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Review

Do cross-bridges contribute to the tension during stretch of passive muscle?

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Abstract

The tension rise during stretch of passive skeletal muscle is biphasic, with an initial steep rise, followed by a subsequent more gradual change. The initial rise has been interpreted as being due to the presence of numbers of long-term, stable cross-bridges in resting muscle fibres. A point of weakness with the cross-bridge interpretation is that the initial stiffness reaches its peak value at muscle lengths beyond the optimum for myofilament overlap. To explain this result it has been suggested that despite the reduced overlap at longer lengths, the closer interfilament spacing and a higher sensitivity of the myofilaments to Ca^{2+} allows more stable cross-bridges to form. Recently the stretch responses of passive muscle have been re-examined and it has been suggested that it is not necessary to invoke cross-bridge mechanisms at all. Explanations based on a viscous resistance to interfilament sliding and mechanical properties of the elastic filaments, the gap filaments, were thought to adequately account for the observed tension changes. However, an important property of passive muscle, the dependence of stretch responses on the immediate history of contraction and length changes, thixotropy, cannot be explained simply in terms of viscous and viscoelastic properties. The review discusses the cross-bridge interpretation of muscle thixotropy and the relationship of passive stiffness to filament resting tension and latency relaxation. It is proposed that cross-bridges can exist in three states; one, responsible for the resting stiffness, requires resting levels of calcium. When, during activation, calcium levels rise, cross-bridges enter a low-force, high-stiffness state, signalled by latency relaxation, before they move to the third, force-generating state. It is concluded that, compared with viscoelastic models, a cross-bridge-based explanation of passive muscle properties is better able to accommodate the currently known facts although, as new information becomes available, this view may need to be revised.

Introduction

Most accounts of mechanical properties of muscle focus on force generation during a contraction. The principal role of muscle is, after all, to provide the forces necessary for maintenance of posture and to initiate movements. The properties of passive muscle, that is, the mechanical changes observed during lengthening and shortening of relaxed muscle, tends to receive less attention. Yet passive properties play as important a role as properties during a contraction. Take, for example, the simple act of flexing the elbow. Flexor muscles contract and shorten in the process stretching the inactive extensors. An important component of the load on the contracting flexors will be the passive stiffness of the stretching extensors.

But the mechanical load represented by stretch of passive muscle is only one consideration. So, for example, signals coming from muscle spindles in the stretched muscles, are likely to be more important than those from the shortening muscle in providing us with information about the position and movement of our arm (Ribot-Ciscar and Roll, 1998), and the action of spindle impulses on motoneurones will add reflex support to any centrally generated motor commands (Macefield *et al.*, 1993). If the muscle is repeatedly stretched and, particularly, if it is generating some active tension during the stretches, this can lead to muscle damage, soreness and subsequent remodelling of the muscle (Jones *et al.*, 1997; Macpherson *et al.*, 1997).

Stretch of a muscle not only expresses itself as tension at the tendon but acts within the muscle as an internal trigger for adaptive processes. It has been known for a long time that maintained muscle stretch, particularly if combined with electrical stimulation, can lead to alterations in the number of sarcomeres in fibres (Goldspink, 1985). More recently, progress has been made in our understanding of the molecular events, triggered by muscle stretch, which lead to growth of developing muscle fibres, to exercise induced hypertrophy and to re-modelling upon damage (Goldspink, 1999).

Finally, while this review focuses on mechanisms at the level of sarcomeres, in the freely moving animal, passive properties will also include those of the tendon, particularly when it is in the 'toe' region of its tension-

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extension relation. So for the first 1% or so of strain of a just-taut tendon there is very little tension developed and stiffness remains low. Further strain results in a steep increase in tension. It means that when a passive muscle and its tendon are stretched, initially most of the movement is taken up by the tendon and only when tension begins to rise are the muscle fibres themselves stretched (for a review see Proske and Morgan, 1987).

The motivation to write this review was provided by the unexpected flare-up of a controversy. It has been known for a long time that the rise in tension seen during slow stretch of a resting skeletal muscle cannot be adequately described in terms of purely elastic behaviour. The tension rise is biphasic and the size of the discontinuity depends on whether or not another stretch had been given in the immediately preceding period. The experiments in 1968 by D.K. Hill, exploring aspects of passive properties, led to the formulation of a comprehensive theory to explain these phenomena. This view, based on the assumption of the presence of cross-bridges between actin and myosin within sarcomeres of resting muscle fibres, was able to explain many of the older observations made on passive muscle, and several made more recently. However, in the past few years it has been realised that a major contributing factor to the passive length-tension relation of muscle is provided by elastic filaments, the gap filaments, spanning each half sarcomere, and composed of the extensible protein titin, which is attached at one end to the Z line and at the other to the thick filament. Titin is now thought to be one important source of passive tension in muscle (Wang *et al.*, 1991). As more knowledge about the elastic filaments has become available it has led to questioning of the existing theories about passive muscle properties. In particular, the ideas about a contribution, if any, to passive properties from cross-bridges have come under scrutiny. This has generated a revival of the debate about the origins of passive muscle stiffness. As often happens in science, we may have been swayed by the freshness and novelty of the new ideas. If the original theory is able to survive this challenge it will be the stronger for it. If not, it must move aside and make room for the new ideas.

Here we sketch out the background leading to the present-day controversy over passive muscle properties. We have taken the view that a comprehensive theory must be able to incorporate all of the known facts, some of which have emerged only recently. It is our conviction that such a discussion is useful, both in laying down the foundations for future experiments and for helping us to acquire a broader view of muscle, one that takes into account all of the properties required for everyday activities.

Background

Short-range elastic component

In response to a slow stretch, resting skeletal muscle does not behave like a purely elastic structure. Liddell and Sherrington (1925), describing the response to stretch of the denervated vastocrureus muscle of the decerebrate cat, noted 'a slightly steeper upgrade of tensile resistance at commencement of the stretch'. Four years later Denny-Brown (1929), also studying the decerebrate cat, commented on the initial steep tension rise in response to stretch of the denervated semitendinosus muscle. He noted that the size of the initial stiffness was smaller in response to the second of two stretches, given three seconds apart, and stated that the 'preliminary rigidity' took less than one tenth of a second to appear. He attributed this behaviour to the muscle fibres themselves since stretching a piece of semitendinosus tendon produced a tension rise with the expected 'viscoid' reaction but no evidence of any preliminary rigidity.

A detailed description of this 'spring-like' elastic resistance at the beginning of a stretch of resting muscle was made by D.K. Hill (1968) recording tension in the frog sartorius muscle in response to stretches with a range of velocities from 0.2×10^{-4} muscle lengths (ML)/s to 5 ML/s. Since this initial stiffness persisted for only a small part of the length change he called it the short-range elastic component (SREC). Hill chose to study passive properties in hypertonic solutions which greatly increased the size of the SREC, presumably because it causes fibre shrinkage. He also noted a rise in permanent resting tension, accompanying the increase in SREC, which formed part of the response to the hypertonicity. He called this the filament resting tension (FRT) and proposed that the drop in tension immediately preceding a contraction, called latency relaxation (LR), was due to a fall in FRT. Hill suggested that the elastic properties of the SREC were due to the mechanical stiffness of a small number of cross-bridges between actin and myosin in sarcomeres of resting muscle. If the velocity of the length change was not too low, the cross-bridges did not significantly detach during the early stages of the stretch so that the tension rise was linearly related to the size of the stretch, justifying use of the term 'elasticity'. Once the elastic limit had been exceeded, the rate of detachment increased rapidly, tension levelling off or falling slightly to give a constant 'frictional resistance'. The term 'frictional' was used since the force developed depended only slightly on the velocity of the length change, in the lower range of stretch rates used. The calculated elastic modulus was found to increase with temperature in the range 6–24°C. Three pieces of evidence were put forward in support of the proposal that LR represented a reduction in FRT. The first was that LR became larger, as did FRT, in a hypertonic solution. Secondly, both were essentially independent of muscle length in hypertonic solution. Thirdly, the calculated elastic modulus for the SREC diminished during the latent period.

Confirmation of SREC-like behaviour at the level of single isolated muscle fibres was provided by the experiments of Lännergren (1971). He showed that the SREC persisted for about 0.2% of the length change,

becoming larger in hypertonic solution and smaller when measured a few seconds after a twitch, taking 3 min to recover fully. In discussing his findings, Lännergren argued that if the SREC and active tension were both due to cross-bridges, stiffness was expected to increase rather than decrease during low-level activation of the muscle. He concluded that the measured elastic modulus might reflect some component of the muscle fibre other than the cross-bridges. The point here is, of course, that during activation the response of the muscle to a slow stretch involves cross-bridges that are actively cycling, leading to a lower effective stiffness than during stretch of SREC bridges in passive muscle which have a much lower turnover rate.

Effect of calcium

The problem was approached from a rather different point of view by Moss *et al.* (1976). They showed that both mechanically skinned and chemically skinned muscle fibres of frog did not exhibit a SREC in normal relaxing solutions containing low calcium concentrations ($[Ca^{2+}]$). However, raising $[Ca^{2+}]$ slightly, to levels just below those required for activation, re-established stretch responses typical of those of living passive muscle. Chemical treatment disrupts the sarcoplasmic reticulum. The fact that in chemically skinned fibres, in solutions with $[Ca^{2+}]$ just below activation levels, a SREC-like behaviour could still be observed was taken as evidence that the sarcoplasmic reticulum was not involved in generating the SREC. It was concluded that one explanation of the data was that calcium was required for the development of SREC, that is, in skinned fibres normal relaxing solutions lowered $[Ca^{2+}]$ to values below those at which cross-bridge formation associated with SREC could occur.

A point of difficulty with a cross-bridge origin of SREC is that its dependence on muscle length does not parallel that of active tension. There is an increase in SREC in a region of reduced myofilament overlap, followed by a fall at very long lengths (Hill, 1968; Sandow, 1970; see also Haugen and Sten-Knudsen, 1981). However it has been known for some time that changing the length of a muscle fibre not only changes the degree of overlap between myofilaments but modifies other processes associated with the contraction. In particular the sensitivity of the myofilments to $[Ca^{2+}]$ changes with muscle length. This was first shown by Endo (1973) on mechanically skinned amphibian muscle fibres. He found that when sarcomere length was increased from 2.0 μm to 2.9 μm, the threshold for contraction shifted towards lower $[Ca^{2+}]$ by a factor of 1.4. More recent work has shown that the entire force-pCa curve is shifted, not just the threshold (for a review see Stephenson and Wendt, 1984). However, Balnave and Allen (1996) reported for single fibres of mouse muscle that while at moderately long lengths Ca^{2+} sensitivity increased above the level at optimum, stretching the muscle further led to a fall in Ca^{2+} sensitivity. It remains uncertain how closely the length dependence of SREC parallels that of changes in Ca^{2+} sensitivity. On the assumption that SREC requires a certain level of sarcoplasmic $[Ca^{2+}]$, if sensitivity of the myofilments to $[Ca^{2+}]$ increases at moderately long sarcomere lengths, this would tend to increase SREC and so oppose any decrease in SREC due to reduced filament overlap. Presumably there is an interplay between these two factors, in addition to any effects attributable to a reduced interfilament spacing, the outcome being a net increase in SREC. At lengths approaching the region of no filament overlap the SREC would be expected to fall.

Latency relaxation

If cross-bridges play a role in the generation of SREC, the tension changes seen during the transition from rest to activity in muscle may provide further supporting evidence for such a view. One aspect of the transition is the small drop in tension that occurs at the onset of a contraction, LR.

For muscles bathed in isotonic solution, LR, like the SREC, was found by Hill and others to increase with muscle length over the range of sarcomere lengths with reducing myofilament overlap 2.4–3.0 μm (Haugen and Sten-Knudsen, 1976; Haugen, 1982). The idea that LR represented the release of FRT was supported by the finding that LR was absent preceding a second twitch when this was elicited a few milliseconds before the peak of the first twitch (Close, 1981). In addition, measurements of Ca^{2+} transients in single frog fibres with the dye Arzenazo III showed that the rise in sarcoplasmic Ca^{2+} and the onset of LR occurred at almost exactly the same time (Close and Lännergren, 1984; see also Claflin *et al.*, 1994). LR was found to be maximal at muscle lengths which corresponded to the point where about one half of the cross-bridge bearing part of the thick filaments was overlapped by thin filaments, that is, about 2.9 μm. This is about the same length at which SREC peaks (Haugen and Sten-Knudsen, 1981). In discussing his results, Close (1981) proposed that the length dependence of LR could be explained if (1) the resting force between filaments was directly related to the amount of overlap and inversely related to the lateral spacing and (2) the amount of interfilamentary force that was discharged during LR increased with the amount of Ca^{2+} bound to the thin filament.

A more recent, detailed study of the muscle length dependence of LR in single frog fibres was carried out by Claflin *et al.* (1990). They measured LR in combination with fibre stiffness, using high-frequency vibration. Measurements made before and during the onset of a contraction showed that the earliest measured stiffness increase always coincided with the start of the fall in tension during LR. This remained true even in the face of changes in LR produced by changing muscle length or from application of drugs. The finding was interpreted as being consistent with the view that LR had a cross-bridge origin. When the muscle twitch was

reduced with the Ca^{2+} release blocker D-600 (methoxyverapamil), the amplitude of LR and the stiffness rise during LR were both reduced much less than peak tension. These findings were used to argue for the existence of two cross-bridge transitions, one from the relaxed state to an intermediate state (high stiffness, low tension) and a second step from the intermediate state to the tension generating state. Both would be controlled by $[Ca^{2+}]$ but the second step would require higher $[Ca^{2+}]$. The time of onset of LR was found to be independent of sarcomere length while the time to peak of LR increased over the length range 2.5–3.2 μm. It was speculated that variations in onset time with sarcomere length, representing the time required for diffusion of sufficient Ca^{2+} to trigger the first transition, might be too small to detect. The higher $[Ca^{2+}]$ required for the generation of tension would be reached more slowly so that the peak of LR would show a measurable length dependence. In summary, the proposal was of cross-bridges which could be in three states, the resting state with low stiffness and producing low levels of tension (FRT), the intermediate state producing no tension but a rise in stiffness, and the active force generating state. Transitions between the three states were controlled by $[Ca^{2+}]$. The Ca^{2+} dependence was in the order, resting < intermediate < force generating. The origin of LR could be thought of as arising from a fall in FRT as a result of a large increase in the number of cross-bridges in the SREC state, leading to a small pushing force. The interpretation we prefer is that there is a conversion of all cross-bridges, including those in the SREC state, into an intermediate, stiff, but non force-generating state.

Additional observations on this subject were made by Morgan *et al.* (1990). They showed for single frog fibres that tension levels during fibre shortening at imposed velocities near the maximum shortening velocity were higher for passive than for active shortenings. Measurements of latency relaxation made in a rapidly shortening fibre showed that it was significantly larger than when measured under isometric conditions. It was concluded that a component of dynamic passive tension, perhaps the FRT, which at some lengths is the dominant component, is abolished by activation. The increase in latency relaxation during shortening would be the result of a pushing action by the stiffened sarcomere.

Figure 1 shows some unpublished data on the relation between muscle stiffness, LR and tension in a single living muscle fibre, using the compounds BDM (2,3-butanedione 2-monoxime) to reduce tension and D-600 to alter available $[Ca^{2+}]$. The D-600 data have been replotted from Claflin *et al.* (1990, Figure 6). When plotted on a double logarithmic scale, both the peak of LR and the increase in fibre stiffness during LR, vary with the size of the twitch, but not proportionately. So when the D-600 has reduced the twitch to 10%, LR and stiffness are still at 40% to 60% of maximum. The new observation reported here is the effect of BDM (Claflin *et al.*, unpublished observations). Both LR and stiffness

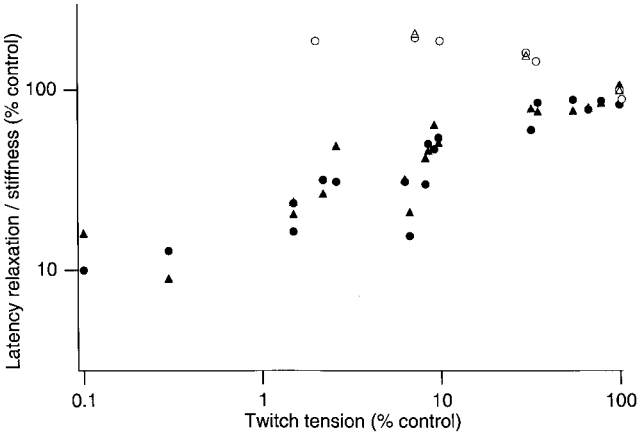

Fig. 1. A plot, for a single frog muscle fibre, of latency relaxation (circles) and stiffness rise during latency relaxation (triangles) both expressed as a percentage of control values, against the size of the twitch, also expressed as percent of control, in the presence of D600 (filled symbols) and BDM (open symbols). All of the measurements with BDM were made while monitoring peak $[Ca^{2+}]$ during the twitch with the dye Mag-fura. Note both axes are on a logarithmic scale. Stiffness was measured with a 2 kHz sinusoidal signal of amplitude equivalent to 0.5 nm per half sarcomere. The stiffness data has been re-plotted from Claflin *et al.* (1990), the BDM data are previously unpublished observations of Claflin, Morgan and Julian.

actually increase above control levels in the presence of BDM and remain there as the twitch falls with increasing BDM concentration. Peak $[Ca^{2+}]$, as indicated by the dye MagFura, fell by only about 10% over this range of force. So while peak tension has been reduced to less than one tenth of its original value, LR amplitude and stiffness rise during LR have doubled in size. To explain this result it is postulated that BDM blocks transition to the force generating step, leading to a large increase of cross-bridges in the intermediate state. The above findings emphasise the difference in action of BDM on the active cycling cross-bridges and on LR and stiffness. The parallel changes seen in LR and stiffness changes during LR indicate a close relationship between these two properties. Additional studies with BDM should be carried out to help further elucidate the intermediate state and its properties.

Thixotropy

Another aspect of muscle that must be accommodated in a comprehensive theory of passive properties is its thixotropic behaviour, that is, contraction and length dependent changes in responses to stretch. At long lengths a resting muscle will be taut regardless of its previous contraction history. At very short lengths, it will always be slack (Gonzalez-Serratos, 1971). However there is an intermediate length range where a muscle can be either taut or slack, depending on the history of contraction and length changes. The presence or absence of slack can dramatically alter the shape of the tension rise seen during stretch of a passive muscle. Slack can be introduced at a particular test length by contracting a muscle at a longer length, letting it relax completely and

then shortening it back to the test length. The slack can be removed by a contraction at the test length (Proske *et al.*, 1993). This is an aspect of passive muscle properties which has commonly been overlooked but which is important for a comprehensive description of muscle. It is a property which is difficult to account for by non-cross-bridge mechanisms of SREC.

The cross-bridge explanation (Lakie *et al.*, 1984; Morgan *et al.*, 1984) is that when a muscle is contracted at a particular length, once the muscle has relaxed, stable cross-bridges form in the fibres at that length to give them their SREC. If the muscle is now shortened, the compressive forces on sarcomeres, stiffened by the presence of the SREC bridges, may lead to detachment of some bridges, but insufficient for the fibre to be able to fully take up the shorter length without falling slack. The persisting cross-bridges presumably exert a pushing action, which opposes passive tension, to generate the slack. Given the postulated very low turnover rate of SREC bridges, the slack may remain for long periods, provided the muscle is left undisturbed. It also means that SREC bridges, when put into compression, do not rapidly detach as do actively cycling bridges, at least for small compressive forces.

When, in the absence of slack, the muscle is stretched, once the 'elastic limit' of the SREC has been exceeded, cross-bridges detach, the rate of detachment depending on the speed of the stretch. Since beyond the elastic limit tension does not fall rapidly to zero, it has been postulated that the bridges must reattach quite quickly. So the process envisaged during progress of a stretch is a continuous detachment and reattachment of cross bridges. It means that when the final length has been reached, the majority of cross-bridges within the muscle fibre will have formed at or near that length. If the muscle is now shortened back to the starting length, slack will develop. Our data suggest that to develop slack fully, the muscle must be held at the longer length for several seconds before returning it to the starting length (Morgan *et al.*, 1984). In a series of repeated stretch-shortening movements, the initial, steep tension rise in response to the second and subsequent stretches is delayed in onset due to the take-up time of the slack and the tension at the yield point is lower because of the slow rate of formation of SREC bridges. In this way the greater compliance of passive muscle after preceding movements is a combination of slack and a reduced number of attached SREC cross-bridges.

In their recent report of the responses of rat muscles to slow stretches, Mutungi and Ranatunga (1996b) describe that the discontinuity in the tension trace, called by them the 'apparent break point', was not reached until the stretch had reached 1–2% of resting length of the muscle, well beyond the 0.2% reported by Hill (1968). We have recently made similar observations on the cat medial gastrocnemius muscle (Whitehead, Morgan, Gregory and Proske, unpublished observations). Here it became apparent that the measured yield point depended on the previous history of contraction and length changes. Stretch of the passive muscle at 1 mm/s, 5 s after an isometric contraction required a length change of 0.6 mm to reach the yield point representing about 3% of the fire length (see Figure 2). Depending on the initial muscle length at which the measurements were made, after a conditioning contraction at a longer length the yield point could be increased to more than double this value, presumably because there was now considerable slack distributed amongst the muscle fibres and this had to be taken up before each fibre reached its yield point. Such an effect must be taken into account whenever responses of passive muscle to stretch are measured. The rather high figure of 3% for gastrocnemius we attribute to the wide range of fibre lengths in the muscle and the long tendon (Walmsley and Proske, 1981).

When a single muscle fibre is contracted isometrically at a sarcomere length just beyond 2.2 μm, and after a few seconds delay is shortened, the shorter length signalled by the transducer is not the actual length adopted by the fibre, which typically falls slack. This shows up as a delay in tension rise after onset of stimulation, a delay which is absent in the response to a second contraction. Presumably the contracting fibre shortens isotonically until the slack is fully taken up and tension can begin to rise. For a single tibialis anterior fibre of the frog the measured slack-removal time was 10 ms representing 0.6 mm of slack (Proske *et al.*, 1993).

Figure 2 shows an example of history dependent slack in whole mammalian muscle. The medial gastrocnemius muscle of the anaesthetised cat was contracted by stimulating its nerve at 60 pps for one second at the test length or at a length 3 mm longer than the test length. The portion of the range of physiological lengths over which these measurement were made, $L_m - 19$ to $L_m - 22$ mm (L_m = maximum body length), was chosen

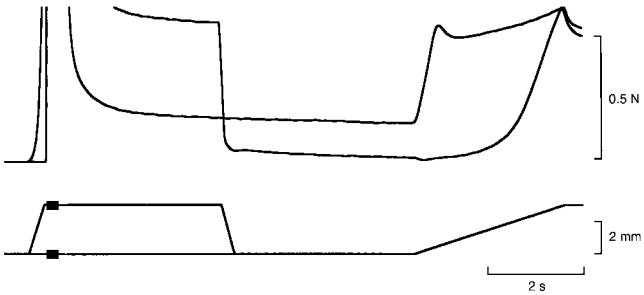

Fig. 2. Muscle history-dependent changes in passive tension in the medial gastrocnemius muscle of the anaesthetised cat. The upper pair of superimposed traces represent tension, the lower pair muscle length. A movement of the length trace upwards indicates muscle lengthening. The filled bars on the length traces indicate the duration of a tetanic contraction at 60 pulses/s. The tension records during the contractions are not shown fully. When the muscle is contracted at the longer length (maximum body length −19 mm), after return to the test length (maximum body length −22 mm) the level of resting tension is close to zero and a slow test stretch of 3 mm at 1 mm/s produced a delay in the rise of tension. When the conditioning contraction is carried out at the test length resting tension is higher and there is no delay in the rise of tension during stretch (Whitehead, Gregory, Morgan and Proske, unpublished observations).

to be near the slack length. When the muscle was contracted at $L_m - 19$ mm and then brought to $L_m - 22$ mm, a slow test stretch of 3 mm at 1 mm/s showed a delay in tension rise when compared with the response after a contraction at $L_m - 22$ mm. The difference in onset time of the rise in tension was 1.6 s, representing the time required to take up about 1.5 mm of slack. Given that the shortening step was 3 mm it means that about half of the shortening was taken up by muscle fibres and the rest went into producing slack.

Notice that after the contraction at the longer length, return of the muscle to the test length brought tension levels close to zero. That is, at this short length there was no passive tension. However after the conditioning contraction at the test length passive tension was higher by 0.14 N which we interpret as representing the FRT of D.K. Hill.

We have emphasised the concept of slack here because it is difficult to explain in terms other than the presence of SREC in muscle fibres. Slack is important because it can develop not only in the ordinary muscle fibres but in the intrafusal muscle fibres of muscle spindles. Given that intrafusal fibres make rather compliant connections with the surrounding extrafusal fibres, they are relatively free to move and readily fall slack, even at intermediate muscle lengths where whole-muscle passive tension has already become appreciable. Slack in intrafusal fibres leads to reduced strain on the sensory endings of spindles. That, in turn, lowers background levels of activity in spindles and reduces spindle stretch sensitivity. These are important factors when considering the role of muscle spindles in spinal reflex action and in proprioception. For a more detailed treatment of this topic see Proske *et al.* (1993).

Present-day controversies

There has been a recent revival of discussion about passive properties of muscle and the question of the cross-bridge origin of passive stiffness. It began with an enquiry about the possible presence of weakly attached cross-bridges in intact resting muscle (Bagni *et al.*, 1992, 1995). In relaxed, skinned muscle fibres rapid cross-bridge cycling had been thought to occur (Brenner *et al.*, 1982), resembling that observed in solution (Chalovich *et al.*, 1981), where the complexes M.ATP and M.ADP.Pi combine with actin. In intact muscle fibres Bagni *et al.* found no evidence of rapidly cycling, weakly attached cross-bridges. The tension responses during high-speed stretches, 2–6% of initial length (L_0) at 2–250 L_0/s were separated into three components. An initial rapid phase of tension rise, designated P_1, was attributed to a viscous element. The value of P_1 increased proportionately with stretch velocity, but with a finite intercept at zero velocity. This intercept was taken to represent the SREC of Hill. The rise in force beyond P_1 was described by a visco-elastic element called P_2 which had a relaxation time constant of 1 ms at 15°C. The final tension, measured 50 ms after the end of the stretch and designated P_3, was assigned to a non-linear elastic element. In skinned fibres Bagni *et al.* reported that all three components, although present, were much reduced in amplitude. However, the intercept of the force:stretch velocity plot for P_1 was now zero, in accord with earlier observations of Moss *et al.* (1976). Effects of altering resting $[Ca^{2+}]$ were not tried.

These observations were extended to mammalian muscle by Mutungi and Ranatunga (1996a,b, 1998). They measured tension and sarcomere length changes in response to ramp stretches of fibre bundles of the slow-twitch soleus and fast-twitch extensor digitorium longus muscles. However they included much slower stretch rates than Bagni *et al.* had used. With slow stretches the slow-twitch muscle showed two distinct tension discontinuities. The fast-twitch muscle showed similar discontinuities but they were much less clearly distinguishable. The first discontinuity, taken to be the P_1 of Bagni *et al.*, occurred near the point where the stretcher had reached maximum velocity and its value was proportional to velocity. This discontinuity became difficult to discern at the lowest stretch velocities. The second discontinuity, the 'apparent break point', was not reached until the stretch had exceeded 1–2% of resting length for slow-twitch muscle, while for fast-twitch muscle it was less than 1%. This component showed a weak dependence on stretch velocity and its limit occurred at tension levels which were more than ten times higher for slow twitch muscle than for fast-twitch muscle. The 'apparent break point' was considered to be part of the P_2 component of Bagni *et al.*, A plot of P_1 tension against stretch velocity, for the range of stretch speeds where it was present, passed through the origin. This was taken to support the view that P_1 had its origin in a purely viscous resistance to filament sliding. It may be recalled that P_1 for Bagni *et al.*, had a finite intercept and this was attributed to the SREC.

There was a striking resemblance between the 'apparent break point' and the elastic limit of the SREC of Hill. However it was argued that this discontinuity had to have another origin since it persisted for much longer than the required 0.2–0.4% of L_0, especially in the slow twitch fibres. Furthermore, slow stretch responses with an 'apparent break point' could be resolved into P_1, P_2 and P_3 components, just as for the tension responses to fast stretches. When the sum of P_1 and P_2 plus a fraction of P_3 was calculated for each stretch velocity the plotted relationship corresponded reasonably well with that for the 'apparent break point'. However, it seems to us that P_1 plus P_2 plus a fraction of P_3 gives values close to the peak tension during stretch so that the comparison being made is between the 'apparent break point' and peak tension. These roughly correspond because there is little further tension change between the apparent break point and the end of the stretch, a slight fall in the case of slow-twitch fibres and a rise for fast-twitch fibres (Mutungi and Ranatunga, 1996b, Figure 5). This anal-

ysis therefore does not directly test the kind of mechanical model being put forward (Bagni *et al*., 1995).

It was concluded that the 'apparent break point' represented the viscoelastic behaviour of the P_2 component. P_2 had a calculated relaxation time of 11 ± 1 ms for fast-twitch fibres and 44 ± 2 ms for slow-twitch fibres, when measured at 10°C. These values were much larger, that is, the relaxation times were much longer than for frog muscle fibres (Bagni *et al*., 1995).

When muscle fibres were stretched to longer lengths (2.5–3.1 μm) all three components grew in size. Adding BDM to the bathing solution depressed active tension but had no effect on the three components. A study of the temperature dependence showed that all three components were reduced at high temperatures, P_2 showing the greatest fall as well as a reduction in relaxation time (Mutungi and Ranatunga, 1998).

On the basis of their findings Mutungi and Ranatunga concluded that the initial, steep tension rise during a stretch, representing P_1, arose from a viscous resistance to interfilamentary sliding. The component P_2 was thought to arise, in part, from the folding and unfolding of titin molecules which made up the gap filament. However such effects have been reported to become significant only near 3 μm sarcomere length (Kellermayer *et al*., 1997) while the visco-elastic behaviour persists down to much shorter lengths, leading the authors to conclude that this was unlikely to be the whole explanation for the origin of P_2. The substantial differences in P_2 values observed between slow and fast twitch muscle were considered to arise from different titin isoforms rather than myosin isoforms, as has been assumed for the origin of other slow/fast differences (Jolesz and Sreter, 1981).

While the rise in tension during a stretch can be adequately described in terms of viscous, visco-elastic and elastic components, such a model encounters difficulties when faced by falls in tension. So, for example, there is a fall in P_2 after the 'apparent break point' (Figure 5 in Mutungi and Ranatunga, 1996b). A related difficulty is the tendency for P_2 to fall to zero at higher temperatures. Cross-bridge models, on the other hand, interpret tension changes during stretch in terms of attachment, stretch and detachment of cross-bridges. Where detachment dominates the response, tension will be seen to fall. Mutungi and Ranatunga did not address the question of muscle history effects.

The case for a cross-bridge origin of SREC and FRT was taken up again by Campbell and Lakie (1998). They measured tension and sarcomere length changes in single fibres and small bundles of fibres from different frog hindlimb muscles, using stretch speeds from 5×10^{-4} to 2 L_0/s. They found that the slope of the initial steep phase of the tension response to stretch during triangular stretch-shortening movements was independent of velocity (elastic) at low velocities and increased with velocity, though less than proportionally, at higher velocities, the transition taking place at about 0.1 L_0/s. The asymptote on the ordinate of the stiffness (Young's modulus):stretch velocity plot was at about 9×10^4 N/m. In response to pairs of triangular stretches the initial tension rise at the beginning of the second stretch was smaller, representing the thixotropic nature of the response. During the interval between the two stretches sarcomeres shortened slightly while resting tension rose back towards its original level, with a time constant of several hundred milliseconds. Rising tension during sarcomere shortening is incompatable with an elastic element. It is, however, compatable with a cross-bridge mechanism, where increasing cross-bridge attachment will raise FRT and shorten the sarcomeres. SREC, as measured by force at the yield point, showed a recovery half time of about 5 s. Finally, neither the measured SREC stiffness nor the force level at the elastic limit of the SREC, extrapolated to zero at zero stretch velocity, emphasising the inadequacy of an explanation based on purely viscous behaviour. However, the observations showed that SREC was not purely elastic either, since stiffness and the elastic limit did increase with higher stretch velocities.

The authors proposed a model, the 'cross-bridge population displacement mechanism' to account for some of the findings. One feature of the model is that in relaxed muscle the actin and myosin filaments are linked by a relatively small number of cross-bridges, each of which behaves as a linear spring for both extension and compression. It is hypothesised that SREC and FRT are generated by a $[Ca^{2+}]$ dependent binding of cross-bridges. The mean cross-bridge displacement of an undisturbed population is slightly positive. This gives rise to the FRT. If SREC stiffness is 1% of the stiffness of tetanically contracting muscle and between 50% and 75% of cross-bridges are attached to thin filaments at any one time during an isometric contraction (Bershitsky *et al*., 1997), it suggests that SREC and FRT involve something less than 1% of the total number of available cross-bridges, assuming, of course, that stiffness of the SREC bridges and of actively cycling bridges are similar. A second point of the Cambell and Lakie model is that unstrained cross-bridges are stable and remain attached for a long time. Strain raises the probability of detachment.

During interfilamentary movement, attachment probability is postulated to increase. This is a necessary requirement since there is not a large fall in force once the stretch has exceeded the elastic limit. The proposal is that detached bridges reattach rapidly to give the required frictional resistance. Attachment rate is low in the absence of movement. The less than proportional increase in elastic limit force with stretch velocity may be due to the greater probability for detachment when cross-bridges are under strain from stretch. The observed differences in time-course for redevelopment of SREC (5 s) and FRT (<1 s), it is proposed, may be related to the relatively rapid detachment of any compressed cross-bridges (negative force) leading to rapid recovery of FRT and the much slower attachment rate, in the absence of further movement, associated with recovery of the SREC.

There is something inherently unsatisfactory, at least in terms of the Huxley (1957) model of cross-bridge properties, in the requirement that SREC cross-bridge turnover rates are high during movement and low in a static muscle. Responses of single frog fibres to paired stretches show that in a resting fibre, it takes up to 3 minutes following the first stretch before something approaching the full size of the initial tension rise has recovered (Lännergren, 1971; Campbell and Lakie, 1998). Yet during movement, turnover rates are required to be high to account for the lack of a large drop in tension at the yield point.

We have considered a way out of this difficulty. We postulate that the reformation rate, after SREC cross-bridge detachment by stretch, remains low, and is, in fact, no faster than the formation rate in the static muscle. Since the sarcomeres in passive muscle fibres are not likely to all resist the stretch with the same strength, some will be stretched more than others. At stretch onset the SREC bridges in the weakest sarcomere will be stretched to their elastic limit. Once the cross-bridges detach, the sarcomere continues to lengthen until its passive tension has risen to the point where the next weakest sarcomere will have its SREC bridges detached. This process continues from the weakest towards the strongest sarcomere in a similar way to the response of actively contracting muscle to stretch (Morgan, 1990). The feature of this kind of model is that tension will not fall to zero beyond the yield point, yet the reformation rate of new SREC bridges can remain low. It means that during a stretch not all SREC bridges have necessarily been detached. A second stretch soon after the first will restretch the sarcomeres with detached bridges, which may have now re-formed a few new bridges to give a much lower yield point.

The same kind of explanation can be applied to the tension changes observed during controlled shortenings of the muscle. Initially, there is a steep drop in tension as sarcomeres go into compression. In the sarcomeres with the least number of SREC bridges a point is reached where compressive forces lead to detachment of the bridges. This is indicated by the end of the steep fall in tension. These sarcomeres then continue to rapidly shorten until opposing passive forces stop the movement. This would be at less than 1.5 μm where thick filaments begin to butt up against Z lines, producing an opposing force. Then the bridges in the next weakest sarcomere detach, and so on. If shortening was uniformly distributed across sarcomeres, following detachment of all SREC bridges, tension should begin to rise during the remainder of the shortening since there would now be no compressive forces opposing the movement.

In their discussion of possible objections to the cross-bridge basis of SREC, Campbell and Lakie considered other structures located between successive Z lines in a sarcomere which might be responsible for the stretch responses. While the available evidence suggests that molecules like titin may bear a considerable proportion of the tension at long muscle lengths, such a mechanism was judged unable to account for the SREC and FRT observed at near slack lengths of the muscle. Furthermore, observations by Tskhovrebova *et al.* (1997) suggest that titin behaves as a *stiffening* spring during extension and not as a *softening* spring, which describes the property of the SREC beyond its elastic limit. The authors concluded, that the existence of bonds between actin and myosin in resting muscle must imply a small but significant ongoing energy consumption for the muscle so that 'the molecular motors of muscle may be idling rather than switched-off when the muscle is relaxed'.

Our current view

While non-cross-bridge models can predict the tension response to passive stretch at higher stretch velocities, they encounter difficulties with slower stretches. Slower velocities are important because they are more likely to lie within the physiological range. So, for example, in a cat walking or running on a treadmill at up to 3 m/s stretching velocity of ankle extensors is less than 4 L_0/s (see Walmsley *et al.*, 1978; Walmsley and Proske, 1981). In addition, alternatives to cross-bridge models must be able to explain the development of slack and other evidence of thixotropy, as well as the events during the latent period. Because of these difficulties we prefer to continue to use explanations based on the presence of cross-bridges. In doing so we do not want to imply that there is no role for the gap filaments but their contribution to passive properties is likely to be small at short muscle lengths so that, at least under these conditions, other explanations must be sought.

Our working hypothesis is that muscle in the resting state has a small number of attached cross-bridges, which are responsible for the SREC. The presence of these cross-bridges requires normal resting [Ca^{2+}] levels. In the relaxing solutions used for skinned fibres, resting [Ca^{2+}] is too low and the fibres are too swollen for SREC to develop. Following activation, as [Ca^{2+}] rises during the latent period, cross-bridges enter an intermediate state characterised by a high stiffness and low force. The tension does not rise but falls, because in this state the bridges do not generate a FRT. As [Ca^{2+}] continues to rise the cross-bridges enter the active, force generating state. BDM interferes with active cross-bridge cycling but does not block the formation of bridges in the intermediate state, nor the increase in their numbers at the onset of activation. This would explain the action of BDM in decoupling LR and the stiffness rise during LR from the development of active tension. The increase in SREC and LR at long lengths is attributed to the change in [Ca^{2+}] sensitivity of the myofilaments at long lengths, perhaps brought about by the closer proximity of the myofilaments to one another.

Our underlying assumption is the existence of a functional relationship between SREC, LR and stiffness

measured during the latent period. Arguments linking tension and stiffness with cross-bridge dynamics have been presented by others (Ford *et al*., 1977, 1981). We take the additional step of including LR and SREC in these considerations. At the same time there does not appear to be any evidence of weakly attached, rapidly cycling bridges in resting intact muscle fibres (Bartoo *et al*., 1997). Once these various assumptions have been accepted it is possible to account for the majority of existing observations on passive muscle. Points of uncertainty which remain include the exact origin of LR. Is it due to a pushing action by SREC cross-bridges, or the result of detachment of tension generating cross-bridges? Further investigation of LR under a variety of conditions, while keeping in mind its possible link with SREC, will help to resolve this issue.

The link between SREC and FRT is more problematical. Both certainly show some similarities in behaviour, including their length dependence and increases in size in hypertonic solutions, but this may just indicate that they are both controlled by Ca^{2+} rather than arising from the same population of cross-bridges. Campbell and Lakie report somewhat different time courses for their development. A direct link between FRT and SREC is difficult to reconcile with LR. It may be that this can be explained in terms of a range of cross-bridge strains. For example, if FRT is the result of a distribution of cross-bridge strains, both positive and negative, and the first of the extra bridges formed during LR form at the negative end of that distribution, a tension fall could accompany the rise in stiffness. This would be consistent with the Campbell and Lakie model.

Concluding comments

The point of view adopted in this discussion is that whatever theory is preferred to account for the various properties of passive muscle, it must provide a comprehensive explanation. New, emerging information about the elastic filaments within sarcomeres has led to a reappraisal of the established view. It may, indeed, turn out that a sizeable portion of the response of passive muscle, over much of its length range, can be attributed to the elastic filaments. Similarly, other non-cross bridge sources of passive forces, viscous friction between the sliding filaments and forces arising from within other cytoskeletal elements are likely to contribute to the observed tension changes during stretch of passive muscle. The main objection, in our view, to a comprehensive explanation in non-cross bridge terms is that it is unable to account for the sizeable, history-dependent component of the passive stretch response. And it is probably this aspect, more than any other, which has important consequences for the behaviour of muscle during everyday activities. As soon as a muscle has begun to relax, its mechanical state will depend on what has happened to it in the immediate period beforehand. Was it just contracted, contracted and then shortened or contracted and then stretched? If, for example, we co-contract our elbow muscles while the arm is held flexed, if the passive arm is then extended, elbow flexor muscles remain taut while extensors fall slack. That, in turn, has consequences for the time of onset of subsequent contractions, for the potency of reflex action in these muscles and for the relaying of information which allows us, in the absence of vision, to locate the position of our limbs in space.

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