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THE INITIAL BURST OF IMPULSES IN RESPONSES OF TOAD MUSCLE SPINDLES DURING STRETCH

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SUMMARY

1. The responses of muscle spindles in the iliofibularis muscle of the cane toad *Bufo marinus* were examined during constant velocity stretch of the passive muscle. Spindles were found to show an 'initial burst' of high frequency impulses at the onset of stretch. Associated with the initial burst was a steep passive tension rise in the whole muscle, the short-range elastic component (Hill, 1968), called here the passive stiffness.

2. The size of the initial burst was found to depend on muscle length in a similar way as whole-muscle tetanic tension.

3. Repetitive stretch was found to reduce both the initial burst and passive stiffness. The time taken for both to return to their control values was 3 and 10 s respectively. If immediately following repetitive stretch the muscle, and hence the spindle, was held stretched for 3 s, the initial burst in response to a subsequent stretch from a shorter length remained reduced in size for 300 s. The depression could be reversed by a brief period of fusimotor stimulation.

4. Hypertonic Ringer solutions were found to increase the initial burst and passive stiffness, while both were reduced in hypotonic solutions.

5. Low concentrations of caffeine (1·5 mM) produced a similar decrease in both the initial burst and the passive stiffness. Calcium-free Ringer solution left the stiffness unchanged, and increased the whole dynamic response of the spindle.

6. Metabolic exhaustion and poisoning of the muscle caused the initial burst to increase while decreasing the active tension.

7. It is concluded that the initial burst is an intrafusal manifestation of the passive short-range stiffness of extrafusal muscle which is thought to be due to the formation of stable cross-bridges between the actin and myosin filaments of myofibrils.

INTRODUCTION

A characteristic feature of the response of the mammalian muscle spindle is a high-frequency burst of impulses at the onset of a stretch, called the 'initial burst' (Jansen & Matthews, 1962). The initial burst is thought to be due to an over-extension of the more compliant sensory region of the intrafusal fibres relative to their poles. It is particularly prominent for primary endings but under certain conditions can also

be seen with secondary endings (Brown, Goodwin & Matthews, 1969). At one stage it was considered that the initial burst represented a response of the spindle to the acceleration at the onset of a movement (Schäfer, 1967). However, a number of features of the response which are inconsistent with this idea have led Jansen & Matthews (1962) to refer to the initial burst as arising from a static frictional force in intrafusal fibres rather than from an acceleration signal (see also Lennerstrand & Thoden, 1968).

More recently the initial burst has been studied in single isolated mammalian muscle spindles where it was considered to arise from a steep initial tension rise in intrafusal fibres at the onset of a ramp stretch (Hunt & Ottoson, 1976). A similar initial stiffness, called the short-range elastic component had been observed previously during stretch of amphibian muscle and had been attributed to the presence of stable cross-bridges between actin and myosin filaments of the myofibrils (Hill, 1968). The current hypothesis is therefore, that the initial burst arises from the presence of stable cross-bridges in intrafusal fibres.

The initial burst is very much reduced in size in response to the second of a pair of closely spaced stretches and the muscle must be left undisturbed for up to 10 s to allow full recovery (Proske & Gregory, 1977). It has been proposed that stable cross-bridges in intrafusal fibres which give rise to the initial burst become detached following muscle stretch but they rapidly reform at the length at which the muscle is held immediately after the stretch. When this corresponds to the length at the onset of a test stretch the initial burst is large. When reformation is at a longer length the burst is small or absent (Morgan, Prochazka & Proske, 1984). It was concluded that an initial burst only occurs when the test stretch stresses stable bridges in the intrafusal fibres.

While the initial burst has frequently been compared with the short-range elastic component of frog muscle, the evidence remains indirect and rather limited. The aim of the experimental series reported here has been to further test this hypothesis. An attempt has been made to study responses with the muscle in isolation in a recording chamber where the composition of the bathing solution could be altered. By this means it was possible to demonstrate that spindles of the iliofibularis muscle of the toad do show an initial burst and that following changes in the osmolarity or in the ionic composition of the bathing solution the size of the burst changes together with passive muscle stiffness in a manner consistent with the hypothesis that both arise from the presence of stable cross-bridges.

METHODS

Experiments were carried out using the iliofibularis muscle of the toad *Bufo marinus*. The muscle, together with its nerve supply, which included the appropriate dorsal and ventral roots, was dissected free of surrounding tissue and then placed in a bath through which an oxygenated Ringer solution flowed. At one end in the bath the muscle was clamped to an isometric tension transducer (Devices Dynamometer 8 oz) while at the other end it was attached to an electromagnetic stretcher supplied with length feed-back. The muscle nerve was lifted from the main chamber through a Vaseline gap into an adjacent paraffin pool. Here dorsal roots were dissected into fine filaments until a filament was obtained which contained only a single functional afferent. Discharges were identified as coming from muscle spindles by a pause in the discharge during a muscle contraction. Stimulation and recording used fine platinum electrodes. In one series of experiments the spindle's motor supply was isolated (see Fig. 4). This was achieved by repetitively stimulating portions of ventral root and

looking for an increase in afferent discharge during the period of stimulation. The ventral root was then subdivided further until a small filament remained which on stimulation had a specific and powerful effect on the spindle.

Measurements were made on the initial burst of spindles in response to a rapid triangular stretch and release movement. The initial burst was measured as the peak value of the instantaneous frequency above a zero base line ('total'). This method was chosen as it was the simplest and most accurate. Since, by definition, the initial burst appears before and above the main dynamic response it could also have been measured as the height of its peak above the dynamic response, rather than its absolute value ('active component'). This would require extrapolating the dynamic response back to the point where the initial burst had occurred. (The assumption here is that the response to stretch is made up of two components, one being due to the rise in intrafusal tension associated with the short-range elasticity and the second due to passive tension.) In practice the more complicated method of measurement proved to be less accurate and in most experiments it did not need to be used as the dynamic response was found to be constant for any particular set of conditions. However, when the experiment involved changes in muscle length (Fig. 2) which altered both the size of the initial burst and the dynamic response it was necessary to adopt the method of extrapolation.

Whole-muscle stiffness was measured as the change in muscle tension ΔP during a length change, Δl. During a stretch the slope of the tension trace was initially steep (see Fig. 1) and then became more shallow after yielding at what is termed the 'elastic limit' (Hill, 1968). The slope of the steep portion, $\Delta P/\Delta l$ was used as a measure of the passive stiffness. Measurements were made on records displayed in expanded form on the screen of a digital oscilloscope (Nicolet Explorer Model 2090) and each value was obtained using the movable cursor. Stiffness was calculated from the average of four individual sets of measurements each carried out on a separate record. Variation in stiffness values between records was less than 10%.

All experiments were performed at the shortest length at which a recognizable initial burst could be obtained from a spindle in response to a triangular stretch. This was generally at lengths just above slack and about 1 mm below the optimum length, well within the physiological range of lengths. The full range from slack to maximum extension was approximately 4 mm. Triangular stretches of 2 mm amplitude were used with a stretch rate of 50 mm/s. This stretch rate was chosen as it was found to elicit the most consistent response. During each experimental sequence the muscle was brought to the test length, conditioned with a rapid stretch and release movement, and then tested with further triangular stretches. After each test the muscle was returned to its slack length.

During control experiments the muscle was bathed in normal toad Ringer solution containing 111 mM-NaCl, 2·5 mM-KCl, 0·1 mM-$KHPO_4$, 2·4 mM-$NaHCO_3$, 1·8 mM-$CaCl_2$ as well as 11 mM-glucose. Ringer solutions were continuously bubbled with a mixture of 95% O_2, 5% CO_2.

Hypertonic solutions were prepared by the addition of sucrose. The addition of 30 g of sucrose per litre of Ringer solution gave an estimated osmotic strength 1·35 times that of normal Ringer solution. Hypotonic solutions were prepared by adding less NaCl per litre of Ringer solution, leaving the concentrations of other constituents unchanged. Addition of only 3·13 g NaCl per litre of Ringer solution gave an estimated osmotic strength of 0·54 times that of normal Ringer solution. Caffeine Ringer solution was prepared by the addition of 1·5 mM-caffeine (0·29 g) to each litre of normal toad Ringer solution. No correction was made for the change in tonicity. Calcium-free solutions were prepared by substituting $MgCl_2$ for $CaCl_2$ with the addition of 3 mM-EGTA per litre plus NaOH (approximately 6 mM) to bring the pH back to that of normal toad Ringer solution (6·8). The solution was made osmotically equivalent to that of normal toad Ringer solution by addition of less NaCl.

For experiments involving metabolically exhausted and poisoned muscles a glucose-free Ringer solution was prepared which contained KCN (2 mM) and iodoacetate (1 mM), corrected for tonicity by the addition of extra NaCl. The solution was continuously bubbled with nitrogen and the muscle was made to twitch every second throughout the experiment.

Before each solution change the consistency of the size of initial burst in response to a test stretch was checked over a period of 10 min. Control values were taken 1 min before the change. It was then necessary to wait at least 2 min, the flushing time of the muscle chamber with the new solution. A further 2 min was required for proper equilibration. All results are represented as percentage change relative to the control response.

Unless otherwise stated all experiments were performed at room temperature, which varied from 18·5 to 23 °C.

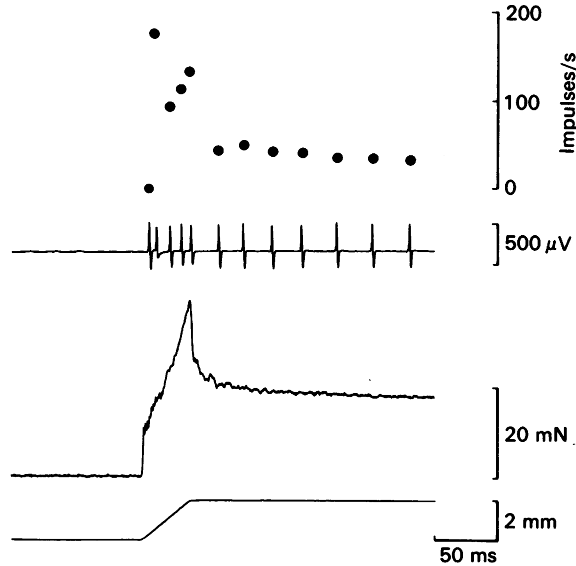

Fig. 1. A typical response from a toad muscle spindle during a ramp-and-hold stretch. The bottom trace represents the length change during the stretch of 2 mm at 50 mm/s. The trace above shows whole-muscle tension during the passive stretch. The second record from the top shows the action potentials recorded from a single spindle in response to the stretch. An instantaneous frequency display of the impulse activity is represented in the top trace. Calibrations are shown to the right of each trace. In this and in subsequent Figures the dots of the frequency display have been re-touched.

RESULTS

Responses were recorded from a total of eighty-three muscle spindles. All showed an initial burst in response to a ramp stretch (Fig. 1) although occasionally it was necessary to stretch the muscle to long lengths (but still within the physiological range) before the burst became detectable. The burst illustrated in Fig. 1 is typical in that it comprised only a pair of impulses at short interval, although occasionally there were three impulses. Notice too that prior to stretch there was no resting discharge which again was typical for these spindles.

Associated with the initial burst was an initial steep rise in tension in the passive extrafusal fibres, representing the short-range elastic component of Hill (1968). After a length change of 0·2–0·3 % of muscle length there was a discontinuity in the tension trace signalling the limit of the short-range stiffness.

Both the size of the initial burst and of the short-range elastic component showed a dependence on stretch rate, the initial burst becoming unmeasurably small below 5 mm/s and continuing to increase at least up to rates of 100 mm/s. However, in this series velocity dependence was not studied systematically.

On the assumption that the initial burst arises from an intrafusal short-range elasticity and that this is due to stable cross-bridges it would be expected that the size of the burst depends on the muscle length. The reason is that it is known that the amount of overlap between actin and myosin filaments determines the number

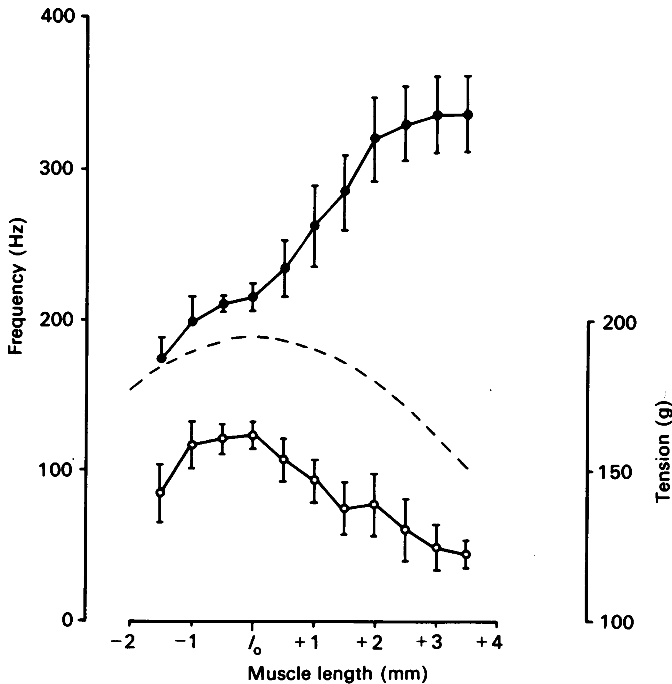

Fig. 2. The effect of different muscle lengths on the size of the initial burst. A plot of impulse frequency and tetanic tension against muscle length. The size of the 'total' initial burst is represented by the filled circles, the 'active component' by the open circles. Each point represents the mean ($\pm$S.E. of mean) from four experiments. The dashed line represents the averaged whole-muscle tetanic (active) tension curve. For each preparation muscle length was related to the length at which a tetanic contraction reached its maximum value (l_0).

of available cross-bridge attachment sites (Gordon, Huxley & Julian, 1966) and presumably therefore the number of stable cross-bridges. We have studied the length dependence of the initial burst in the experiment illustrated in Fig. 2.

An increase in muscle length not only raised the frequency of the initial burst but elevated the entire dynamic response. It was therefore decided to make two measurements, that of the absolute peak of the burst and the value of the peak after subtraction of the estimated frequency of the dynamic response at that time, the 'active component' of the initial burst (see Methods). It was found that the active component showed a length dependence similar to that of the whole-muscle tetanic (active) tension. The absolute value of the burst, on the other hand, increased at all muscle lengths.

Early during the experiments it was found that the size of the initial burst of spindles was reduced in response to the second of a pair of closely spaced stretches, in much the same way as is seen with mammalian spindles. A simple interpretation of this observation is that the first stretch stresses and then results in detachment of stable bridges in the intrafusal fibres and bridges have not had time to reform when the second stretch is applied. By increasing the interval between the conditioning and test stretches it is possible to plot the time course of reappearance of the initial burst

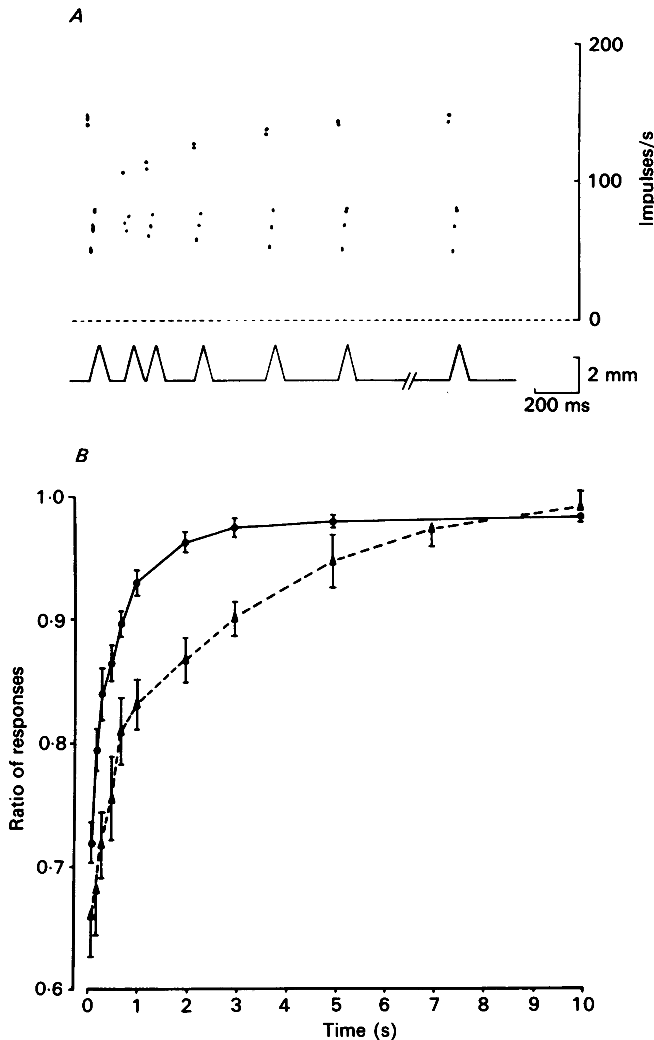

Fig. 3. *A*, the effect of repetitive stretch on the response of a single muscle spindle. The top trace shows an instantaneous frequency display of superimposed responses to pairs of triangular stretches (2 mm at 50 mm/s, bottom trace) separated in time by 100–3000 ms. *B*, the time course of recovery of the initial burst (circles), and of the short-range stiffness (triangles) following a stretch. This was obtained by plotting the ratio of the response to a second stretch to that of the first from data of the kind shown in *A*. Each point represents the mean ($\pm$ S.E. of mean) of observations from seven experiments for the initial burst and from five experiments for the short-range stiffness.

(Fig. 3). At the same time the passive stiffness was measured and this too was seen to show a depression followed by gradual recovery. It required an interval of 3 s for full recovery of the initial burst and 10 s for recovery of the stiffness. This is a little faster than the recovery time for mammalian spindles (Proske & Gregory, 1977).

In a recent series of experiments on after-effects of repetitive muscle stretch and of fusimotor stimulation on the responses of mammalian spindles Morgan *et al.* (1984)

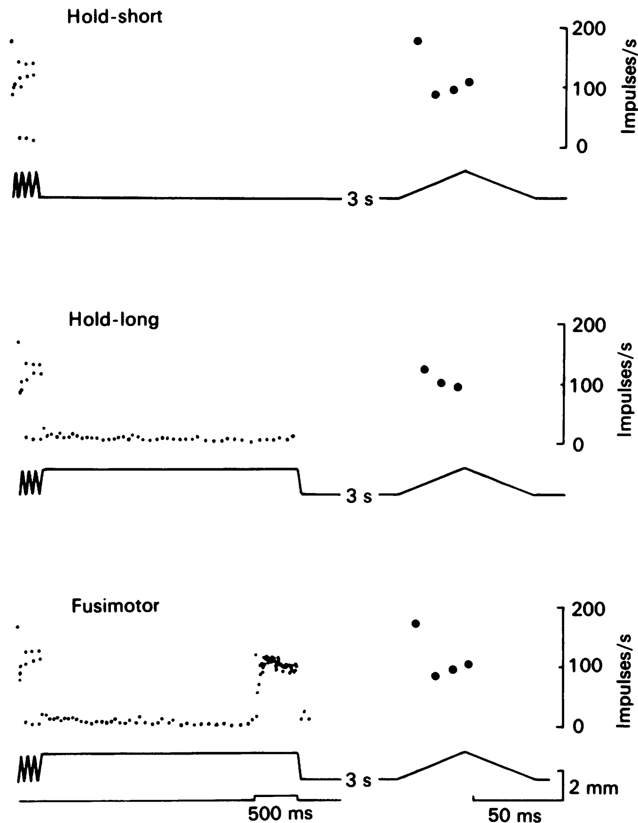

Fig. 4. The effect of muscle length and fusimotor stimulation on recovery of the initial burst. The top record shows the response of a spindle to a test stretch (NB expanded time scale) 6 s after a series of alternating stretch–release movements with the muscle returned to its rest length after the movements (hold-short). The middle record shows the response to the same test stretch but after the muscle had been held stretched for 3 s after the conditioning movements (hold-long). The bottom record shows the response to the test stretch when a brief period of fusimotor stimulation (500 ms, at 150 Hz) was given just prior to release from hold-long. The upper trace in each pair of records represents the instantaneous firing rate of the spindle, the lower trace, length. The trace at the bottom of the Figure indicates the period of stimulation.

found that the size of the test response depended very much on the length the muscle was held at immediately after a conditioning stretch or period of fusimotor stimulation. A large test reponse, and incidentally also a large initial burst in response to the test stretch was observed only when after conditioning the muscle was returned to a length corresponding to the starting point of the test stretch. The interpretation was that only when cross-bridges reformed at a length where they would be stressed at the onset of the test stretch would there be a large initial burst.

The same kind of experiment has been tried with toad spindles (Fig. 4). First, the muscle was stretched repetitively using a series of rapid conditioning stretches. At the end of these the muscle was either held at the short length (hold-short) for 6 s before a test stretch was applied or held at the long length for 3 s and then returned

to the short length for the remaining period (hold-long). With the hold-short condition there was always a prominent initial burst in response to the test stretch. The burst following hold-long was reduced in size. If, however, the spindle's motor supply was stimulated just prior to release from hold-long, depression of the initial burst could be reversed (Fig. 4, bottom panel). This effect was not simply due to the extrafusal contraction produced by stimulation of the motor axon since it persisted when all extrafusal contraction had been blocked by curare. (The spindle motor supply comes from collaterals of axons innervating extrafusal muscle.) When fusimotor stimulation was applied after a hold-short sequence there was no increase in size of the initial burst beyond the value reached in the absence of stimulation, suggesting that the fusimotor effect was not due to some separate mechanism.

The interpretation of these experiments assumes that stable cross-bridges become detached during a series of stretches or fusimotor stimulation and then reform over the next 3 s. If, after the stretches the muscle was held at the short length, cross-bridges would form there and a test stretch would immediately stress them leading to a large initial burst. When the bridges formed at the long length, release back to the short length introduced slack in the fibres and a test stretch starting at the short length would not immediately stress them and so the initial burst was depressed. However, if bridges made at the long length subsequently became detached by activation of the fibre following fusimotor stimulation, and the muscle was then brought back to its short length, all new stable bridges would reform here and the burst would reappear.

The next question posed was, do bridges formed at the long length remain there permanently so that the initial burst in response to a stretch starting at the short length continues to be depressed? In fact it gradually returned to its control size provided the muscle was left undisturbed for 5 min (Fig. 5). The precise time of recovery depended on the muscle length at which the measurements were made. In comparison 20–30 min were required for recovery of the initial burst of mammalian spindles (Morgan *et al.* 1984, Fig. 5).

Hill (1968) proposed that stable cross-bridges formed spontaneously between actin and myosin filaments in the resting muscle. He showed that if muscle fibres were made to shrink by placing them in a hypertonic solution the short-range elasticity, representing stable cross-bridges, increased presumably because in the shrunken fibre myofilaments would lie closer to one another and therefore have more opportunity for interaction (Brandt, Reuben, López & Grundfest, 1964). The opposite effect was observed with fibres made to swell by bathing them in a hypotonic solution.

With an isolated preparation the same kinds of tests were available to us and Fig. 6 shows the changes in both the passive stiffness and in the initial burst following superfusion of the muscle with either a hypertonic or hypotonic solution. In the hypertonic solution stiffness increased by up to 150 % while the initial burst increased by only 10–15 %. This difference may simply have arisen because the spindles were buried deep in the muscle and were not exposed to the full osmotic gradient while the stiffness change arose from a superficial layer of extrafusal fibres. Why the same differential effect did not occur in the hypotonic solution remains unclear; here both the initial burst and stiffness fell by about 40 %. Perhaps when muscle fibres swell, the diffusion barrier between regions inside and outside of the muscle is reduced. Alternatively there may have been some direct effect of the hypertonic solution on

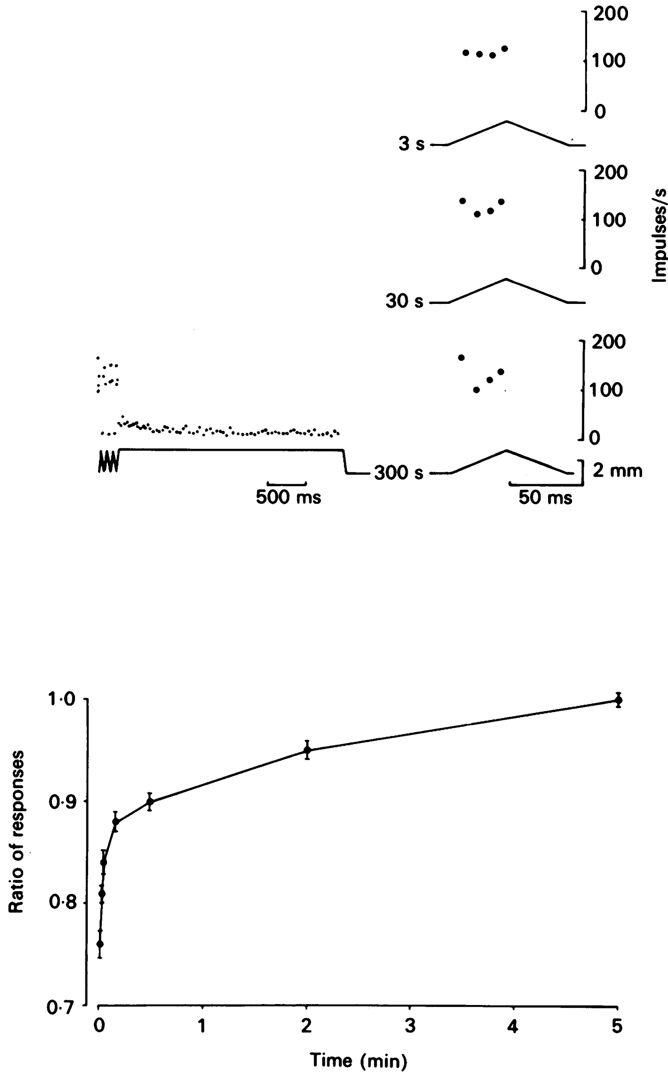

Fig. 5. The time course of recovery of the initial burst. Top, the response of a spindle to a test stretch (NB expanded time scale) 3, 30 and 300 s after release from a 3 s period of hold-long immediately following a series of conditioning stretches. In all records the top trace represents the instantaneous firing rate, the bottom trace length. Bottom, the time course of reappearance of the initial burst after hold-long. This was obtained by plotting the ratio of the test response to that of the control, hold-short, value at different time intervals after release from hold-long. Each point represents the mean (±S.E. of mean) for observations from seven experiments.

receptor excitability. However, this was not manifest in any obvious way such as alteration in the resting discharge rate.

A central question which remains unanswered is why stable cross-bridges form in the first place. An obvious possibility is that following a period of activity not all of the free calcium is taken up by the sarcoplasmic reticulum and sufficient remains to allow a few cross-bridges to form (but see also Discussion). According to this

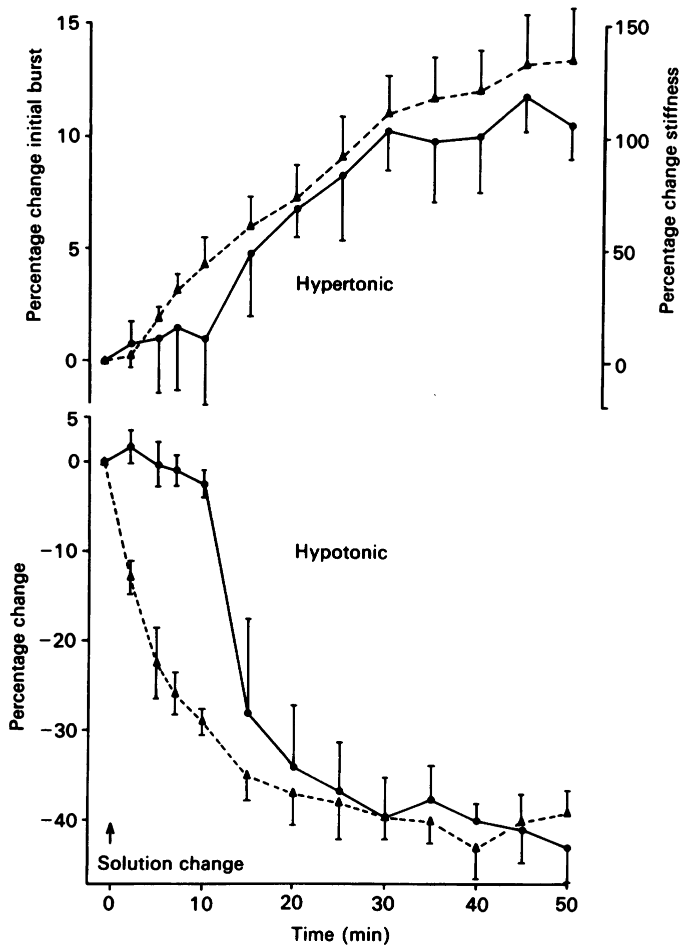

Fig. 6. The effect of changes in osmolarity. Top, the percentage change in the initial burst (circles) and short-range stiffness (triangles) relative to their control levels (NB different ordinates) at different times after a solution change to hypertonic Ringer solution (1·35 times normal). Each point represents the mean (±S.E. of mean) for four observations. Bottom, the percentage change in the initial burst (circles) and the short-range stiffness (triangles) after a solution change to hypotonic Ringer solution (0·54 times normal). Each point represents the mean (±S.E. of mean) for values from three preparations.

hypothesis any change in sarcoplasmic calcium should be accompanied by a change in the passive stiffness and for the spindle by a change in the initial burst.

It is well known that caffeine, at concentrations of less than 2 mM, potentiates muscle twitches (Sandow & Brust, 1966). This is thought to be due to release of calcium from the sarcoplasmic reticulum which acts to prolong the active state. The effect of adding 1·5 mM-caffeine to the bathing solution is shown in Fig. 7. Both the passive stiffness and the initial burst were seen to decrease by 20%.

The alternate experiment of testing the effect of lowering calcium concentrations was also tried. López, Alamo, Caputo & Di Poli (1983) have shown that the frog sartorius muscle bathed in calcium-free Ringer solution containing 3 mM-EGTA

shows a significant reduction of free intracellular calcium in muscle fibres. Prolonged immersion of the toad muscle in calcium-free Ringer solution produced no change in passive stiffness and an elevation of the entire response of the spindle to stretch including both its resting discharge and the size of the dynamic response. When these changes were taken into account there remained no real increase in the size of the initial burst.

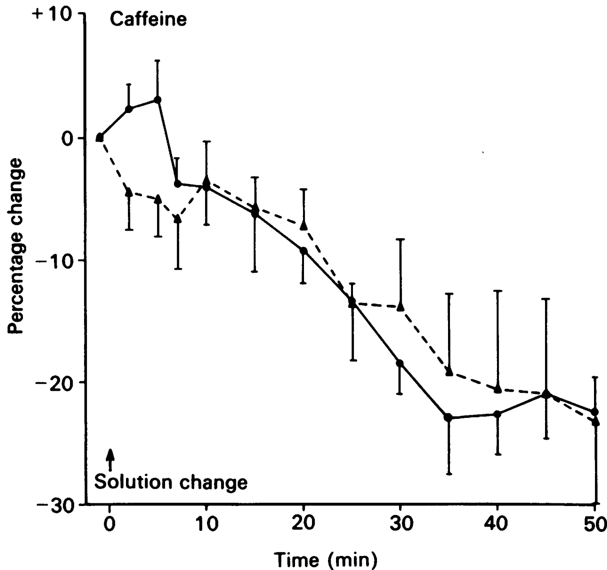

Fig. 7. The effect of caffeine. The percentage change in the initial burst (circles) and the short-range stiffness (triangles) relative to their control values, at different times after a solution change to caffeine Ringer solution (1·5 mM). Each point represents the mean (±S.E. of mean) for values from four preparations.

A characteristic feature of stable cross-bridges is, in fact, their stability. While the experiments described earlier suggest that detached bridges reform relatively rapidly, once formed they seem to remain attached for long periods. In an actively contracting muscle the rate of cross-bridge cycling is two or three orders of magnitude faster, the actual value differing from one muscle to another, as expressed by its maximum shortening velocity. The principal factor which is thought to determine contraction speed is activity of the enzyme actomyosin ATPase (Barany, 1967). In other words the rate of splitting of ATP is an important determinant of the lifetime of a cross-bridge. Furthermore it is known that muscles depleted of their stores of ATP enter a state of 'rigor'; in the absence of ATP the actin–myosin link can no longer be broken. It is conceivable that the presence of stable cross-bridges represents a low level of rigor in the muscle. If this is so, then any lowering of ATP levels in the muscle should increase the incidence of stable cross-bridge formation.

A method by which ATP levels in frog sartorius muscles have been lowered by as much as 80% is described by Fink, Hase, Lüttgau & Wettwer (1983). Metabolic exhaustion was achieved using glucose-free Ringer solution, bubbled with nitrogen

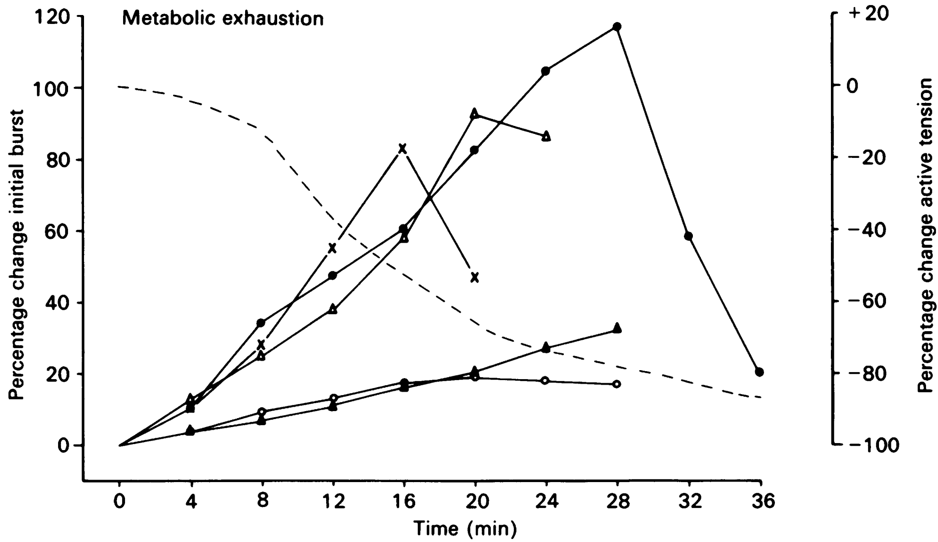

Fig. 8. The percentage change in the initial burst relative to its control value at different times after commencement of metabolic exhaustion and poisoning of the muscle. Each symbol represents the response from a different spindle; the dashed line represents the average percentage change in the active tetanic tension from the five preparations studied.

and activating the muscle at regular intervals. As well, the metabolic poisons cyanide and iodoacetate were added. Under these conditions it was found in our experiments that there was a progressive drop in the whole-muscle tetanic tension and this was accompanied by both an increase in the passive stiffness as well as an increase in the initial burst (Fig. 8). The rise in passive stiffness was very large but was not measured systematically in this series. Interestingly, not only the initial burst increased but there was an elevation of the whole response to stretch including both static and dynamic components. However, unlike the experiments in calcium-free solution, when these changes were taken into account there still remained a significant increase in the initial burst itself.

The experiment was carried out on five separate occasions and each time tetanic tension was seen to drop to about 20 % of its original value. On three occasions the spindle showed a progressive increase in the initial burst by up to 120 % of its original value over a period of 15–20 min. It then gradually began to fall, the drop in the burst being accompanied by a decrease in the whole response to stretch. Finally the spindle showed no response to stretch whatever even though it continued to maintain a high resting discharge ($>$ 10 Hz). On two occasions the increases in the initial burst were smaller, reaching peaks of only 20–30 % above control values. Such differences in behaviour may again be due to diffusion barriers between the inside and outside of the muscle.

DISCUSSION

While much of the evidence remains indirect it is now widely accepted that the initial steep tension rise seen during stretch of passive muscle, the short-range elastic component, is due to the presence of cross-bridges between actin and myosin, formed spontaneously, and remaining attached for long periods. It can be readily accepted that the same kind of phenomenon occurs in intrafusal fibres which, after all, are striated muscle too. The experiments described in this paper provide substantial additional support for the existence of a short-range elastic component and therefore stable cross-bridges in intrafusal fibres.

It is, of course, difficult to make inferences about muscle mechanics from discharge patterns of a stretch receptor. The peak frequency of the afferent response is not likely to be directly related to the peak intrafusal tension; (for primary endings of mammalian spindles the best fit could be obtained from an arbitrary mixture of tension and its first-order derivative, Lewis & Proske, 1972). An even more serious problem arises in the experiments with solution changes. Low calcium, while on the balance without effect, had to be judged against a background of changes in the resting discharge and stretch response. It is well known that calcium is implicated in transduction processes and specifically for amphibian spindles one component of the generator potential has been attributed to calcium mechanisms (Ito & Komatsu, 1979). Similarly metabolic exhaustion and poisoning is likely to interfere with sensory transduction which is known to depend on energy in the form of ATP. It has been reported that changes in osmotic pressure alter the responses of frog spindles to stretch (Ottoson, 1965) so that here too there is the problem of looking at a receptor discharge rather than directly at intrafusal tension changes. Despite these many uncertainties clear-cut effects could be observed in the size of the initial burst. The fact that these changes were paralleled by similar changes in the extrafusal short-range elasticity, provides substantial additional support for the idea that the burst arises from an intrafusal short-range elasticity.

Length dependence

Interpretation of the observations presented in Fig. 2 is not as straight forward as appears at first sight. Detailed measurements have been made on length dependence of the short-range elastic component in single muscle fibres (Haugen & Sten-Knudsen, 1981). It was found that the short-range elasticity reached its peak at lengths well beyond the optimum for muscle contraction (see also Hill, 1968). The explanation put forward suggested that while at long lengths the short-range elasticity would be expected to decrease in direct proportion to the reduced overlap between myofilaments, at the same time there was likely to be a greater probability of stable cross-bridge formation and therefore a higher stiffness due to the decreased interfilament spacing.

In our experiments changes in passive stiffness with muscle length were not followed closely because of the complication introduced by the passive tension. It was, however, noticed that passive stiffness increased over the whole range of muscle lengths as did the peak value of the initial burst. The 'active component' of the burst however, reached its peak at the optimum length for a tetanic contraction. We

feel that this is unexpected in view of the length dependence of the passive stiffness predicted from single fibre measurements. Two possible factors may help to account for our observations.

First, the length–tension curve for active tension involved contraction of the whole muscle and consequently stretch of series elastic elements which in a muscle like iliofibularis with its prominent tendon and pennate fibre arrangement are likely to be large. Stretch of the tendon would allow the fibres to shorten so that the measured optimum length for a contraction is likely to be longer than if series elasticity was small as it will be in the passively stretched muscle. Consequently the whole-muscle optimum is likely to represent a length in the passive muscle where sarcomere spacing is already beyond the point of maximum overlap. A second factor is that intrafusal fibres are likely to have fewer sarcomeres than extrafusal fibres since they are striated along only part of their length. This too would result in a shift to the left of the intrafusal length–tension curve. It may be worth mentioning here that unlike mammalian spindles, the intrafusal fibres of amphibian spindles run the whole length of the muscle (Gray, 1957).

Changes in tonicity and ionic composition

The changes in passive stiffness and the initial burst in hypertonic solutions are consistent with similar observations made by Hill (1968) and by Lännergren & Noth (1973) on the short-range elastic component. It is known that shrinking or swelling of muscle fibres produced by osmotic gradients alters the filament spacing (Brandt *et al.* 1964) and it seems plausible that this will alter the probability of formation of stable cross-bridges.

An observation which supports the idea that resting calcium levels are responsible for the presence of a short-range elasticity is that chemically skinned skeletal muscle fibres show almost no short-range elasticity in low calcium solutions (relaxing solution containing 10^{9} M-calcium, Moss, Sollins & Julian, 1976). Here there may be the additional complication that skinned muscle fibres swell and therefore reduce their interfilament spacing. Our negative result in low calcium may simply mean that we did not achieve a significant drop in sarcoplasmic calcium levels. The effect of caffeine, on the other hand, is at first sight the opposite to what might have been expected. If caffeine increases free calcium levels the number of stable cross-bridges might have been expected to increase. Our observed decrease in initial burst and passive stiffness is consistent with the findings of Länergren (1971) and can be most simply explained by proposing that caffeine triggers a low level of active cycling of cross-bridges. Evidence suggests that stable cross-bridges must first be detached before they can contribute to active cycling. Hill (1968) proposed that 'latency relaxation' (the small drop in tension immediately preceding active contraction) was due to a drop in 'filament resting tension' which Hill attributed to stable cross-bridges (see also Haugen, 1983).

Recent experiments using stiffness measurements (Brenner, Schoenberg, Chalovich, Greene & Eisenberg, 1982), biochemical techniques (Chalovich & Eisenberg, 1982) and X-ray diffraction analysis (Brenner, Yu & Podolsky, 1984), have suggested that in resting muscle cross-bridges may form and that this is not due simply to resting levels of calcium. It may be that calcium is not involved in cross-bridge binding itself but in the force-generating step. Once the power stroke is com-

pleted, in the presence of ATP, the bridges detach from actin. The action of caffeine would therefore be to trigger the force-generating step of the cycle and subsequent detachment of bridges. A remaining point of uncertainty is that a rise in stiffness could only be detected by Brenner *et al.* (1982) in solutions of low ionic strength and using very high stretch rates. It may be therefore that the mechanism for cross-bridge formation under these conditions is related to but not identical with the passive short-range elasticity of normal resting muscle.

Our findings from metabolically exhausted and poisoned muscles are that there is a large increase in both the passive stiffness and initial burst. This poses the question, should stable cross-bridges be considered as representing a low incidence of rigor complexes? Rigor bridges result in the absence of ATP from persisting attachment following the force-generating step. The essential feature of stable cross-bridges is that their attachment and detachment is not accompanied by measurable generation of force. We are therefore inclined to think that these two states are seperate and distinct and that metabolic exhaustion, by raising the number of rigor complexes simply adds to the passive stiffness generated by stable cross-bridges.

Conditioning stretches

Repetitive stretch was found to decrease the initial burst and passive stiffness which required about 3 and 10 s respectively before they returned to their control values. Thus it seems that there is a finite formation rate for stable cross-bridges which may well be the same for extrafusal and intrafusal fibres, taking into account likely differences in their mechanical connexions within the muscle; intrafusal fibres are highly non-uniform in their structure and for any one spindle there are only three or four fibres compared with several hundred extrafusal fibres. It is of interest that Proske & Gregory (1977) found a similar difference in time course in the cat soleus muscle. Lännergren (1971), on the other hand, found the recovery time for the short-range elasticity of single frog fibres to be 3 min. The difference between his finding and our observation is most probably due to the testing procedure used by Lännergren: his test stretch was applied at the final length following completion of the conditioning stretch. The rising passive tension at the longer length may affect stable cross-bridge formation and thereby prolong the time course.

The length at which the muscle was held during the period of reformation markedly changed the time course of reappearance of the burst. If the muscle was held stretched following conditioning stretches the time for recovery was increased from the minimum value of 3 s to 300 s. The explanation for this difference is as follows: after detachment of stable bridges by the conditioning stretches, if the muscle is then immediately returned to its rest length all stable bridges reform at that length and a subsequent test stretch will stress them to produce a large initial burst. If on the other hand during the period of cross-bridge formation the muscle is held stretched then it seems plausible that when it is subsequently returned to its rest length the intrafusal fibres, having become comparatively rigid at the longer length by virtue of the presence of stable bridges, may buckle as the bridges resist passive shortening of the fibres. Consequently a subsequent test stretch must first take up slack produced by buckling before it will begin to stress bridges and therefore there will be no initial burst at the onset of the stretch (Morgan *et al.* 1984).

The fact that under these conditions it took 5 min for the initial burst to recover

suggests that the stable bridges in the buckled fibre gradually detach, allowing the fibre to shorten passively. This process continues until all parts of the fibre have returned to their normal length. It can be postulated that with the fibre in a buckled state there are passive compressive forces acting on segments of rigid fibre. Our observations suggest that the higher these passive forces the more rapid the detachment and return to the rest length: when the experiment illustrated in Fig. 5 was repeated at longer muscle lengths, the recovery time fell from 5 min to 30 s. It may be that once there are no longer sufficient passive forces on the fibre stable bridges remain permanently attached. Alternatively there may continue to be a low resting rate of turnover of stable bridges. We do not yet have sufficient data to be able to distinguish between these two alternatives.

To conclude, the hypothesis suggesting that the initial burst is due to an intrafusal short-range elasticity and hence arises from the presence of stable cross-bridges is strongly supported by the findings of this study. In the toad these bridges reform within 3 s, after being detached by stretch, but require up to 5 min of spontaneous cycling to return the intrafusal fibre to its rest length. For this reason they are termed stable cross-bridges, although the reason underlying their apparent stability remains unclear. This leads to the more general question of the formation and mechanical properties of cross-bridges in resting muscle compared with cross-bridges arising from the normal activation cycle. It is unlikely that a method as indirect as measurement of an initial burst of spindles is likely to resolve such fundamental questions.

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