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EFFECT OF LENGTH AND CAFFEINE ON ISOMETRIC TETANUS RELAXATION OF FROG SARTORIUS MUSCLES

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Summary

In an isometric tetanus of frog sartorius muscle the total relaxation time increased linearly with change in length from 0.7 to 1.4 times rest length. Maximal rate of relaxation, measured from the time derivative (dp/dt) of tension decay, decreased with both decrease and increase from rest length in correlation with the generated tetanus tension. Stretching the muscle did not significantly affect the times to maximal rate, positive and negative inflexion points but greatly increased the time to total relaxation from the negative inflexion point. Caffeine at 2 mM, acting on muscles at rest length, also slowed the relaxation and decreased the maximal rate of tension decay. However, caffeine increased the times to maximal rate, positive and negative inflexion points without significantly affecting time to total relaxation from the negative inflexion point. These results suggest that caffeine slows an earlier step in relaxation, while stretch slows a later step. It is proposed that muscle relaxation is a two step process: an initial step that is regulated by the rate of Ca^{2+} uptake by sarcoplasmic reticulum, and a later step that is mostly controlled by the speed of dissociation of remaining cross-bridges.

Introduction

The physiological mechanisms underlying muscle relaxation are not fully understood. Relaxation cannot be explained by simple diffusion of substance from sensitive areas of the myofibrils [1], or by a change in the compliance of

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the muscle fibers [2]. Hartree and Hill [1] suggested that muscle relaxation must be related to an underlying physicochemical reaction.

In view of the role of Ca^{2+} in contraction-relaxation cycle of muscle fibers [3—7], the rate of Ca^{2+} uptake by sarcoplasmic reticulum was suggested to be the regulating factor determining the rate of relaxation [8—10]. However, even the fastest rate of Ca^{2+} uptake by sarcoplasmic reticulum in isolated condition or in skinned muscle fibers is too slow to account for the rate of relaxation in living muscle fibers [7,11]. On the other hand, myoplasmic free Ca^{2+}, as monitored by murexide [12] and aequorin [13—15] falls off much sooner than muscle tension.

Alternatively, the speed of dissociation of actomyosin cross-bridges was proposed to be the rate-limiting factor in relaxation [16]. This hypothesis was bsed on a significant correlation between the rate of adenosine triphosphate (ATP) hydrolysis, which is involved in cross-bridge dissociation [17]; and the half-time of exponential decay of tension [16]. But the time constant of the exponential part of relaxation may not reflect cross-bridge dissociation since, during this phase the muscle fiber is no longer in an isometric state [18,19]. In addition, use of ATP utilization as an index of cross-bridge dissociation may not be specific since ATP is involved in uptake of Ca^{2+} by sarcoplasmic reticulum as well [20,21].

Since relaxation involves both uptake of Ca^{2+} by sarcoplasmic reticulum and dissociation of cross-bridges, present studies were aimed at determining the relative role played by these two processes in regulating the rate of muscle relaxation. The analysis was based on alterations in the time course of isometric tetanus relaxation caused by changes in muscle length and exposure of muscle to caffeine. These studies, besides confirming the reported slowing of relaxation by stretch [1,2], and caffeine [8]; vividly showed that these two agents exert their slowing effect on two different phases of tension decay.

Methods

Sartorius muscles of the frog, *Rana pipiens*, were used for these studies. The muscle was isolated under oxygenated Ringer's medium containing 116.8 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 2.0 mM Tris buffer (pH 7.2), and 0.029 mM D-tubocurarine chloride. Following 1 h equilibration, the muscle was mounted in a vertical recording chamber containing the Ringer's medium that was continuously bubbled with oxygen and maintained at 20 ± 0.2°C.

General methods of stimulation and tension recording of muscles were similar to those described previously [23]. Isometric tetani were elicited by applying massive supermaximal shocks of 0.3 ms duration at 200 Hz for 0.3 s using a pair of platinum plate electrodes flanking the muscle. Tension was monitored by a RCA 5734 transducer and displayed on a Tektronix 565 dual-beam oscilloscope. The first beam of the scope traced the entire course of the tetanus tension on a slow sweep, and the second beam displayed the relaxation part on a high speed delayed sweep that was electronically switched between two channels: one to record the tension drop and the other to register the rate of tension drop obtained from a differentiating circuit having a time constant of 20 ms.

The length of a muscle stretched taut without bearing measurable resting tension was designated as rest length. At 10 min intervals, a tetanus response was elicited at each of the lengths corresponding to 10% increments from 0.7 to 1.4 times rest length. After each length change resting tension on the muscle was noted. In some of the experiments, the responses were recorded in sequence first in normal Ringer's medium at 1.0 and 1.3 times rest length and then following 10 min exposure to 2 mM caffeine at rest length.

Results

Typical recordings of the tetani obtained from a muscle at various lengths are shown in Fig. 1. In each frame trace A shows the entire tetanus tension on a slow sweep, while traces B and C respectively show the time course of tetanus relaxation and the first derivative (dp/dt) of the relaxation part on faster sweeps. In order to directly compare the relaxation kinetics at various lengths, the plateau tension during tetanus relaxation latency, that is involved between the last shock and the start of relaxation, was taken as 100% and times to different percent levels of tension fall were measured. These times were increased with increment in length from 0.7 to 1.4 times rest length (Fig. 2). There was a bigger increase in total relaxation time than in half relaxation time

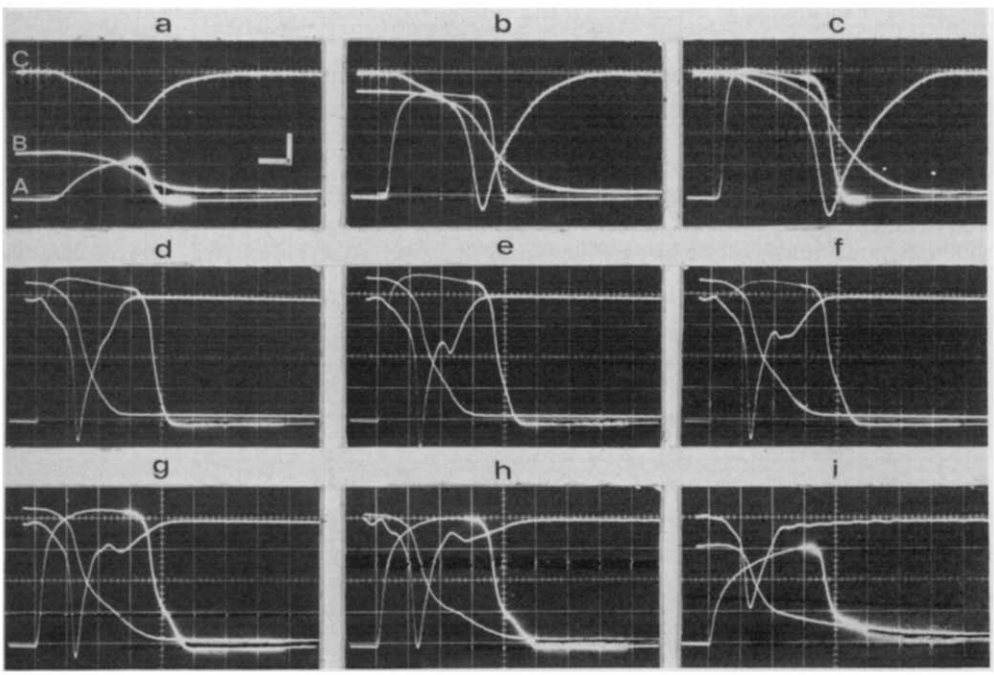

Fig. 1. Typical recordings of isometric tetanus responses of a frog sartorius muscle set at lengths of: a 0.7, b 0.8, c 0.9, d 1.0, e 1.1, f 1.15, g 1.2, h 1.3, and i 1.4 times rest length at 20°C. In each frame trace A shows the entire tetanus (calibration: horizontal 100 ms, vertical 25 g), trace B shows the relaxation part on an extended delayed sweep (calibration: horizontal 20 ms for frames a, b, c and 50 ms for frames d through i, vertical 25 g), trace C shows the time derivative; dp/dt, of trace B (calibration: horizontal is same as for trace B, vertical 0.5 g/ms).

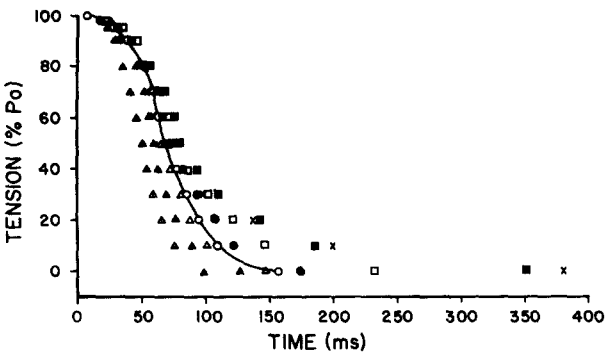

Fig. 2. Relaxation curves after normalization with respect to tetanus tension produced by the muscle at lengths of: △ 0.7, ▲ 0.8, △ 0.9, ○ 1.0, ● 1.1, □ 1.2, ■ 1.3, X 1.4 times rest length. Each point represents a mean obtained from 5 muscles at 20°C.

(Fig. 2, Table I). There was a strong correlation between total relaxation time and muscle length ($r = 0.939$, $P < 0.001$). The method of least squares [24] was used to fit a straight line to the data giving:

Total relaxation time = 399.4 × muscle length − 211.0 ms.

Sandow et al. [8] reported that tetanus relaxation of variously treated muscles follows a generalized 'canonical curve', when times to different levels of tension fall were normalized with respect to maximal tension and the half relaxation time (see also Ref. 25). The canonical curve was thought to represent the first-order kinetics of Ca^{2+} uptake by sarcoplasmic reticulum

TABLE I

EFFECT OF LENGTH ON ISOMETRIC TETANUS RELAXATION OF FROG SARTORIUS MUSCLE AT 20°C

Values are given as mean ± S.E. obtained from 5 muscles. In each of the muscles, tetanus responses were elicited at 10 min intervals at the indicated fractions of the rest length. Statistical significance of a differ-

	Length of the muscle (rest length = 1.0)		
	0.7	0.8	0.9
Tetanus tension (g)	17.6 [c] ± 4.9	55.9 [b] ± 8.2	80.7 [b] ± 9.3
Half relaxation time (ms)	51.3 [b] ± 2.2	59.5 [b] ± 2.9	67.4 ± 3.4
Total relaxation time (ms)	108.8 [b] ± 3.6	127.8 [c] ± 11.3	147.0 ± 13.5
Rate at + inflexion (g/ms)	0.209 [a] ± 0.062	0.330 [a] ± 0.064	0.374 ± 0.049
Time to + inflexion (ms)	27.4 [a] ± 2.6	31.9 [a] ± 2.0	38.1 ± 1.6
Tension at + inflexion (%p_0)	85.5 ± 2.6	88.2 ± 4.5	91.9 ± 0.9
Maximal rate (g/ms)	0.419 [b] ± 0.135	1.459 ± 0.298	1.771 [c] ± 0.294
Time to maximal rate (ms)	49.5 [c] ± 2.9	56.1 [a] ± 3.2	64.0 ± 3.8
Tension at maximal rate (%p_0)	49.0 [c] ± 3.2	56.6 [d] ± 2.3	60.2 [c] ± 1.1
Rate at − inflexion (g/ms)	0.149 [a] ± 0.058	0.722 ± 0.220	0.891 ± 0.160
Time to − inflexion (ms)	76.6 ± 5.6	76.4 ± 6.8	87.2 ± 8.4
Tension at − inflexion (%p_0)	11.2 ± 2.5	9.7 ± 3.3	12.2 ± 4.6
Difference of total relaxation time and time to − inflexion	28.3 ± 2.2	51.6 ± 5.4	59.8 ± 6.4

approximating a single exponential curve [8]. In the present studies, the 'canonical' pattern was observed in relaxation of muscles at or below rest length, while at stretched lengths the latter 30% of relaxation clearly dissociated from it (Fig. 3). Semilogarithmic plots of the relaxation showed single exponential decay of the latter 70% of tension [2,10,16] in muscles at or below rest length. At stretched lengths the final 20% of tension decay clearly deviated from the exponential curve.

Analysis of the derivative records (trace C in each frame of Fig. 1) showed that after tetanus relaxation latency the rate of relaxation increased slowly to a point of positive inflexion, then it rapidly reached the maximum, and later declined first quickly to a point of negative inflexion and then slowly to zero value. As shown in Table I, the rate at positive inflexion was about 0.48 g/ms at rest length and it was significantly reduced by bigger changes in length. Positive inflexion occurred at about 37 ms at rest length and significantly earlier at shorter lengths. By this time about 8% of tetanus tension decayed at rest length and smaller tension decayed at longer lengths. Maximal rate of relaxation was about 1.85 g/ms at rest length and it decreased with change to either side of rest length. There was a strong correlation ($r = 0.86$, $P < 0.001$) between maximal rate of relaxation and tetanus tension produced at different lengths. Using least squares method a straight line was fitted to the data giving

Maximal rate of relaxation = 0.0197 × tetanus tension − 0.0061 g/ms.

Maximal rate occurred at about 62 ms at rest length and it was attained earlier on 20% or greater decrease in length and later with 40% stretch. By this time about 44% of tension decayed at rest length while more tension decayed at shorter lengths and less tension dropped at longer lengths. The rate at

ence from the value at rest length, was calculated by paired *t*-test and is indicated as: a ($P < 0.05$), b ($P < 0.02$), c ($P < 0.01$) and d ($P < 0.001$).

1.0		1.1		1.2		1.3		1.4	
91.8	± 11.1	96.3 [a]	± 11.0	92.1	± 11.1	79.7 [a]	± 12.3	68.7 [c]	± 11.1
69.7	± 3.6	73.5	± 6.4	74.6	± 4.4	81.1	± 13.4	64.5	± 7.5
157.6	± 11.7	174.0 [b]	± 12.6	238.8 [d]	± 25.3	343.6 [c]	± 33.6	384.7 [d]	± 5.1
0.402	± 0.056	0.374	± 0.060	0.346	± 0.068	0.195 [b]	± 0.079	0.205 [a]	± 0.029
37.0	± 0.9	38.5	± 2.6	38.5	± 2.7	32.2	± 2.4	36.9	± 5.9
92.1	± 0.9	89.3	± 1.8	91.7	± 2.3	97.0 [b]	± 0.8	97.1 [b]	± 0.8
1.852	± 0.295	1.865	± 0.318	1.573 [a]	± 0.307	1.341 [c]	± 0.309	1.125 [d]	± 0.211
61.8	± 3.6	62.7	± 2.7	65.7	± 3.6	68.2	± 4.1	72.5	± 5.9
66.6	± 1.8	68.4	± 1.9	71.6	± 4.4	71.3	± 1.9	70.0	± 1.1
0.830	± 0.141	0.685 [b]	± 0.142	0.496 [b]	± 0.097	0.295 [b]	± 0.095	0.293 [c]	± 0.056
91.0	± 7.1	96.8	± 2.8	107.0	± 7.5	114.0 [a]	± 4.9	111.5 [a]	± 5.2
16.3	± 9.9	27.2	± 2.5	29.4	± 2.9	32.6	± 3.9	33.6 [a]	± 2.4
66.6	± 11.1	77.2	± 10.3	136.8 [b]	± 17.4	229.8 [c]	± 29.8	277.9 [d]	± 5.5

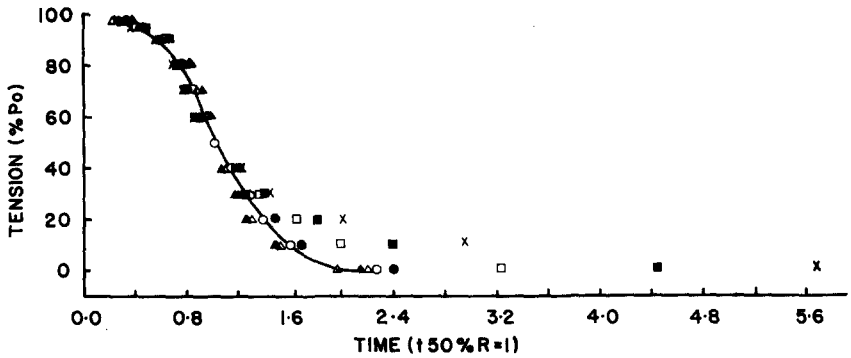

Fig. 3. Relaxation curves in Fig. 2 were replotted after normalization of the times with respect to half relaxation time (t(50% R)) that was taken as 1.0. Different lengths of the muscles are represented by the same symbols as in Fig. 2. Notice that at stretched lengths the last 20% of relaxation falls clearly out of the 'canonical curve'.

negative inflexion was about 0.83 g/ms at rest length and it decreased with stretch. Negative inflexion occurred at about 91 ms at rest length and significantly later at 30% or greater stretch. By this time about 84% of tension decayed at rest length and significantly smaller tension dropped at 30% or greater stretch.

Stretch-induced slowing of relaxation obviously occurred after the negative inflexion point, since stretching the muscle by 40% increased the time to negative inflexion by about 20% while it increased the total relaxation time by more than 100% (Table I). Hence the difference between times to total relaxation and the point of negative inflexion should represent the part of relaxation that was greatly affected by muscle length. Accordingly this difference got progressively bigger with stretch (Table I).

At 1.1 times rest length a brief hump appeared at the point of negative inflexion (see the decaying phase of trace C in Fig. 1e) clearly reflecting the shoulder that is barely seen on the direct trace of tension decay. It appeared clearly in rather thick muscles (i.e. weight to length ratio of $\geqslant 2.3$) than in thin ones and when the initial tension was between 0.1 and 0.9 g. With further increase in length, time to appearance of the hump increased and its magnitude decreased (Fig. 1f to g). At 1.4 times rest length, a discrete hump was not seen (Fig. 1i). In the present studies the hump was observed generally at later times than was reported by Huxley and Simmons [18,19] possibly due to use of whole muscles in our experiments.

Just like stretch, caffeine also increased the total relaxation time and decreased the maximal rate (Fig. 4). However unlike stretch, caffeine significantly increased times to points of positive inflexion, maximal rate and negative inflexion without affecting the difference in times to total relaxation and negative inflexion (Fig. 4, Table II). Absolute values of certain parameters presented in Table II are slightly different from those given in Table I. This could be partly due to seasonal variations, since earlier experiments were performed in January while the latter in June. However, these studies do show that caffeine slowed the relaxation by affecting an earlier phase, while stretch caused the slowing by affecting a later phase of tension decay.

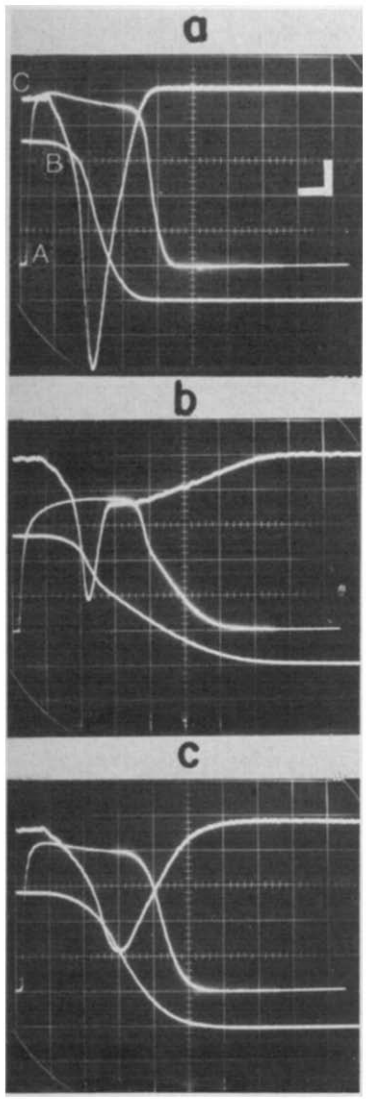

Fig. 4. Isometric tetanus responses obtained from a frog sartorius muscle tested at 20°C in normal Ringer's first at rest length (a), then at 1.3 times rest length (b); and finally following a 10 min equilibration in 2 mM caffeine Ringer's at rest length (c). Trace A shows entire tetanus (calibration: horizontal 100 ms, vertical 20 g), trace B shows the relaxation part (calibration: horizontal 50 ms, vertical 20 g), and trace C shows the first derivative of trace B (calibration: horizontal 50 ms, vertical 0.25 g/ms). Notice a clear shift in time to maximal rate of relaxation in caffeine Ringer's as against in normal Ringer's.

Discussion

In view of Hartree and Hill's suggestion [1] the time course of relaxation could be determined by: (1) rate of ATP utilization, (2) amount of Ca^{2+} released, (3) rate of Ca^{2+} uptake by sarcoplasmic reticulum and (4) speed of dissociation of cross-bridges.

Changes in length towards either side of rest length decreased the rate of

TABLE II

EFFECT OF STRETCH AND CAFFEINE ON ISOMETRIC TETANUS RELAXATION OF FROG SARTORIUS MUSCLES AT 20°C

Values are mean ± S.E. obtained from 4 muscles tested first at 1.0 and 1.3 times rest length in normal Ringer's medium and then following a 10 min exposure to 2 mM caffeine Ringer's at rest length. Significance of a difference from the value at rest length in normal Ringer's was calculated by paired *t*-test and is indicated as: a ($P < 0.05$), b ($P < 0.02$), c ($P < 0.01$).

	Normal rest length	Normal 1.3 × rest length	Caffeine rest length
Tetanus tension (g)	106.2 ± 12.8	90.2 ± 8.9	94.0 ± 9.1
Half relaxation time (ms)	77.6 ± 4.7	103.8 [c] ± 3.2	122.9 [c] ± 5.3
Total relaxation time (ms)	179.4 ± 8.7	339.4 [c] ± 28.8	275.0 [b] ± 12.5
Rate at + inflexion (g/ms)	0.392 ± 0.049	0.300 ± 0.069	0.258 ± 0.044
Time to + inflexion (ms)	40.3 ± 6.2	43.8 ± 2.1	59.4 [b] ± 1.9
Tension at + inflexion (%p_0)	92.7 ± 1.3	93.8 ± 1.1	92.5 ± 1.5
Maximal rate (g/ms)	1.784 ± 0.242	1.269 [c] ± 0.252	0.834 [c] ± 0.139
Time to maximal rate (ms)	67.8 ± 2.1	73.3 ± 1.2	109.5 [c] ± 3.1
Tension at maximal rate (%p_0)	62.9 ± 3.4	72.2 ± 2.0	60.9 ± 1.8
Rate at − inflexion (g/ms)	0.625 ± 0.127	0.447 ± 0.045	0.263 [b] ± 0.089
Time to − inflexion (ms)	109.4 ± 2.8	103.1 [a] ± 2.9	186.9 [c] ± 13.6
Tension at − inflexion (%p_0)	18.7 ± 7.1	50.7 [c] ± 3.9	13.6 ± 3.9
Difference of total relaxation time and time to − inflexion (ms)	70.0 ± 9.9	236.2 [c] ± 31.7	88.4 ± 13.5

phosphorylcreatin, thus ATP utilization [27], and the amount of Ca^{2+} released [14,15] on tetanic stimulation. While these alterations can explain the observed decrease in the maximal rate of relaxation with change in length towards either side of rest length, they can not account for the monotonic increase in total relaxation time with length. This suggests that rate of ATP utilization and amount of Ca^{2+} released may not be involved directly in determining the time course of relaxation.

The rate of relaxation was suggested to be regulated by the rate of Ca^{2+} accumulation by sarcoplasmic reticulum [8—10], and alternatively by the speed of dissocation of cross-bridges [16]. Both of these theories presume that relaxation proceeds as a single exponential involving a first order chemical reaction. However, in the exponential analysis as much as 30% of the initial part of relaxation is excluded. In addition, assumption of a single exponential decay of tension does not agree with the time course of relaxation observed in stretched muscles.

These studies show that stretching the muscle slows the relaxation especially after the point of negative inflexion, while caffeine slows it right from the start upto the point of negative inflexion without significantly affecting the later part. Caffeine releases Ca^{2+} from sarcoplasmic reticulum [22] by facilitating the Ca^{2+}-induced Ca^{2+} release [11] leading to the maintenance of higher levels of Ca^{2+} in the myoplasm for longer time. This leads to an apparent decrease in uptake of Ca^{2+} by sarcoplasmic reticulum and thus to a slowing in relaxation. Since this slowing occurred mostly upto the point of negative inflexion it may be inferred that this point on the derivative trace might mark the time by which most of the released Ca^{2+} is pumped back into the sarcoplasmic reticulum. It has been suggested that decrease of Ca^{2+} concentration from a

peak value to less than 10^{-6} M, could occur in 100 ms in a muscle at 15°C [11,14]. This time which could be less at 20°C as used in the present studies, agrees very well with the time to the point of negative inflexion (91 ms at rest length) observed in the present studies.

Then the part of relaxation following the point of negative inflexion may be controlled by a process other than Ca^{2+} uptake. Stretching the muscle significantly slowed this part of relaxation. This slowing may not be due to slowed uptake of Ca^{2+} by sarcoplasmic reticulum since preliminary tests showed that 2 mM caffeine did not affect this later part of relaxation even in a muscle stretched to 1.3 times rest length, and stretch did not cause a slowing in the exponential decay of the Ca^{2+} transient as revealed by the aequorin pulse [15]. In addition, stretching the muscle, which distorts the junctional region between the terminal cisternae of the sarcoplasmic reticulum and the transverse tubules [30] decreasing Ca^{2+} release during activity [15,30], may not cause a significant distortion of the longitudinal elements since their surface area per unit volume of the fiber is about 170% greater than that of the terminal cisternae [31]. Since longitudinal elements are suggested to be the sites of Ca^{2+} uptake [32], this process may not be significantly altered in stretched muscles. The occurrence of a relaxation shoulder ([1,8,18,19,26] and present results) which seems to involve shortening of some sarcomeres while others are lengthening [19,26,28] may lead to the slowing of relaxation. Though the mechanism underlying this non-uniform behaviour of sarcomeres is not understood, Edman and Flitney [29] reported that the amplitude of both elongation and shortening of sarcomeres during relaxation decreased with increase in length of the muscle fiber. Similarly in the present studies, the magnitude of the hump decreased with increase in muscle length, although the relaxation time following the point of negative inflexion greatly increased. This suggests that the slowed relaxation at stretched lengths may not be significantly due to the non-uniform behaviour of sarcomeres.

The other alternative that can explain this slowed relaxation is that stretch may retard the speed of dissociation of cross-bridges. There is no direct evidence for this effect. However, recent X-ray diffraction studies [33] suggest that dissociation of cross-bridges is a slow process continuing well past the total fall of tension. Thus Yagi et al. [33] showed that in frog sartorius, following 1 s tetanic stimulation at 4°C, tension fell to resting level within 0.4 s while; 80% of the myosin heads rapidly returned to the thick filaments in about 1 s and the remaining 20% slowly returned over several seconds. Though X-ray diffraction pattern implies only spatial proximity without necessarily indicating cross-bridge activity, the rapid phase of the return occurred almost simultaneously with fall of tension [33] and might represent the dissociation of cross-bridges. The kinetics of this rapid return did not change significantly even after applying a correction for the non-uniformity in sarcomere lengths [33]. Hence it may be inferred that the time course of relaxation following the point of negative inflexion may be regulated by the speed of dissocation of remaining cross-bridges, and that at stretched lengths this process may be somehow slowed. Thus it may be suggested that during isometric tetanus relaxation, the earlier part of tension decay may be regulated by the rate of Ca^{2+} uptake by sarcoplasmic reticulum and the later part by the speed of dissociation of remaining cross-bridges.

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