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0014-4754/85/081010-08$1.50 + 0.20/0

Current problems in smooth muscle mechanics

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Key words. Smooth muscle; mechanics; length-tension relation; force-velocity relation; mechanical transients; cross-bridge properties.

The primary aim of studies on muscle mechanics is to establish relations between variables such as length, force, stiffness, velocity, and time in the relaxed state and during contraction. It is often useful to describe these relations by fitting them to mathematical equations or in terms of analogue models, but the ultimate goal must be to explain the relations on the basis of the biochemical and ultrastructural properties of the tissue.

Among the mechanical relationships that have been studied in muscle research the *length-tension relation* is the most well known as it received attention already in the end of the 19th century. However, a proper understanding of its characteristic features was not obtained until the launching of the sliding filament mechanism of contraction in the mid 1950ies, and accurate quantitive comparisons between active force and filament overlap in the sarcomer were first reported in 1966[8].

Detailed determination of the length-active tension curve in smooth muscle is particulary difficult due to the fact that there is an appreciable passive force already at the length (L_0) where active force is maximal and that passive force rises steeply above this length. This is exemplified by figure 1 taken from the work of Herlihy and Murphy[11] on pig carotid smooth muscle. Note that the spread of data increased very much above L_0 and that the descending curve could not be followed down to zero force. Mulvany and Warshaw[20] studied length-tension relations of arterial smooth muscle from small vessels and used a quick release technique to separate passive and active forces above L_0. They found that the descending part of the active curve was reasonably straight and extrapolated to zero force at about 1.8 L_0.

It is considered that the early compliant part of the passive length-tension curve in blood vessels reflects the stretching of elastin fibers and that the later, stiffer part is due to collagen[21]. If it is true that the smooth muscle cells in spite of their cytoskeleton of intermediate filaments do

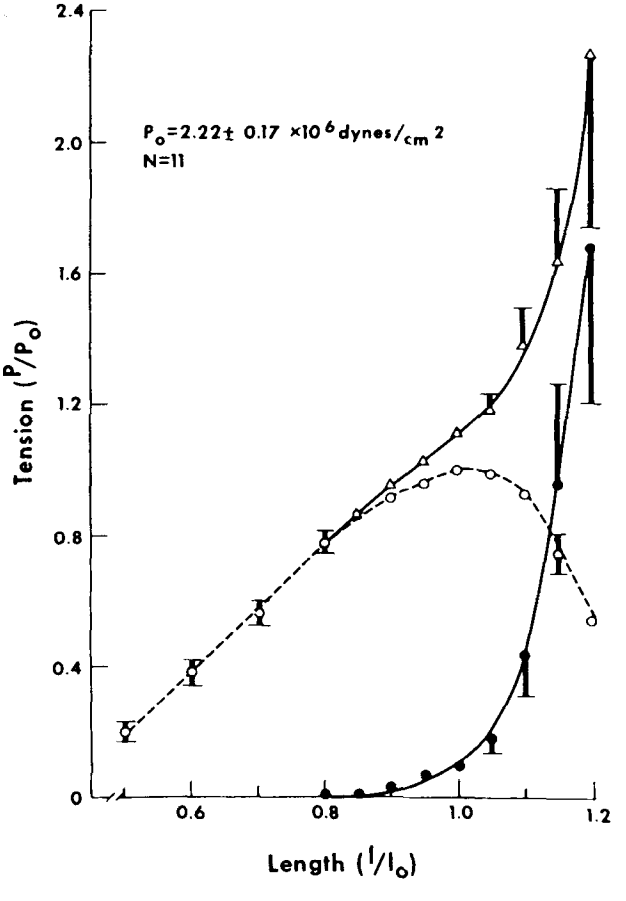

Figure 1. Length-tension relations of carotid artery smooth muscle. The top curve (triangles) shows tension as a function of length for the potassium-depolarized muscle in physiological salt solution containing 5 mM $CaCl_2$. The bottom curve (solid circles, solid line) is the resting tension curve. The difference between the two curves is the active length-tension curve (open circles, broken line) (from Herlihy and Murphy[11]).

not contribute appreciably to the passive force of the intact tissue we should be able to get a reliable picture of the active force curve of the contractile machinery from studies of isolated smooth muscle cells. Force measurements on isolated cells from moluscan smooth muscle showed that passive force was indeed minimal over most of the working range of the cell[13]. The active force curve was fairly symmetric and extrapolated to zero at about $2 \times L_0$. We seem to need studies on vertebrate smooth muscle where cell lengths are determined in the intact tissue at different degrees of stretch and where isolated cells from the same tissue are then studied at the respective lengths so that we can tie tissue and cell mechanics together in a quantitative manner. The fact that stretching of smooth muscles gives a proportionate increase of cell and tissue length[22] provides a solid basis for this type of experiment.

I have found only one report on smooth muscle where the length active tension curve shows a distinct plateau with sharp corners and again the results come from a moluscan preparation, the ABRM, as studied by Cornelius and Lowy[3]. This muscle of course has a different filament arrangement than vertebrate smooth muscle.

Therefore, in summary, we must admit that we still have difficulties in describing the length-tension relation of vertebrate smooth muscle adequately. However, with further progress on the structural side both with electron-microscopy and light microscopy we should finally get an explanation of the relationship also in this contractile system.

The *force-velocity relation* was first appreciated by muscle physiologists in the 1920ies and 30ies. It is usually described by Hill's equation for a rectangular hyperbola with the parameters a/P_0 and b, although systematic deviations from the hyperbolic curve have been reported for experimental data both at the highest and the lowest force levels[5,6]. Extrapolation of the hyperbola to zero force gives the maximal velocity of isotonic shortening, V_{max}, a value which correlates with the maximal ATPase activity of the actomyosin and probably reflects the kinetics of the mechanical cross-bridge cycle.

There are different ways in which the force-velocity curve may be obtained experimentally. The most common procedures are to look at afterloaded contractions or to perform isotonic quick releases. Both methods have their advantages and disadvantages, but it is important to realize that the results may come out differently. Velocities measured in afterloaded contractions of smooth muscle at graded loads will most likely represent different degrees of activation and different lengths of the contractile element. The data points therefore will not represent one single instantaneous force-velocity curve but will be taken from different members of the family of force-velocity relations through which the muscle goes during activation and internal shortening. These particular problems are circumvented by subjecting the muscle to quick releases at a given point in time during the isometric twitch. Figure 2 shows that data from afterloaded contractions of portal vein extrapolate to a much lower V_{max} than the data from quick releases in the rising phase of the isometric contraction[9]. The major problem with quick releases on smooth muscles is that the shortening velocity does not really stay constant over any period of time. There is a gradual decay of velocity with shortening so that one actually has to pick a standard but somewhat arbitrary point in time after the release where measurements are made. Studies of isotonic quick release responses with techniques that allowed high time resolution showed a biexponential decrease of the shortening velocity[10,15]. The early transient component will be considered below, but the subsequent slower phase was attributed to the force-velocity characteristics of the contractile element after adaptation to the new load. It was conceivable that the late exponential decay of shortening velocity after the release was simply due to the influence of the length-active tension relation since the experiments were done at lengths $< L_0$[10]. However, other factors may contribute to the phenomenon.

Additional techniques are available for the study of the force-velocity curve. One can estimate contractile element shortening velocity from the rate of change of isometric force if the load extension curve of the series elasticity is first established. One can also carry out isovelocity stretches and releases on the contracted muscle and measure the forces during such imposed length changes. Again the force-velocity relations obtained with this method can differ from those obtained with other techniques[14]. The so called 'slack test' method[4] is an attractive way of measuring V_{max} directly without having to extrapolate from other force-velocity data. When used on smooth muscle at least, attention has to be paid to variations in the results due to changes in the size of the release step[1].

In summary, the force-velocity relation and the V_{max} value provide interesting information on contraction kinetics but it is important to be aware of the variations that can be caused by the measuring techniques; for instance, moderate differences in V_{max} between different

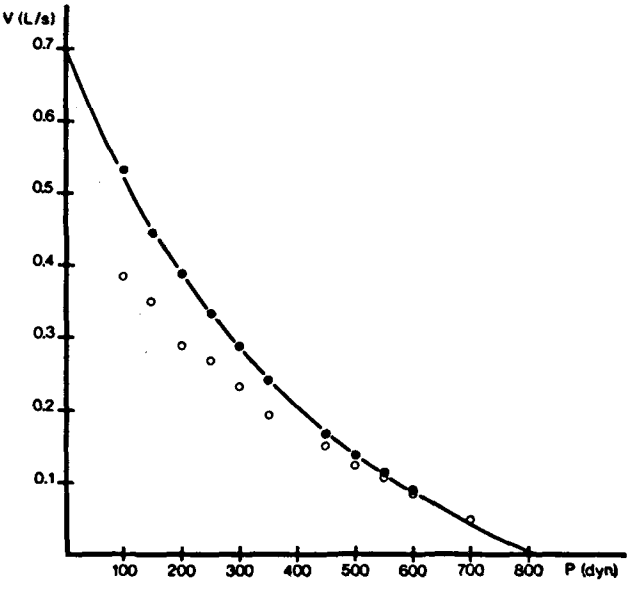

Figure 2. Force-velocity curves in phasic contractions of rat portal vein obtained by two different methods. Open circles: afterloaded isotonic contractions. Filled circles: quick release at instantaneous tension of 630 dyn. Solid line fitted to quick release points by Hill's equation with $a/P_0 = 0.69$, $b = 0.48$ lengths/s. Peak isometric tension 810 dyn (from Hellstrand and Johansson[9]).

smooth muscles should not be overemphasized particularly if the results originate from separate laboratories. The length-tension relation and the force-velocity relation both represent steady-state situations in the muscle. Studies of striated muscle mechanics in the recent decade have been more concerned with the *transient responses* to sudden perturbations in length or force[2,12] considering that such responses place greater constraints on models of contraction and can be more helpful in differentiating between alternative models. Studies of such transients obviously require good time resolution and minimal distortion from levers, transducers and recording systems.

Our own studies on portal vein and urinary bladder smooth muscle have mainly been concerned with isotonic velocity transients[10,15]. As exemplified by figure 3, the shortening that followed the immediate elastic recoil could be separated into two exponential phases of which the fastest had a time constant of 15–30 ms at 37 °C. The amount of shortening associated with this process correlated with the size of the force step and reached a maximum of about 1.2% of the muscle length. We considered the possibility that this transient reflected a cross-bridge response in analogy with interpretations from skeletal muscle. Its amplitude of 1.2% would then reflect the operation range of the cross-bridge within the half sarcomer and the figure seemed rather similar to values from skeletal muscle. The slower time course of the smooth muscle transient compared with skeletal muscle seemed to fit with the lower V_{max} of the smooth muscle and the finding of Marston and Taylor[16] of a long-lived state of actin-myosin association in the biochemical cross-bridge cycle of the latter tissue.

Transient events of similar type following step changes in length or load have been examined in arterial smooth muscle and in isolated amphibian smooth muscle cells by Mulvany[19], and by Warshaw and Fay[23], respectively. Results from these studies will be presented in their contributions to this issue.

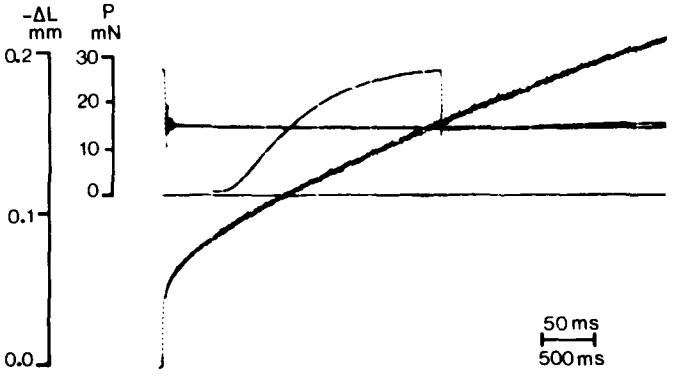

Figure 3. Oscilloscope recordings of force and change in length from experiment on AC- stimulated preparation of urinary bladder smooth muscle. Slow sweep shows the time course of the isometric contraction and the load step elicited by release of the isotonic lever. Fast sweep, triggered by the release, illustrates the force step and the change in length. The most rapid responses, not clearly visible on the film, have been indicated by dotted lines. Zero force baseline indicated by thin horizontal line.

The change in length is seen to consist of a rapid recoil and a subsequent shortening, the velocity of which decreases rapidly within the first 50 ms and more slowly thereafter. Modified from Hellstrand and Johansson[10].

A different kind of transient event was studied in frog sartorius by Flitney and Hirst[7]. When subjected to isovelocity stretches during contraction this muscle showed an early steep rise in force followed after about 1.2% lengthening by a distinct yield, most clearly seen with faster stretches. This yield point was associated with a sudden elongation of the sarcomeres. It corresponded to a filament displacement of 11–12 nm and this was thought to represent the range of movement over which the cross bridge could remain attached. A similar response was seen by Moss et al.[18] in cardiac muscle.

In smooth muscle this type of experiment has been done by Meiss[17] on mesotubarium and by us on portal vein[14]. In the latter tissue the two phases of the force response are clearly visible but the break point is not very distinct probably due to extracellular series compliance. In mesotubarium Meiss found a break point corresponding to 1.7% of the muscle length and with reservations regarding the lack of precise structural information he came up with a figure for single cross-bridge deformation of 18.5 nm in smooth muscle. This would probably be an interesting kind of experiment to do also in the isolated smooth muscle cells.

In summary, studies of smooth muscle mechanics have revealed mechanical relations and transient phenomena which in principle bear much resemblance to corresponding events in striated muscle. This suggests that we may be dealing with similar mechanisms of contraction in the two tissues. However, from a quantitative point of view smooth muscle mechanics still struggles with lack of precision in some measurements and with difficulties in associating mechanical responses with structural properties. Particular caution is required in attempts to use time courses and magnitudes of transient mechanical events to identify characteristics of individual cross-bridge structures in the smooth muscle.

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Energetics and regulation of crossbridge states in mammalian smooth muscle

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Key words. Smooth muscle energetics; light chain phosphorylation; crossbridges; calcium.

We have been studying the relationships between mechanical output and chemical energy input in smooth muscle in order to gain an understanding of the mechanisms by which energy derived from high energy phosphates is transduced into mechanical work. We have done this by determining the mechanical characteristics and associated energy utilization in smooth muscles under different mechanical conditions including rest, during an isometric tetanus, relaxation and isovelocity stretch. We have also attempted to learn how contractile activity may be regulated, specifically with respect to the role of phosphorylation of the 20,000-dalton light chain of myosin. The results to be summarized here represent a synthesis of work we have done in recent years and our current views on the existence and regulation of crossbridge states in smooth muscle.

Methods

The taenia coli muscle of immature virgin female rabbits was isolated and divided into three segments about 15 mm long and weighing about 15 mg. The segments from each muscle were subjected to similar or different experimental designs and compared internally. All of the methods for dissection, the apparatus for measurement of isometric tension, isovelocity stretch and maximum velocity of shortening, procedures for measurement of high energy phosphate usage and degree of myosin light chain phosphorylation have been described in detail previously[12,14,15,32,33] and will not be repeated here.

Briefly, the muscles were bathed in plexiglass chambers containing flowing, oxygenated Krebs bicarbonate solution (in mM/l, NaCl, 118; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; $CaCl_2$, 1.9; and glucose, 11; bubbled with a mixture of 95% O_2, 5% CO_2, pH 7.4) at 21 °C, and were allowed to equilibrate for 2 h at 2×10^{-2} Newton prior to any experimental maneuvers. The muscles were stimulated supramaximally in a transverse field with platinum-platinum chloride electrodes and 10 V rms 60 Hz AC pulses. For measurement of high energy phosphate usage, recovery reactions of glycolysis and respiration through which ATP synthesis occurs were blocked by treatment of the muscles in a Krebs solution lacking glucose and containing 0.5 mM iodoacetic acid and 5.0 mM sodium fluoroacetate under anaerobic conditions. The validity of this method has been described previously[12,32].

According to the particular experimental design, the muscles were quickly freeze-clamped at liquid nitrogen temperature, and the frozen tissues were extracted with 0.5 N $HClO_4$, neutralized and analyzed by liquid chromatography for ATP and ADP and spectrophotometrically for phosphocreatine (PCr) and total creatine (Ct) (see Butler et al.[12] for details). High energy phosphate usage ($-\Delta \sim P$) is calculated as the sum of the changes in PCr and ATP contents and is expressed relative to the total creatine content which is 2.7 μmoles/g wet wt[12].

The degree of myosin light chain phosphorylation was determined from tissues frozen and extracted in a manner identical to that used for metabolites. The perchloric acid insoluble material was dissolved in a solution containing 9.0 M urea, 5% (wt/vol.) 2-mercaptoethanol, 1.5% pH 5–7 and 0.5% pH 7–9 ampholines (LKB Instruments), and subjected to isolectric focussing followed by electrophoresis in sodium dodecyl sulfate in the second dimension[14]. The second dimension gel was stained with Coo-