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Length dependence of the contractility of pig detrusor smooth muscle fibres

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Abstract Evidence on the length dependence of the contraction velocity of smooth muscle fibres is contradictory. Nevertheless, a thorough understanding of this dependence is essential for a correct urodynamic diagnosis of voiding problems. We studied muscle fibres of pig urinary bladders ($n = 23$). Force-velocity relations were measured at different muscle lengths with a stop test technique. This method involves measuring force generation of electrically stimulated muscle fibres during controlled shortening from a pre-shortening length at a pre-set velocity to a fixed stop-length. We normalized the length dependence of the measured properties to slack length, optimum length (the length at maximum isometric force generation), and passive force. Isometric force was found to be length dependent with an optimum length of 290 ± 68% of the slack length ($n = 11$, $P < 0.05$). The maximum shortening velocity was 0.37 ± 0.14 s − 1 related to the slack length and 0.13 ± 0.05 s − 1 related to the optimum length and was not length dependent ($n = 16$, $P < 0.05$). Slack length is preferable to normalize the length dependence of smooth muscle.

Introduction

Muscle contractility may be defined by the relation between the exerted force and the shortening velocity. This relation can mathematically be described by a hyperbolic curve [8]. This may be rewritten in normalized form with three independent parameters [10, 15]. One of these, the ''curvature'' of the relation may, when the muscle is stimulated supramaximally, be considered constant so that two parameters, the isometric force and the maximum shortening velocity, remain to characterize muscle contractility fully [8, 10, 13 19, 23].

For skeletal muscles it is known that the isometric force depends on the overlap between the myosin and actin filaments [3, 9] and thus on the stretched length of the preparation, and that the maximum shortening velocity is constant over a large range of sarcomere lengths [1]. It is often assumed that the same is true for smooth muscle. It has been confirmed that the isometric force measured in smooth muscle fibres of the urinary bladder is length dependent [12, 13, 22]. It also seems that the maximum shortening velocity of smooth muscle actin is independent of the number of cross-bridges [24]. However, few studies have been made on the influence of stretched fibre length on the maximum shortening velocity, and the results are contradictory. In 1977, Uvelius [23] reported that the maximum shortening velocity of strips of rabbit urinary bladder increased dramatically with stretched length. Griffiths et al [4] reported, on the basis of measurements in pig urinary bladder strips, that it is reasonable to assume that the maximum shortening velocity is constant. In 1991, Malmqvist, Arner and Uvelius [13] reported that in skinned human bladder muscle, the stretched length did not influence the maximum shortening velocity. In our laboratory, we found in a number of pilot experiments in 1997 that in muscle fibres of the pig urinary bladder the maximum shortening velocity increased with stretched length at small lengths, but stabilized at higher lengths [10]. There is thus no consensus on the length dependence of the maximum shortening velocity of the urinary bladder wall. On the other hand, this topic is clinically relevant. The length dependence of the maximum shortening velocity of the urinary bladder wall is reflected in the dependence of the maximum flow-rate of volunteers and patients on the volume voided. This dependence does not conform to the current models of urinary bladder contractility [16, 17], and there is

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reason to believe that this deviation may be diagnostically relevant [15, 20]. It was therefore decided to study the length dependence of the shortening velocity of the urinary bladder in vitro as a basis for a more detailed understanding of the clinical value of the dependence of maximum flow-rate on voided volume.

One possible reason for the contradictory results obtained thus far, may be the difference in the measurement methods used. In our present study, the maximum shortening velocity was measured using the stop test method. This method involves measuring force generation of electrically stimulated muscle fibres during controlled shortening from a pre-shortening length at a pre-set velocity to a fixed stop-length. It was first described by Griffiths et al. in 1979 [4] and was further applied in our laboratory in 1985 [18] to quantify the contractility of the urinary bladder. Until our pilot experiments in 1997 [10], it had not been used to systematically study the length dependence of the maximum shortening velocity of the urinary bladder. More often the quick release method is used to measure the maximum shortening velocity [13, 23]. However, the stop test has the advantage that the force at a certain shortening velocity is measured at a fixed muscle length and can be corrected for passive force, which makes it more suitable for measuring the length dependence of smooth muscle contractility. The length dependence of the force-velocity relation can thus be determined at a large range of stretched fibre lengths.

One problem in studies on the length dependence of smooth muscle contractility is that in contrast to the favourable situation in striated muscles where the sarcomeres can be seen and their length measured, contractile elements are not easily identified and that good and reliable estimates for their number and degree of overlap are missing. A contractile element in this study is defined as the smallest part of a muscle fibre, which is able to generate force, an element comparable to a sarcomere in skeletal muscles. In smooth muscle, a well-defined measure for fibre length may be used as an estimator for the number of contractile elements that are linked in series. The slack length, the smallest length at which a minimum passive force is measured, and the optimum length, the length at which the isometric force is at its maximum, have both been suggested as such an estimator [12, 25]. The stretched fibre length related to the slack length or optimum length can then be used as an estimator for the overlap between the filaments in the contractile elements. Passive force has also been suggested as an estimator for the overlap between the filaments [12, 25]. When we studied pig urinary bladder fibres, we first evaluated which length normalization method was best, by relating maximum isometric force to passive force, to the optimum fibre length and to the slack length. Subsequently, we used this normalization method to measure the length dependence of the maximum shortening velocity.

Methods and materials

Tissue preparation, incubation and stimulation

Urinary bladders of freshly killed pigs were obtained from the local slaughterhouse. Longitudinal muscle fibres of about 2 by 0.2 mm were cut from the bladder wall with the help of a binocular microscope. The fibres were transported to an organ bath (Fig. 1) containing 0.3 ml Kreb's solution, which was refreshed at a rate of 0.8 ml/h. The Kreb's solution was of the following composition: NaCl, 118 mM; KCl, 4.7 mM; $NaHCO_3$, 25 mM; KH_2PO_4, 1.2 mM; $CaCl_2$, 1.8 mM; $MgSO_4$ 1.2 mM; glucose, 11 mM; pH 7.4; aerated with 95% O_2/5% CO_2. The fibres were clamped between two pairs of tweezers, one linked to a KG3 force transducer connected to a BAM3 amplifier (Scientific Instruments, Heidelberg, Germany). The other pair of tweezers was attached to a length controller [10]. The length controller made it possible to shorten the fibres at a constant velocity to a pre-set stop length and later re-stretch them to the pre-shortening length. The measurement was controlled using a computer equipped with a PCL818 A/D converter driven by modified multi-channel data acquisition and measurement control software (MKR computer programme: developed by the CID Department, University Hospital, Rotterdam). Temperature was kept at 37.5 ± 0.5 °C using a 200-μm diameter thermocouple (Omega) controlling a halogen lamp (Philips, 12 V, 20 W, 6°, type 6483). The muscle fibres were stimulated by direct depolarization of the muscle cells with an electrical field generated by applying alternating biphasic pulses with an amplitude of 6 V, a duration of 4 ms, and a repetition rate of 125 Hz to two platinum electrodes. The repetition rate was optimized in earlier studies. [5]. An increased voltage resulted in a minor increase in force, but an

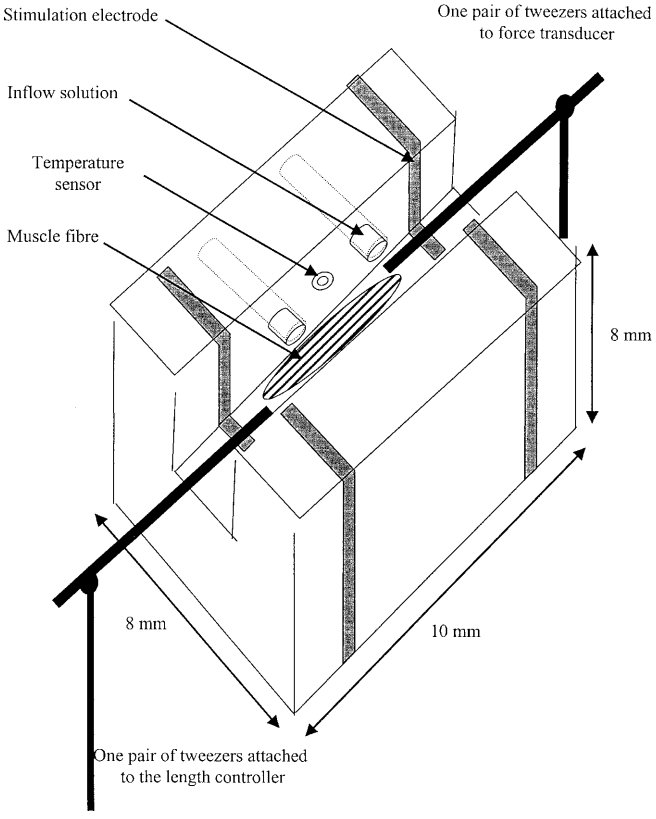

Fig. 1 A schematic drawing of the organ bath. The fibre is clamped between two pairs of tweezers. The groove containing the fibre is filled with Kreb's solution

increased risk of damaging the muscle fibre, as evidenced by a faster decline in isometric force between subsequent measurements. The duration of stimulation depended on the type of measurement.

Twenty minutes after mounting the fibres, the slack length (l_s), the minimum length at which a passive force change (± 10 μN) in response to a slight change in length (± 10 μm) became visible, was determined. Next, the fibre was stretched to its first measurement length. After another 10 min the first measurement was made. Between two measurements a resting period of 10 min was allowed. The measurements were stored in the computer and later analysed with the help of the computer programmes MKR and Matlab.

Force-length relation

The muscle fibres ($n = 11$) were electrically stimulated until the force started to decrease. The time needed to reach a maximum force was between 10 and 25 s and depended on the muscle fibre, the stretched fibre length, and the contraction history. Passive force was measured just before the onset of stimulation and subtracted from the total force to calculate the active force response. The maximum active force was called the isometric force (F_{iso}). Isometric force values were determined at increasing fibre lengths. The fibre length at which the maximum isometric force ($F_{iso.max}$) was measured was called the optimum length (l_0). After the fibre had been stretched over its optimum length, it was shortened to see if the isometric force increased again. Some fibres were re-stretched several times to test if the maximum isometric force occurred at the same stretched fibre length. After completion of the measurements, the fibres were reset to the slack length and embedded in Epon, cut in slices of 0.5 μm, and coloured with toluïdine blue. The maximum active stress was calculated by dividing the maximum active force by the cross-sectional area, which was measured at five different locations along the fibre and averaged. Epon has been reported to cause a volume change of 15% in single muscle fibres [2], thus causing a possible inaccuracy of the calculated stress up to this value.

The passive and isometric forces were normalized by dividing them by the maximum isometric force ($F_{pas.norm}$ and $F_{iso.norm}$). The isometric and passive force values measured at the same length were averaged for each strip. The results from the different fibres were averaged using linear interpolation. The stretched fibre length was expressed as a percentage of the slack length (% l_s) and as a percentage of the optimum muscle length (% l_0). To establish the dependence of the isometric force on the passive force, the normalized data of the 11 muscle fibres were combined and sorted in classes, each containing 20 observations. As other studies [12] only measured at increasing passive forces, we separately plotted the values of the increasing part of the first length cycle in each fibre. The results of different fibres were averaged using linear interpolation.

Force velocity relation

The maximum shortening velocity was measured using a stop test method [4, 10]. The fibre was first stretched to a pre-shortening length (l_{start}). After 10 min, when the passive force was stable, the fibre was shortened with a pre-set velocity to the stop length (l_{stop}) without stimulation to measure the passive force response (Fig. 2, dashed line). The fibre was then re-stretched at a velocity of 10 μm/s to the pre-shortening length. When the passive force was stable again and of the same magnitude as before, the fibre was stimulated and when the isometric force was reached, shortened with the same pre-set velocity to the stop length. The stimulation continued until a maximum force could be identified: the recovery force (F_{rec}) (Fig. 2, dotted line). The total duration of the stimulation was between 30 and 90 s. The active force response was calculated by subtracting the measurement without stimulation from the measurement with stimulation (Fig. 2, drawn line). The active force during shortening (F_{short}) at the stop length was divided by the recovery force at the same length to give the relative force (F_{rel}) for that shortening velocity at that stop length.

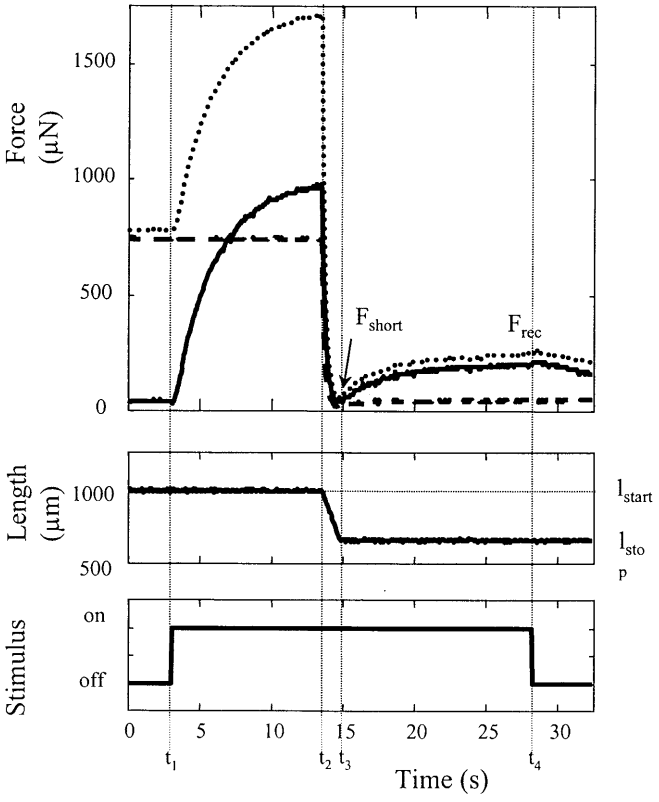

Fig. 2 An example of a force velocity measurement using the stop test technique. At t_1 the stimulation started, the total force (*dotted line*) rose to a maximum value, while the passive force (*dashed line*) remained constant. The *drawn line* represents the active force (the total force minus the passive force). The shortening started at t_2 and both total and passive force dropped. At t_3 the shortening stopped, the active force at t_3 is the force during shortening at the stop length and is called F_{short}. Subsequently the total force rose to a maximum called the recovery force (F_{rec})

To test the effect of the shortening duration on the relative force, three muscle fibres were shortened with a shortening velocity of 100 μm/s to a stop length of 150% of the slack length for a shortening duration that varied between 0.2 and 3 s. The results were averaged using linear interpolation.

Sixteen muscle fibres were used to measure the length dependence of the maximum shortening velocity. At each stop length, three different shortening velocities were applied twice, first in increasing and subsequently in decreasing order. These velocity values were chosen, evenly distributed along the expected force velocity curve. In a few cases more shortening velocity values were applied; this resulted in a small increase in accuracy of the fit (data not shown). The shortening duration was kept constant at 2 s, so that different pre-shortening lengths were used at different shortening velocities. The measured force-velocity data were fitted with a Hill curve [8], which was rewritten in such a form that it related the relative force to the shortening velocity [4, 10]. In this form this relation contains three parameters, the relative isometric force, the curvature a/F_{iso}, and the maximum shortening velocity. The relative isometric force at zero shortening is one by definition. The curvature of the hyperbolic relation a/F_{iso} was fixed at 0.25, as fitting a relation with such a fixed curvature resulted in only a slightly higher sum of squared deviations than fitting a relation with a variable curvature [10]. Therefore, only one adjustable parameter remained: the maximum shortening velocity. Each muscle fibre was measured at 2–5 different stop-lengths in random order. To normalize the maximum shortening velocity, it was divided by

the slack length or by the optimum length for those fibres at which the latter could be determined. The maximum shortening velocities of the various fibres were averaged using linear interpolation.

Statistical analysis

Values are expressed as mean ± the standard deviation. Force and maximum shortening velocity values at various fibre lengths were compared using a *t*-test. The dependence of the maximum shortening velocity on the stretched fibre length was also tested using the Friedman rank test. A significance level of 0.05 was used.

Results

The muscle fibres ($n = 23$) had a slack length between 5.0 and 8.0 mm and a diameter between 150 and 250 μm. Four fibres were used to measure the force length relation only, 16 were used for the length dependence of the force velocity relation. In 7 of these, an optimum length could be identified and these were also used to measure the force length relation. Three fibres were used to measure the effect of the shortening duration on the relative force.

The force length relation

The maximum isometric force was 2556 ± 1090 μN at a muscle length of 290 ± 68% of l_s. The maximum active stress was 6.6 ± 2.7 $N \cdot cm^{-2}$. When the mean normalized isometric force was plotted as a function of the length relative to slack length, it showed a broad maximum (Fig. 3, upper panel). The fibres used only to measure the force length relation showed a higher optimum length and a higher isometric force than the fibres used to measure the length dependence of the shortening velocity (Table 1, $P < 0.05$). The relative passive force at the optimum length did not differ significantly between the two groups. When the fibre length was normalized to the optimum length instead of the slack length, the mean force length relation showed a more reproducible maximum (Fig. 3, lower panel). Seven fibres were stretched beyond the optimum length several times. After shortening or re-stretching the fibre, the optimum length was often found at a different fibre length (Fig. 4, upper panel). When the muscle fibre was shortened after stretching it beyond the optimum length, the maximum isometric force generally increased (Fig. 4, lower panel); in two out of three cases in which the fibres were stretched for a third time, the isometric force decreased. In two fibres (data not shown), the fibres were stretched far beyond the optimum length; upon shortening the isometric force did not increase, but remained constant.

The passive force increased continuously with stretched fibre length in each fibre and became larger than the isometric force at a length slightly greater than the optimum muscle length (Fig. 3, lower panel). At the optimum muscle length, the passive force was 1627 ± 834 μN, which is 68 ± 31% of the isometric force. The passive force showed hysteresis: it decreased faster than it increased when the fibre length was cycled (Fig. 5). At

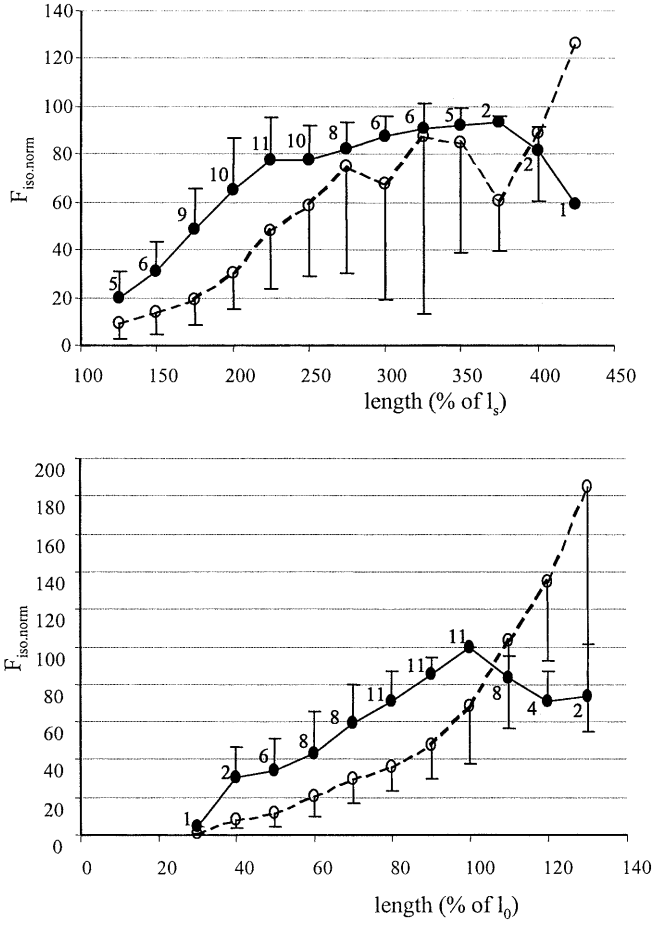

Fig. 3 The force length relation of pig urinary bladder smooth muscle. The upper panel shows the mean normalized isometric (●) and passive force (○) as a function of the fibre length relative to the slack length. In the lower panel the forces are plotted as a function of the fibre length relative to the optimum length ($n = 11$). The error bars represent the standard deviation and the numbers at the markers indicate the number of fibres in which a measurement was made at this length

Table 1 The effect of taking a number of measurements at a fibre length shorter than the optimum length before determining the optimum length, on the position and the magnitude of the maximum isometric force. For the fibres in group 1, the optimum length was determined straightaway; for the fibres in group 2, one or two force velocity measurements were made at a smaller fibre length before the optimum length was determined (*$P < 0.05$)

	Group 1	Group 2
Number of fibres	4	7
l_0 (% of l_s)	364 ± 10	248 ± 15*
$F_{iso.max}$ (μN)	3317 ± 245	2122 ± 423*
$F_{pas.max}$ (μN)	2111 ± 543	1351 ± 215
$F_{pas.max}$ (% of $F_{iso.max}$)	62 ± 13	72 ± 13

repeated stretching and relaxing of the fibre, the hysteresis sometimes disappeared. During the first increase in fibre length, the normalized isometric force was uniquely related to the passive force (Fig. 6, upper panel), with an optimum at 80% of the maximum isometric force and a coefficient of variation of 10%. When the fibre was

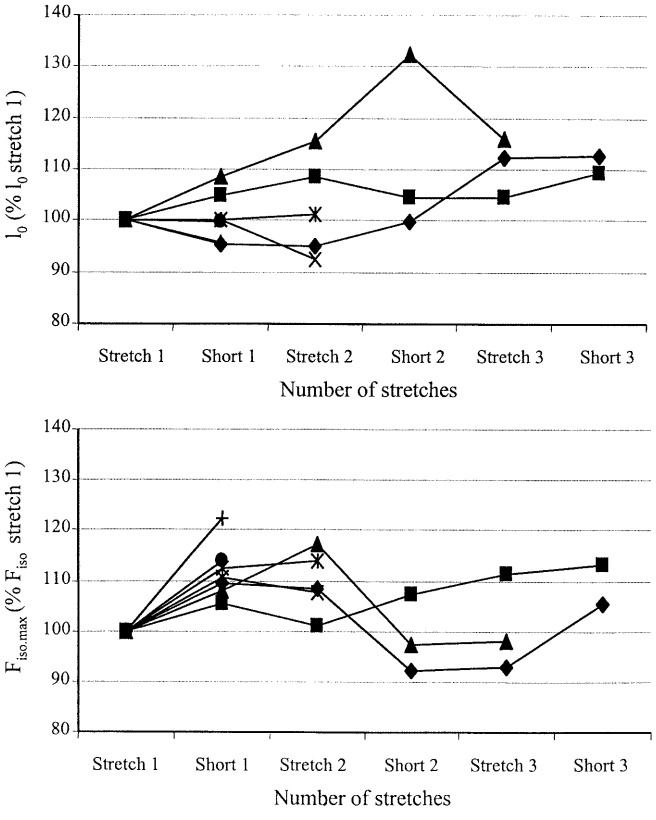

Fig. 4 The upper panel shows the effect of repeated stretching and shortening on the optimum length. Lengths are expressed as a percentage of the initially measured optimum length. The change in isometric force during shortening and re-stretching is given in the lower panel. The isometric forces are related to the first measured maximum isometric force. (7 fibres, 13 re-stretches)

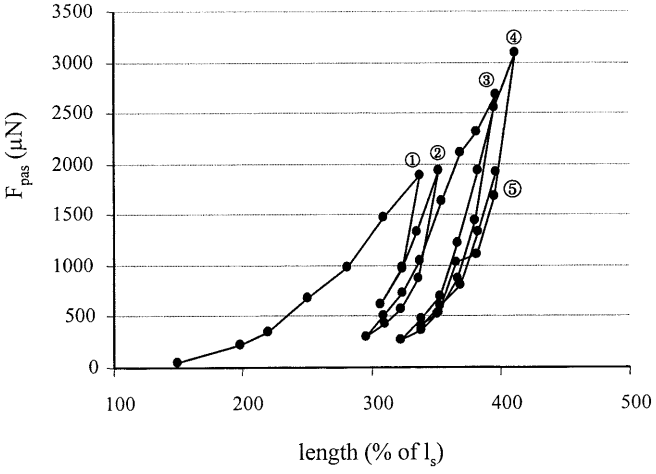

Fig. 5 An example of hysteresis of the passive force. At the second and fourth increase in length, the passive force was much lower than at the previous stretch. At the third and fifth increase in length, there was no hysteresis; the passive force was almost the same as at the previous stretch

re-stretched several times, there was a large variation in passive force values. When all values were pooled, the relation between passive and active force showed a coefficient of variation of 38% (Fig. 6, lower panel).

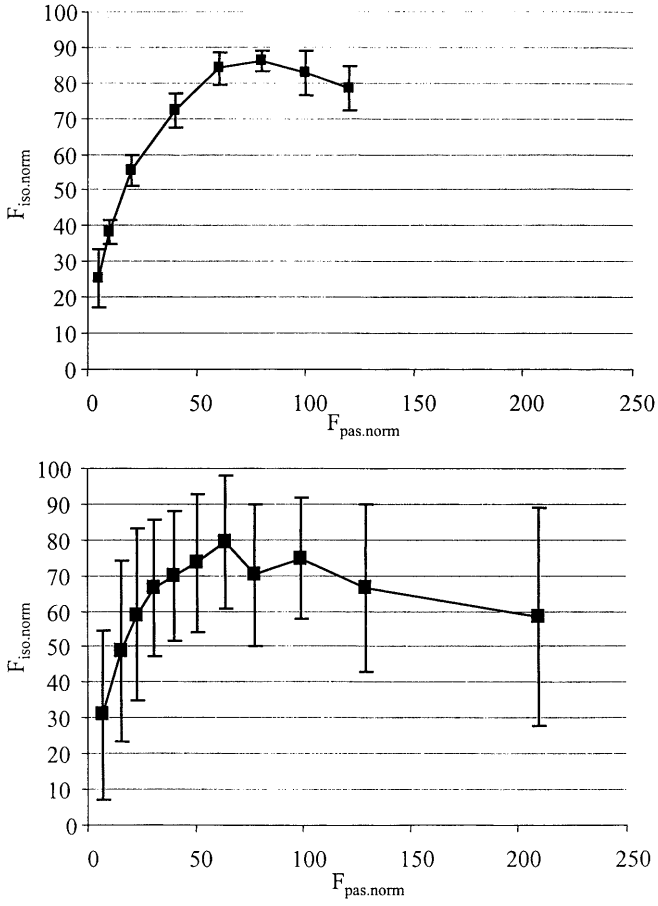

Fig. 6 The isometric forces were plotted as a function of the passive force. In the upper panel the normalized isometric force values measured only during the first increase in fibre length are plotted. The lower panel shows all measured isometric force values measured during repeated stretch and shortening cycles in 11 preparations

The force velocity relation

The relative force decreased with increasing shortening duration until a minimum was reached after about 2 s (Fig. 7). The recovery force did not depend on the shortening duration. In further measurements, 2 s was therefore taken as shortening duration. The coefficient of a regression line fitted to the recovery force as a function of the shortening velocity (Fig. 8) was very small, so that it may be considered independent of it. Figure 9 shows an example of a complete force velocity relation measured in one fibre.

The maximum shortening velocity relative to the slack length was 0.37 ± 0.14 s^{-1} (16 fibres, 55 different stop lengths). Some fibres showed a decrease in shortening velocity when the fibre length was decreased and an increase when it was re-stretched, but the mean shortening velocity at short fibre lengths was not significantly different from the shortening velocity at greater fibre lengths (Fig. 10, upper panel). A Friedman's rank test was also done to test for a length dependence and measurement order dependence of the

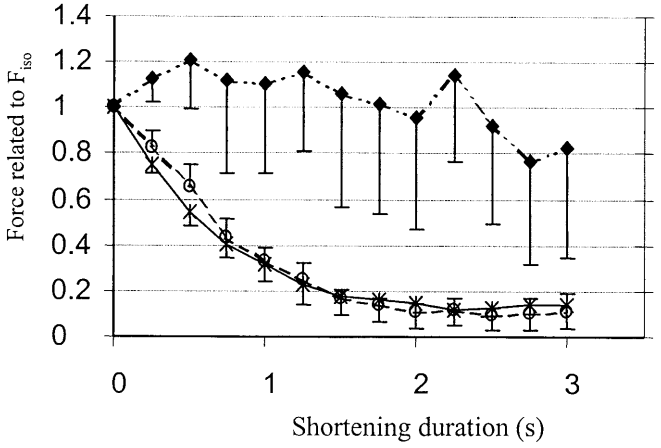

Fig. 7 The effect of shortening duration on the recovery force (◆), the force during shortening at the stop length (○), and the relative force (∗) ($n = 3$). The forces are related to the isometric force at the stop length measured without prior shortening. The shortening velocity was 100 μm·s^{-1}

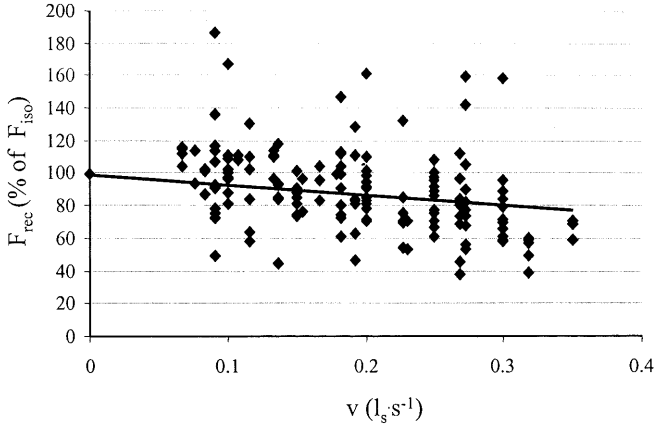

Fig. 8 The effect of the shortening velocity on the recovery force. All measured values are plotted. The regression line was: $F_{rec} = -66.483 * v + 100$ $r^2 = 0.1513$

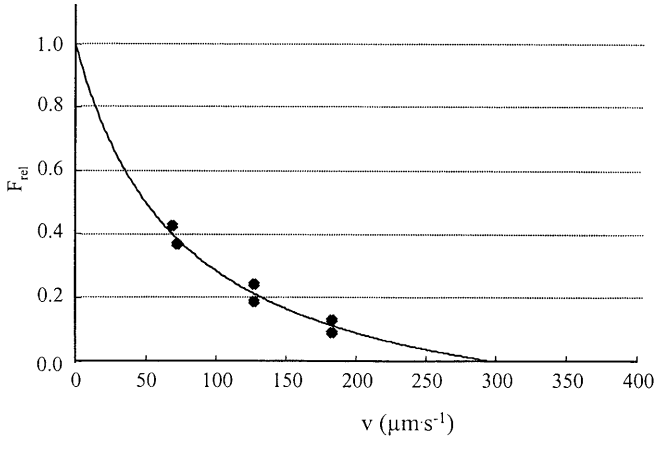

Fig. 9 The force velocity relation of one muscle fibre at one stop length. The drawn line represents a relative Hill equation with a relative force of 1, an a/F_0 of 0.25, and a maximum shortening velocity of 298 μm·s^{-1}

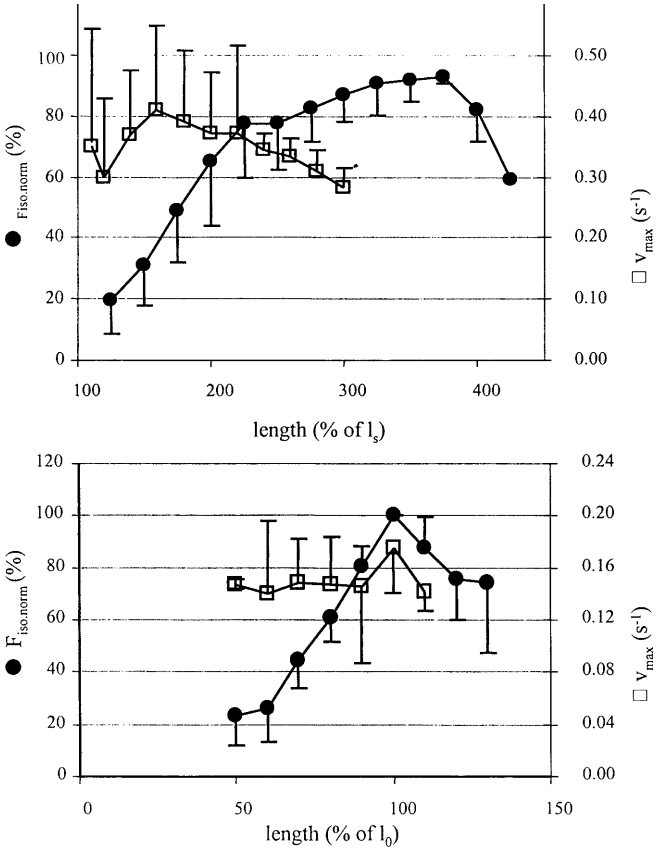

Fig. 10 The length dependence of smooth muscle contractility. The upper panel shows that in contrast to the isometric force (●), maximum shortening velocity (□) is not length dependent. Both length and velocity are related to the stretched length normalized to the slack length ($n = 11$ for F_{iso}, and $n = 16$ for $v_{max,}$ measured in different fibres). The lower panel shows length and velocity data measured in the same fibres and related to the stretched length normalized to the optimum length ($n = 7$)

muscle fibres. The results did not significantly differ from a random distribution. In seven fibres the length dependence of the maximum shortening velocity and the isometric force were measured simultaneously. In those fibres, both the maximum shortening velocity and the isometric force were plotted relative to the optimum fibre length (Fig. 10, lower panel). The maximum shortening velocity relative to the optimum muscle length was 0.13 ± 0.05 s^{-1}. For the same seven fibres, the maximum shortening velocity relative to the slack length was 0.30 ± 0.1 s^{-1}. In these fibres, the coefficient of variation was the same when related to the slack and optimum length (32 and 35%, respectively).

Discussion

Force length relation

The maximum isometric force of smooth muscle fibres of the pig urinary bladder is length dependent, as was found earlier in strips of the same preparation [4, 14]. The

optimum muscle length found in the present study was 290 ± 68% of the slack length (Fig. 3). This is in the same range as the optimum muscle length of bladder smooth muscles of hamster, rat, guinea pig, rabbit, and cat [12]. Furthermore, our maximum active stress (6.6 ± 2.7 $N \cdot cm^2$) is in the same range as the stress values measured in that study, but smaller than those measured by Uvelius (rabbit) [22]. The mean passive force increased with the stretched muscle length and was at the optimum muscle length approximately 70% of the isometric force, as was also found in the other species [12]. In our data the mean passive force (Fig. 3 upper panel) showed a depression at 275% and at 375% of l_s, which is caused by fibres for which the optimum isometric force was reached at a smaller fibre length and which were therefore not measured at larger fibre lengths.

When a number of measurements at a fibre length below the optimum length were taken before determining the optimum length, the maximum isometric force was smaller and was found at a smaller length, compared to the group for which the optimum length was first determined (Table 1). This may have been caused by damage to the muscle fibres. However, as the isometric forces at all fibre lengths were smaller in these fibres, it is more likely coincidental. At re-stretching a muscle fibre, the maximum isometric force was often found at a different fibre length (Fig. 4). Both results indicate that the force-length relation depends on the contraction history [6]. The maximum isometric force increased when the fibres were shortened after reaching the optimum length; this has also been found by Tammela [21].

Length dependence of maximum shortening velocity

In our study, we found that the maximum shortening velocity of the muscle fibres of the pig urinary bladder did not depend on the stretched fibre length when this was related to the slack length (Fig. 10, upper panel) or to the optimum length (Fig. 10, lower panel). This is in line with the assumption we made earlier,when applying the same method in strips of urinary bladder [4].

Our results are in accordance with measurements on skinned muscle fibres [13], but contradict measurements in intact muscle fibres [23], which showed a comparable length dependency of isometric force and maximum shortening velocity. The difference between these two studies may have been caused by the excitation contraction coupling mechanism [13]. As our study was made in intact muscle fibres, results comparable to Uvelius [23] were expected; however, the opposite was found to be true. There are two obvious differences between the two studies in the stimulation and measurements methods used. We used electrical stimulation whereas Uvelius [23] used K^+ stimulation. As electrical stimulation of muscle fibres comparable to ours resulted in the same isometric force as K^+ stimulation [11], the different stimuli probably did not cause the different results. As for measurement methods, we used the stop test method to measure force velocity data, whereas Malmqvist et al. [13] and Uvelius [23] used the quick-release method. Using this method, muscle fibres are released to different afterloads at different fibre lengths, so that the maximum shortening velocity is not measured at a constant fibre length, as in the stop test. This makes the quick release method less suitable for measuring the length dependence of contractility parameters. On the other hand, the stop-test method has the disadvantage that the muscle has to be stretched to large pre-shortening lengths. Another disadvantage of the quick release method is that it does not allow for correction for passive-force changes, which makes it especially unfit for measurements in smooth muscle where passive force amounts to 68 ± 31% of the active force at the optimum length. In our analysis, we assumed that the active and passive forces are independent and linearly add up to a total force, which is probably a simplification, as there may be an active response of the muscle fibre during unstimulated shortening. If we had not corrected our data for passive-force changes, the relative force would have been greater, and this would have resulted in a greater maximum shortening velocity. As at greater fibre lengths passive forces are higher, not correcting for passive force would result in greater maximum shortening velocities at greater fibre lengths, like those found by Uvelius [23].

Our results thus agree with the measurements on isolated smooth muscle actin and myosin filaments that suggest that variations in filament length and differences in the number of parallel coupled cross-bridges do not alter the maximum shortening velocity. In skeletal muscles it was found that only very short and very long sarcomere lengths influenced the maximum shortening velocity [1]. The data of Warshaw [24] in smooth muscle suggest that although increased actin filament length provides more opportunities for cross-bridge interaction, actin velocity is independent of the number of cross-bridges. This is also seen in skeletal muscle [7].

How to estimate the length of the contractile elements in smooth muscle?

For skeletal muscles, it is known that the isometric force depends on the overlap between the contractile filaments, and that the maximum shortening velocity depends on the number of sarcomeres [3, 9]. It seems likely that the same is true for smooth muscles [1], but unfortunately, the smooth muscle equivalent of sarcomeres that we call contractile elements in this study-cannot simply be identified. One possibility for estimating the number of contractile elements in this muscle type is to take a specific, well-defined muscle length [25]. We tested the optimum length and slack length as estimators for the number of contractile elements. We found that the optimum length depended on the contraction history: after re-stretching the same fibre, a different value was found. The coefficient of variation was the same if the shortening velocity of the complete fibre was related to

optimum length or to the slack length, so that the slack length and the optimum length were approximately to the same extent good or bad estimators of the number of contractile elements. As the procedure for determining the slack length has the lowest chance of damaging the muscle fibre, it is preferable above the optimum length.

A means of measuring the overlap between the contractile elements is the stretched fibre length divided by the number of contractile elements. As a good estimator for the number of contractile elements in the preparation is missing, we attempted in this study to relate the contractile properties measured to the passive force exerted by the muscle. When the fibre was stretched to increasing fibre lengths for the first time, the isometric force uniquely depended on the passive force. However, on successive stretch episodes the passive force dropped while the isometric force stayed at the same value or even increased (Figs. 5, 6). This makes the passive force a poor estimator for the overlap between the contractile filaments.

We also tested the stretched fibre length normalized to the optimum length and slack length as an estimate for the overlap between the contractile elements. Zderic [25] found that in rabbits, a fixed length of 250% of the slack length was a good estimation of the optimum length and found only a 10% error in maximum isometric force when this estimation was used. In our study, the averaged difference between optimum isometric force and the force at 250% of the slack length was 30%, which makes, in our view, a fixed percentage of the slack length a bad estimate for the optimum length.

Conclusion

To compare results measured in fibres of different lengths at different stretched lengths, estimators for the number of contractile elements and the overlap between the contractile filaments are necessary. We have tested three candidates for such an estimator: passive force, slack length, and optimum length. We conclude that slack length is the best parameter for normalizing measurements of the length dependence of smooth muscle. Using this normalization, we found that the maximum shortening velocity of smooth muscle is, in contrast to the isometric force, not length dependent. It follows that the dependence of maximum urinary flow-rate on voided volume that can be measured in volunteers and patients cannot be explained on the basis of muscular mechanics [16, 17, 20]. Further research into the mechanism causing this discrepency may lead to new non-invasive diagnostic tools.

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