanus along with the extension of the capsule region (segment 3). The reason for this non-uniform contractile behaviour is unclear at the present time. A most likely cause would be that the isomyosin composition varies along the length of the muscle fibres as previously demonstrated in extrafusal frog muscle fibres (Edman *et al.* 1985, 1988; Lutz *et al.* 1998).

Intracellular Ca^{2+} transient

Simultaneous measurements of isometric force and intracellular free calcium concentration were performed during twitch and tetanus in eight muscle spindle preparations using fluo-3 as a calcium indicator. The time course of the free calcium concentration in the myoplasm, $[Ca^{2+}]_i$, was determined from the fluo-3 signal by accounting for the on- and off-rate constants for the binding of calcium to the dye as described in Methods. As illustrated in Fig. 7 the Ca^{2+} transients recorded in the muscle spindles had the same general characteristics as those derived in ordinary frog muscle fibres (Caputo *et al.* 1994). The Ca^{2+} transient during the single twitch (Fig. 7*C*) thus formed a distinct spike that reached its peak already 2–3 ms after the onset of tension rise in accordance with previous observations in extrafusal fibres (Sun *et al.* 1996). Similar to the situation

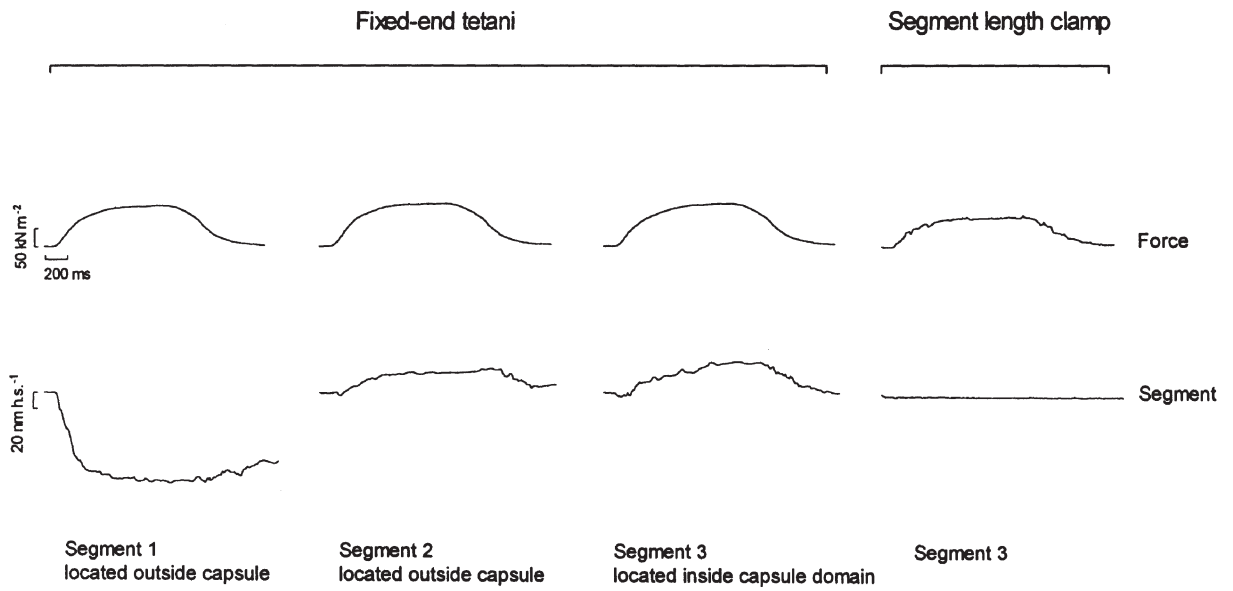

Figure 6. Example records illustrating segmental differences in contractile behaviour along the length of a muscle spindle

Left panels, repeated fixed-end tetani with corresponding length records from three marked segments located outside (segments 1 and 2) and inside (segment 3) the capsule domain. Right panel, tetanic force during length clamp of capsule segment. Shortening of segments downwards. Note that the capsule segment elongates during the fixed-end tetanus and that the force produced by this segment during length clamp is lower than that in fixed-end tetanus. Also note that extracapsular segment 1 shortens greatly whereas extracapsular segment 2 elongates. Temperature: 1.5 °C.

in ordinary muscle fibres (Cannell, 1986; Caputo *et al.* 1994) a secondary 'hump' of the Ca^{2+} transient was, in some preparations, identified during the later part of the relaxation phase, the hump being most clearly developed during the tetanus relaxation (Fig. 7*D*). This secondary rise of the Ca^{2+} transient has previously been shown by Caputo *et al.* (1994) to coincide with the pseudoexponential phase of relaxation, i.e. the phase starting at the 'shoulder' of the force myogram and being associated with non-uniform changes in sarcomere length within the muscle fibre (Cleworth & Edman, 1972; Edman & Flitney, 1982). The coincidence in time between the secondary Ca^{2+} rise and the pseudoexponential phase of relaxation was also apparent in the muscle spindles. This is illustrated in Fig. 7*B* and *D* in which the 'shoulder' in the tetanus myogram and the onset of the secondary rise during the Ca^{2+} transient are indicated by arrows that closely coincide in time.

During tetanic stimulation the Ca^{2+} transients induced by the separate stimuli were largely fused, the peaks of the individual responses forming a serrated upper contour of the compound Ca^{2+} signal (Fig. 7*D*). The mean $[Ca^{2+}]_i$ reached during the tetanus plateau was 1.7 ± 0.1 μM in the eight muscle spindle preparations studied. This is approximately half the $[Ca^{2+}]_i$ recorded under equivalent conditions in ordinary frog muscle fibres using the same recording technique (Caputo *et al.* 1994; Sun *et al.* 1996).

DISCUSSION

The intrafusal muscle fibres can be presumed to play an essential role in the motor control of skeletal muscle (e.g. Katz, 1961; Stein, 1974; Hunt, 1990), as these fibres are able to modulate the degree of strain of the stretch receptor of the muscle spindle and so affect the afferent output of the receptor. The precise mode of action of the spindle fibres has so far been difficult to evaluate, however, in part due to lack of information concerning the kinetic properties of these fibres. For example, a higher velocity of shortening of the spindle fibres, relative to that of the extrafusal fibres, would create conditions for the spindle fibres to be in the lead of the regular fibres and govern the degree of strain of the stretch receptor throughout a contraction. On the other hand, if the spindle fibres are slower than the surrounding muscle fibres, the strain of the stretch receptor initially set up by the spindle fibres would be steadily reduced, and possibly cancelled altogether, as the regular muscle fibres start to contract at a greater speed.

In the present study the contractile performance of isolated frog muscle spindles has been explored using techniques that have enabled recording of active force and shortening velocity both from the spindle as a whole and from discrete segments of the spindle. In this way it has been possible to characterize the contractile properties,

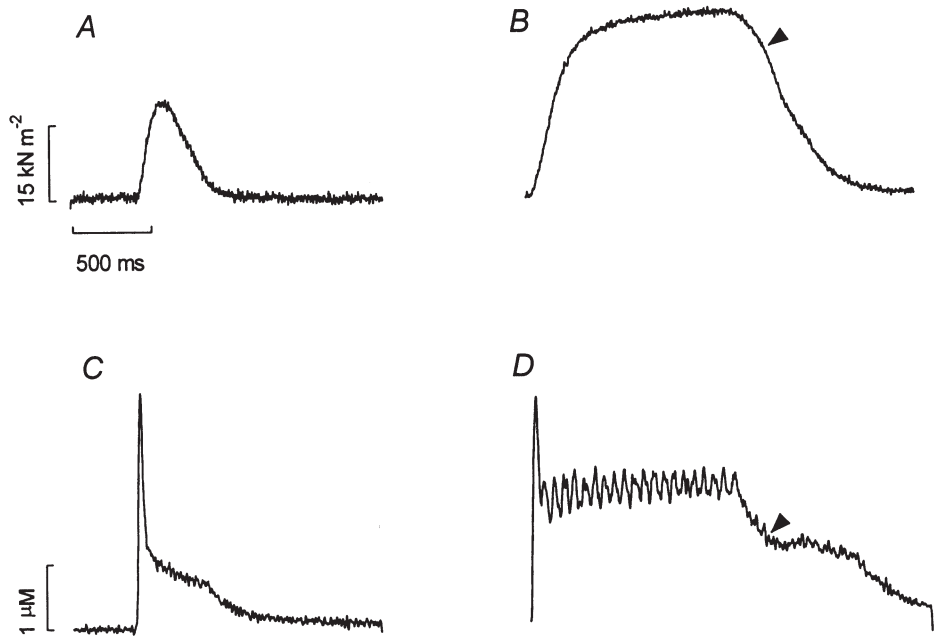

Figure 7. Simultaneous recordings of isometric force and intracellular Ca^{2+} transients during twitch and tetanus in muscle spindle

The Ca^{2+} transient has been transformed from the fluo-3 signal as described in Results. Note the spike-like response during the single twitch (*C*) and after the first stimulus during tetanus (*D*). Also note that during the relaxation phase, both during twitch and tetanus, the decay phase of $[Ca^{2+}]_i$ is forming a secondary 'hump' that is most distinct during tetanus relaxation. The onset of the secondary rise of $[Ca^{2+}]_i$ coincides with the shoulder of the relaxation phase during the tetanus (indicated by the arrows). Temperature: 2.3 °C.

separately, inside the capsule region (where the stretch receptor is located) and outside the capsule domain. The measurements have been performed during supramaximal field stimulation, and the recordings therefore represent the mechanical response of all fibres collectively.

The results show that the force–velocity relationship of spindle fibres has the same characteristic biphasic shape as previously demonstrated for ordinary striated muscle fibres (Edman, 1988). That is, the force–velocity relation has two distinct curvatures located on either side of a breakpoint near 80 % of the isometric force. The force–velocity data could be fitted well with the biphasic equation previously described (see Methods, eqn (1)), and the numerical values of the parameters describing the two curvatures were in good accord with those derived from ordinary frog muscle fibres. These results thus support the view that the mechanical performance of the intrafusal fibres is not fundamentally different from that of the extrafusal fibres in the muscle.

The similarity between the intra- and extrafusal muscle fibres also applies to the intracellular Ca^{2+} transient in the two categories of fibres. The Ca^{2+} transient after a single stimulus formed a distinct spike starting during the latent period and reaching its peak very soon (2–3 ms) after the onset of tension rise in accordance with our previous observations on frog muscle fibres (Sun *et al.* 1996). Further in similarity with regular muscle fibres (see Cannell, 1986 and Caputo *et al.* 1994), there was a secondary rise of the Ca^{2+} transient during the relaxation phase of the tetanus starting at the 'shoulder' of the force myogram and coinciding with the pseudoexponential phase of relaxation. Evidence derived from extrafusal muscle fibres strongly suggests that the 'calcium hump' is ultimately caused by non-uniform sarcomere movements along the muscle fibre leading to enhanced dissociation of calcium from the troponin binding sites (see Caputo *et al.* 1994 for further discussion).

No conclusive information has been presented in previous studies as to whether the active force of muscle spindles differs from that of regular muscle fibres. Results presented by Diete-Spiff (1967) and Jahn (1968), based on measurements on cat and frog muscle spindles, were interpreted to show that the force producing capability of the intrafusal fibres, normalized to the cross-sectional area, does not differ markedly from that of regular muscle fibres. A similar conclusion was later reached by Fukami (1984) in measurements on four cat muscle spindles that were tetanized by indirect stimulation. The techniques used in the present study, including length-clamp recordings of discrete segments of the muscle spindle, have enabled a more precise comparison of the isometric force in intra- and extrafusal fibres than was feasible in previous work. Our results demonstrate that the intrafusal fibres of frog skeletal muscle are less capable of producing active force than are the regular muscle fibres. In fact, the maximal tetanic force produced by the fibres of a given muscle spindle was found to be merely one-third to one-half of the average force per cross-sectional area produced by extrafusal fibres at similar sarcomere lengths. Assuming that the myofilament lengths are not greatly different in the extra- and intrafusal fibres, these findings provide evidence that the force produced per unit filament overlap is substantially lower in the spindle fibres.

The present study also demonstrates for the first time that the spindle fibres, in addition to their lower contractile force, also shorten at a considerably lower speed than the extrafusal fibres. The maximum speed of shortening of the spindle fibres was thus found to be approximately half the value recorded under similar conditions in extrafusal fibres. It could be argued that the lower velocity recorded from the spindle is due to slowness of shortening within the capsule region where the movement might be thought to be hampered by passive structures. This possibility could be excluded by measuring the speed of shortening simultaneously from the spindle as a whole and from a marked segment outside the capsule region. The results of the two measurements were in excellent agreement (see Results) suggesting strongly that the recorded low speed of shortening is a genuine feature of the contractile system of the intrafusal muscle fibres. It is worth pointing out that the maximum speed of shortening recorded from a multi-fibre preparation like the spindle does actually represent the speed of shortening of the *fastest* fibre(s) if the measurement is based on data close to zero load (see Josephson & Edman (1988). It is thus fully conceivable that the spindles contain fibres whose speed of shortening is even lower than that recorded from the preparation as a whole.

The basis of weaker force and lower speed of shortening in spindle fibres

The results provide evidence that the frog muscle spindles do not become fully activated during supramaximal tetanic stimulation in standard Ringer solution. This is indicated by the finding that the maximum tetanic force may be further increased (by approximately 16 %) by exposing the spindle to caffeine in a twitch potentiating concentration. It is well established that caffeine enhances the release of activator calcium from the sarcoplasmic reticulum (Sandow, 1965; Weber & Herz, 1968; Herrmann-Frank *et al.* 1999). The fact that the tetanic force is elevated by caffeine thus strongly suggests that the amount of calcium released under standard (caffeine-free) conditions is inadequate to activate the contractile system maximally. It is of interest to note, however, that even in the presence of caffeine the force produced by the muscle spindle is still clearly lower than that produced by extrafusal fibres. Submaximal activation is therefore only partially responsible for the lower force produced by the spindle fibres.

Inadequate activation of the contractile system can probably also be ruled out as the main cause of the low speed of shortening of the spindle fibres. The maximum speed of shortening has previously been shown in experiments on frog extrafusal fibres (Edman, 1979) to be quite insensitive to the state of activation of the contractile system, and there is no reason to believe that the situation is different in intrafusal fibres considering the similarities in force–velocity behaviour between the two fibre species (see earlier). It is thus probably safe to conclude that the slowness of shortening and the low isometric force of the spindle fibres both reflect inherent properties of the contractile system in these fibres.

Substantial differences in the myosin heavy chain (MyHC) composition between intrafusal and extrafusal muscle fibres have been documented in several previous studies of adult mammalian skeletal muscles (see Walro & Kucera, 1999). The evidence suggests that the MyHC composition is quite similar in the two categories of fibres during the early stages of myogenesis. However, whereas the embryonal 'slow' forms of MyHCs (designated slow-developmental, embryonic and neonatal MyHCs) are largely retained during the postnatal period in the intrafusal fibres, the extrafusal fibres develop additional, 'faster' types MyHC isoforms during maturation that partly replace the original slow types of isoforms. The MyHC subunit-I which is present in slow adult extrafusal fibres is also found to exist in adult mammalian intrafusal fibres. The slow forms of MyHCs thus seem to be in majority in mammalian spindle fibres (for further details, see recent reviews by Soukup *et al.* 1995; Pette & Staron, 1997; Walro & Kucera, 1999). The myosin isoform composition of frog extrafusal fibres has previously been studied in considerable detail and has been related to the contractile properties of the muscle fibres (e.g. Lännergren & Hoh, 1984; Edman *et al.* 1988; Rowlerson & Spurway, 1988; Spurway & Rowlerson, 1989; Lutz *et al.* 1998, 2002). By contrast, there is no detailed information on the isomyosin composition of amphibian intrafusal muscle fibres. The present results make it seem likely, however, that slower myosin heavy-chain subunits are also the predominating isoforms in frog spindle fibres.

The force produced by the spindle fibres is negligible in relation to the total force delivered by the muscle, and it is difficult to see any functional significance of the fact that the spindle fibres are capable of producing less force per cross-sectional area than the extrafusal fibres. There is reason to believe, however, that the *longitudinal differences* in active force along the spindle are of the utmost importance to the function of the spindle. Our results show that a greater force is produced in the extracapsular portion of the spindle than in the capsule segment itself. This was demonstrated by measuring force from length-clamped segments located inside the capsule area and outside the capsule domain, respectively. The lower force in the capsule portion was also apparent during fixed-end tetani. The polar regions could then be seen to shorten while the capsule segment was regularly stretched. The relative 'weakness' of the capsule region may be regarded as an essential feature of the muscle spindle and would indeed seem to be a prerequisite for the proper use of the spindle organ in the muscle. By this arrangement the stretch receptor, and therefore also its signal output, may be varied in a graded way by adjusting the contractile activity of the spindle appropriately (see further below).

The cause of the lower contractile strength within the capsule segment still remains unclear. As pointed out earlier, the intrafusal fibres pass through the capsule without any obvious change in diameter and sarcomere length that could explain the lower force output in this region. The possibility exists that the longitudinal differences in contractile strength are based on axial differences in the isomyosin composition. Such differences have indeed been observed in mammalian intrafusal fibres (Pedrosa *et al.* 1989; Kucera *et al.* 1992) but their functional significance has not yet been explored. Another possible cause of the lower contractile strength of the capsule segment is based on the fact that there is a non-uniform distribution of the nuclei in the intrafusal muscle fibres within the capsule. Thus, as demonstrated in frog muscle spindles (Katz, 1961; Karlsson *et al.* 1966) there is an accumulation of nuclei in one or more regions along the individual muscle fibres inside the capsule, and these heavily nucleated parts ('reticular regions') are interspersed with 'compact zones' where nuclei are sparse. In the reticular regions less space is available for myofibrils and these regions are accordingly found to contain fewer myofilaments per cross-sectional area as compared to the compact zones and the regions outside the capsule (Katz, 1961; Karlsson *et al.* 1966). The reticular regions of the fibres, therefore, can be presumed to be substantially weaker. Since the reticular regions of the individual fibres are irregularly distributed within the capsule, the force-producing capability of the entire capsule segment is likely to be lower than that of the extracapsular portions of the spindle.

The mechanical properties of the intrafusal fibres in relation to the physiological role of the muscle spindle

From the results of this study certain general conclusions can be drawn concerning the function of the muscle spindle *in situ* in the muscle. As already pointed out, the lower force-producing capability of the capsule segment may be regarded as a fundamental property of the spindle. By this feature it is possible to adjust the extension of the stretch receptor in a graded way by varying the state of activation of the spindle. This mechanism will be most effective during isometric contraction, when the ends of the spindle are restrained to move, as the weaker capsule/receptor segment under these conditions will take

up most of the shortening produced by the stronger portions of the spindle. The degree of stretch of the receptor segment will become progressively smaller, however, as the spindle is allowed to shorten at increasing speeds, since the pulling force on the stretch receptor will be steadily reduced at higher velocities of shortening (see force–velocity relationship, Fig. 5). Indeed, when the spindle shortens at its maximum speed, the receptor segment will not be subjected to any extending force at all. It is of interest to note in this connection that the spindle fibres are considerably slower than the extrafusal fibres. As pointed out earlier, the maximum speed of shortening of the spindles is merely half the value of the regular muscle fibres. This means that already when the muscle shortens at a moderate speed, the intrafusal fibres will be unable to generate any force to affect the stretch receptor. The muscle spindles may thus only be expected to serve in the control of muscle activity during eccentric (forced lengthening) and static (isometric) contractions and, with decreasing effectiveness, during slow muscle shortening. Apparently the spindles are not designed to affect rapid muscle shortening.

REFERENCES

Boyd, I. A. (1976). Time courses of activity in intrafusal fibres in isolated cat muscle spindles. *Journal of Physiology* **254**, 23–24.

Cannell, M. B. (1986). Effect of tetanus duration on the free calcium during the relaxation of frog skeletal muscle fibres. *Journal of Physiology* **376**, 203–218.

Caputo, C., Edman, K. A. P., Lou, F. & Sun, Y.-B. (1994). Variation in myoplasmic Ca^{2+} concentration during contraction and relaxation studied by the indicator fluo-3 in frog muscle fibres. *Journal of Physiology* **478**, 137–148.

Cleworth, D. R. & Edman, K. A. P. (1972). Changes in sarcomere length during isometric tension development in frog skeletal muscle. *Journal of Physiology* **227**, 1–17.

Diete-Spiff, K. (1967). Tension development by isolated muscle spindles of the cat. *Journal of Physiology* **193**, 31–43.

Edman, K. A. P. (1979). The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. *Journal of Physiology* **291**, 143–159.

Edman, K. A. P. (1988). Double-hyperbolic force–velocity relation in frog muscle fibres. *Journal of Physiology* **404**, 301–321.

Edman, K. A. P. & Flitney, F. W. (1982). Laser diffraction studies of sarcomere dynamics during 'isometric' relaxation in isolated muscle fibres of the frog. *Journal of Physiology* **329,** 1–20.

Edman, K. A. P. & Lou, F. (1990). Changes in force and stiffness induced by fatigue and intracellular acidification in frog muscle fibres. *Journal of Physiology* **424**, 133–149.

Edman, K. A. P., Radzyukevich, T. & Kronborg, B. (2000). Contractile properties of isolated muscle spindles from frog. *Biophysical Journal* **78**, 120A.

Edman, K. A. P. & Reggiani, C. (1984). Redistribution of sarcomere length during isometric contraction of frog muscle fibres and its relation to tension creep. *Journal of Physiology* **351**, 169–198.

Edman, K. A. P. & Reggiani, C. (1987). The sarcomere length–tension relation determined in short segments of intact muscle fibres of the frog. *Journal of Physiology* **385**, 709–732.

Edman, K. A. P., Reggiani, C., Schiaffino, S. & Te Kronnie, G. (1988). Maximum velocity of shortening related to myosin isoform composition in frog skeletal muscle fibres. *Journal of Physiology* **395**, 679–694.

Edman, K. A. P., Reggiani, C. & Te Kronnie, G. (1985). Differences in maximum velocity of shortening along single muscle fibres of the frog. *Journal of Physiology* **365**, 147–163.

Fukami, Y. (1984). Active force and sensory response of single isolated cat muscle spindles *in vitro*. *Journal of Neurophysiology* **52**, 1131–1139.

Gordon, A. L., Huxley, A. F. & Julian, F. J. (1966). Tension development in highly stretched vertebrate striated muscle fibres. *Journal of Physiology* **184**, 143–169.

Herrmann-Frank, A., Lüttgau, H.-C. & Stephenson, D. G. (1999). Caffeine and excitation–contraction coupling in skeletal muscle: a stimulating story. *Journal of Muscle Research and Cell Motility* **20**, 223–237.

Hill, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proceedings of the Royal Society* B **126**, 136–195.

Hunt, C. C. (1990). Mammalian muscle spindle: Peripheral mechanisms. *Physiological Reviews* **70,** 643–663.

Jahn, S. A. (1968). Static elasticity of isolated muscle spindles of the frog and tension development of their intrafusal muscle fibres. *Acta Physiologica Scandinavica* **74**, 384–393.

Josephson, R. K. & Edman, K. A. P. (1988). The consequences of fibre heterogeneity on the force–velocity relation of skeletal muscle. *Acta Physiologica Scandinavica* **132**, 341–352.

Karlsson, U., Andersson-Cedergren, E. & Ottoson, D. (1966). Cellular organization of the frog muscle spindle as revealed by serial sections for electron microscopy. *Journal of Ultrastructure Research* **14**, 1–35.

Katz, B. (1961). The terminations of the afferent nerve fibre in the muscle spindle of the frog. *Philosophical Transactions of the Royal Society* B **243**, 221–240.

Kucera, J., Walro, J. M. & Gorza, L. (1992). Expression of type-specific MHC isoforms in rat intrafusal muscle fibers. *Journal of Histochemistry and Cytochemistry* **40**, 293–307.

Lännergren, J. & Hoh, J. F. Y. (1984). Myosin isoenzymes in single muscle fibres of *Xenopus laevis*: analysis of five different functional types. *Proceedings of the Royal Society* B **222**, 401–408.

Lutz, G. J., Cuizon, D. B., Ryan, A. F. & Lieber, R. L. (1998). Four novel myosin heavy chain transcripts define a molecular basis for muscle fibre types in *Rana pipiens*. *Journal of Physiology* **508**, 667–680.

Lutz, G. J., Sirsi, S. R., Shapard-Palmer, S. A., Bremner, S. N. & Lieber, R. L. (2002). Influence of myosin isoforms on contractile properties of intact muscle fibers from *Rana pipiens*. *American Journal of Physiology – Cell Physiology* **282**, C835–844.

Minta, A., Kao, J. P. Y. & Tsien, R. Y. (1989). Fluorescent indicators for cytosolic calcium based on rhodamine and fluorescein chromophores. *Journal of Biochemical Chemistry* **264**, 8171–8178.

Pedrosa, F., Butler-Browne, G. S., Dhoot, G. K., Fischman, D. A. & Thornell, L. E. (1989). Diversity in expression of myosin heavy chain isoforms and M-band proteins in rat muscle spindles. *Histochemistry* **92**, 185–194.

Pette, D. & Staron, R. S. (1997). Mammalian skeletal muscle fiber type transitions. *International Review of Cytology* **170**, 143–223.

Rowlerson, A. M. & Spurway, N. C. (1988). Histochemical and immunohistochemical properties of skeletal muscle fibres from *Rana* and *Xenopus*. *Histochemical Journal* **20**, 657–673.

Sandow, A. (1965). Excitation–contraction coupling in skeletal muscle. *Pharmacological Reviews* **17**, 265–320.

Smith, R. S. (1964). Contraction in intrafusal muscle fibres of *Xenopus laevis* following stimulation of their motor nerves. *Acta Physiologica Scandinavica* **62**, 195–208.

Soukup, T., Pedrosa-Domellöf, F. & Thornell, L. E. (1995). Expression of myosin heavy chain isoforms and myogenesis of intrafusal fibres in rat muscle spindles. *Microscopy Research and Technique* **30**, 390–407.

Spurway, N. C. & Rowlerson, A. M. (1989). Quantitative analysis of histochemical and immunohistochemical reactions in skeletal muscle fibres of *Rana* and *Xenopus*. *Histochemical Journal* **21**, 461–476.

Stein, R. B. (1974). Peripheral control of movement. *Physiological Reviews* **54,** 215–243.

Sun, Y.-B., Lou, F. & Edman, K. A. P. (1996). The relationship between the intracellular Ca^{2+} transient and the isometric twitch force in frog muscle fibres. *Experimental Physiology* **81**, 711–724.

Walro, J. M. & Kucera, J. (1999). Why adult mammalian intrafusal and extrafusal fibers contain different myosin heavy-chain isoforms. *Trends in Neurosciences* **22**, 180–184.

Weber, A. & Herz, R. (1968). The relationship between caffeine and contracture of intact muscle and the effect of caffeine on reticulum. *Journal of General Physiology* **52**, 750–759.

Acknowledgements

This work was supported by grants from the Swedish Medical Research Council (projects 14X-184 and 14X-08664, K.A.P.E). T.R. was on study leave from The Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St Petersburg, Russia and was supported by a Visiting Scientist Fellowship from the Swedish Institute. K.A.P.E. would like to thank Professor A. Arner for kindly providing office and laboratory facilities.

Author's present address

T. Radzyukevich: Department of Molecular and Cellular Physiology, University of Cincinnati, 231 Albert Sabin Way ML 573, Cincinnati, OH 45267-0576, USA.