Effect of a Brief Stretch on Time Course of Shortening after a Quick Release in Guinea Pig Tenea Coli

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Abstract: To examine the origin of velocity deceleration in the shortening of smooth muscle, guinea pig tenea coli which contracts isometrically in a high-potassium solution was released quickly (1st release), restrretched to the original length, kept at this length for 0.06–0.2 s and quickly released again (2nd release). The muscle shortened isotonically under the same very-light load after the 1st and 2nd releases. The length change of the muscle during shortening was examined using a video camera. The rapid shortening phase, seen after the 1st release, which followed the instantaneous, elastic recoil and lasted for about 0.3 s, disappeared after the 2nd release. This effect was prominent when the stretch duration was short. The time course of the slow phase of shortening subsequent to the rapid one was not affected by stretching. These results suggest that the rapid shortening phase is due largely to adjustment of the cross-bridges between the thick and thin filaments to the change from an isometric to an isotonic contraction, while the slow phase reflects a steady turnover in the cross-bridges. The velocity of the slow shortening phase reached a maximum before the isometric force peaked and decreased as the activation level was lowered by reducing the external Ca²⁺ concentration from 2.5 to 0.5 mM. This suggests that the steady turnover rate of the cross-bridges depends on the phase of contraction and level of activation. The cause of the gradual velocity decrease in the slow phase of shortening was discussed. [Japanese Journal of Physiology, 46, 457–463, 1996]

Key words: contraction, guinea pig, tenea coli.

The kinetics of the cross-bridges between thick and thin filaments in muscles has been studied widely by examining the transient changes in shortening velocity or force at the time of sudden changes in contraction state and the velocity of steady-state shortening [1–3]. In mammalian smooth muscles, the time course of shortening after a quick release is complicated by extracellular connective tissues and/or intracellular inert structures [4–6], and possibly by the development of “latch” cross-bridges [7]. The velocity of shortening after an instantaneous recoil of the series elastic component upon release decreases greatly with time. The inert structures may act as a parallel as well as a series elastic component and accelerate or decelerate the shortening due to the active turnover of the cross-bridges.

A hyperbolic relation between the velocity of steady shortening and the load on it, which characterizes the muscle energetics [8], seems to stand at any phase of shortening following the elastic recoil after quick release in smooth muscles [9]. Hellstrand and Johansson [4] reported that the rapid shortening phase immediately after elastic recoil is insensitive to the phase of contraction in the smooth muscle of the urinary bladder, while the subsequent slow shortening phase is sensitive, suggesting that the latter reflects a steady turnover in the cross-bridges. Since the rapid shortening phase is sensitive to temperature [4, 5], it is not ascribed merely to the recoil of a damped elasticity but also to adjustment of the cross-bridges to the change from an isometric to an isotonic contraction. However, the time course of the rapid shortening phase is quite different from that of the velocity transient in skeletal muscle, which consists of a half- or one-cycle of oscillatory shortening. It is uncertain...
whether the velocity transient takes a different time course in smooth muscle from that in skeletal muscle or how long it lasts.

The maximum shortening velocity ($V_{\text{max}}$) is useful to assess the rate of steady turnover in the crossbridges. The $V_{\text{max}}$ is usually estimated using either a hyperbolic load-velocity relation or a slack test [10]. In either case, it is necessary to repeat a quick release from the same force level several times to estimate the $V_{\text{max}}$. It is not easy to measure the $V_{\text{max}}$ using such methods under several different conditions with the same preparation in smooth muscle however.

We studied the origin of shortening after quick release with guinea pig tenea coli applying a transient stretch during shortening, and by comparing the time course of shortening before and after the stretch using a video camera to follow the change in muscle length. Based on the results, the turnover rate in the crossbridges was compared between the rising phase and peak of an isotonic potassium-induced contracture (K-contracture) and between high and low levels of activation. This paper describes these results.

**MATERIALS AND METHODS**

Male guinea pigs weighing about 400 g were sacrificed after anesthetizing with diethyl ether. A strip of tenea coli was isolated and trimmed in a modified Tyrode solution (in mM: NaCl, 118.5; KCl, 4.2; CaCl$_2$, 2.5; KH$_2$PO$_4$, 1.2; NaHCO$_3$, 14.5; MgSO$_4$, 1.2; and glucose, 11.0 (pH 7.2–7.4)) oxygenated well with a 95% O$_2$ and 5% CO$_2$ gas. The strip was kept stretched slightly at room temperature until use, usually within 3–4 h after isolation. An aluminum foil T-shaped clip with a hole was attached to each end of the muscle strip, which was 5–8 mm long, 1–1.5 mm wide, and about 0.5 mm thick. It was placed in an experimental chamber (30×15×7 mm) with a trough (2.5 mm deep, 1 mm wide, and 20 mm long) on one side. One end of the strip was connected to a force transducer (Norway, AE801; resonant frequency, 1 kHz) and the other end to a 30 cm long, surgical nylon thread (0.05 mm in diameter), which extended out of the chamber through the trough and was attached to the distal end of a 6 mm long-extension from the shaft of a servomotor (General Scanning, G120D). The selection of the nylon thread was critical for this experiment. The force was recorded on an ink-writing oscillograph (Nihon Kohden, WI-621G) together with the signal from the servomotor.

The muscle strip was set just above the slack length ($L_0$) and activated by introducing an isotonic potassium solution, in which all NaCl was substituted with KCl, into the chamber after draining. The strip was released at the required phase of resulting K-contracture by quickly displacing the extension of the servomotor. The rotation of the servomotor caused about 1 mm displacement of the distal end of the extension within 5 ms. This amount of displacement was enough to reduce the active force to zero and allow shortening of the muscle to an extent sufficient for the present purpose (Fig. 1). The force record showed little rise in force during muscle shortening under the load due to frictional force caused by the slow displacement of nylon thread through the narrow trough in the chamber. The frictional force, as measured by moving the

![Fig. 1. Shortening curves obtained by quickly releasing a guinea pig tenea coli at the peaks of four successive K-contractures. Measured muscle lengths (circles) are connected by lines for convenience in this and subsequent figures. The curves without initial shortening due to elastic recoil are shifted vertically for comparison. The relative force levels are 1.0, 1.0, 0.93, and 0.87 from the top to bottom. The records were obtained in this order. Note that the later phase of shortening is slower in the lower two curves than the upper curves. Insets: Original records of the first contracture, in this and the next figure: L, displacement of the servomotor; upward step indicates release; and F, force record. The force after release is very close to zero. The slow time base was changed to fast one where shown by the arrowhead; 1 min and 1 s for the time scale, respectively. An isotonic K solution was applied where shown by the upward arrow.]
Shortening of Tenea Coli

RESULTS

Time course of shortening after a quick release

At the peak of the K-contracture, the tenea coli was subjected to a quick release. The release was accompanied by a large amount of shortening due to the passive recoil of the series elastic component. This was followed by a phase in which the shortening velocity decreased greatly (rapid phase) and, subsequently, a phase of slow shortening (slow phase). The rapid phase usually lasted 0.03–0.3 s after the release and the slow phase occurred thereafter. The time course of shortening was quite reproducible when the muscle was released from the contraction peaks at the same force level (Figs. 1a, b and 3B). The reduction in force level due to fatigue resulted in decreased velocity in the slow shortening phase without any appreciable change in the rapid phase (Fig. 1c and d).

Effect of a brief stretch

A brief stretch was applied during isotonic shortening after a quick release in such a manner that the isotonically contracting muscle was stretched back to the original length, kept at this length for 0.12 s and then released again (Figs. 2A and 4). It was confirmed, using the video camera, that both shortening upon re-

![Fig. 2. The effect of brief stretching on the time course of shortening. Stretching was applied (A) after and (B) just before the quick release of a muscle. Insets: Original records; T, 1 s time marks; and up- and down-ward deflections in L indicate release and stretching of the muscle, respectively. In A, the 1st shortening curve is replotted on the 2nd one. Replootted curves are shown by closed circles with broken lines in this and subsequent figures. Single and double arrows indicate 1st and 2nd releases, respectively. In B, the 1st (1) and 2nd (2) shortening curves of A are replotted.](image-url)
lease and lengthening upon stretching was distributed uniformly along the entire length of the muscle strip. The shortening before and after the stretching will hereafter be called the 1st and 2nd shortenings, respectively. When the 1st shortening curve was superimposed on the 2nd one (Figs. 2A and 3) or vice versa, the two curves did not match. The 2nd shortening curve appeared to underlie the 1st one especially during the rapid shortening phase following the elastic recoil. The amount of elastic recoil was greater in the 2nd shortening than in the 1st shortening. It appears that a component which disturbs the elastic recoil is lost in the 2nd shortening.

Figure 2B illustrates a shortening curve when stretching was applied just before the initial release. A comparison of the time course of shortening with and without prestretching shows that the two curves take a very similar time course (open and closed circles in the rapid shortening phase in Fig. 2B). Therefore, the change in the rapid phase at the time of stretching during shortening is not due to the stretching maneuver but to the interruption of shortening by the stretching procedure. A prolongation of the stretching period appeared to restore the rapid phase to some extent as shown in Fig. 3.

The time course of the slow phase of the 1st shortening was compared to that of the 2nd shortening to examine the effect of stretching on the slow phase. When the slow phases in the 1st and 2nd shortening curves were superimposed in series on the curve of a long-lasting shortening without interruption by stretching, as shown in Fig. 2B, they matched very well. This suggests that the slow phase in the 2nd shortening is a continuation of the 1st one. This was also suggested by the experiment in which the effect of timing of the stretch on the time course of shortenings.

Fig. 3. The effect of prolonged stretching on the time course of shortening. A and B were obtained from the same preparation. Closed circles with broken lines, 1st shortening curve replotted on the following 2nd curve. Small open circles with broken lines in B, a replot of the 1st shortening curve of A.

Fig. 4. Effect of the stretching time on the time course of shortening. A and B were obtained from the same preparation. In A, the 2nd shortening curve of B is replotted. In B, the 1st shortening curve of A (1) and 2nd shortening curve of B (2) are replotted.

ing was examined. In Fig. 4, where the stretch is applied 0.12 and 0.3 s after the quick release, the slow phase in the 2nd shortening in the latter can be matched to that of the former if shifted vertically to some extent (closed circles in Fig. 4A). When the slow phase in the 2nd shortening was retraced after and in series with the 1st shortening, the entire curve appeared smooth as if it was a single shortening curve (closed circles with broken lines in Fig. 4B). Thus, all of the evidence is in accord with the theory that the time course of the slow shortening phase is not affected by stretching.

Provided that the extension of the series elastic component is the same before the initial quick release and the one after stretching (i.e., the same amount of elastic recoil in the 1st and 2nd shortenings), the above results suggest that the rapid phase is due largely to the adjustment of the cross-bridges to the change from isometric to isotonic contraction. The slow shortening phase is thought to reflect a steady turnover in the cross-bridges under a very light load.

Dependence of the shortening velocity in the slow phase on contraction phase and activation level

The cross-bridge turnover rate can be compared by measuring the $V_{max}$ among muscles of the same type. Since the muscle shortened under a load less than 1% of $P_o$ in this study, the shortening velocity in the slow phase is thought to be close to the $V_{max}$. The average velocity of shortening 0.3–1.0 s after release was measured and defined as the velocity in the slow phase ($V_s$). Then, the cross-bridge turnover rate was compared among the different states of contraction by measuring the $V_s$.

The dependence of the $V_s$ on the phase of a K-contraction was examined by releasing the muscle during the rise in force and at the peak. As shown in Fig. 5, the $V_s$ during the rise in force was greater than that at the peak in two out of five preparations, equal to that in another two and slightly smaller than that in the remaining one. Taking into account that release was not made at exactly the same phase in the contractions of the five preparations and that the load increase is relative as the level of the isometric force just before the release decreases, this result may be taken to indicate that the cross-bridge turnover rate, as judged from the $V_s$, reaches a maximum before the force reaches $P_o$ and declines slightly thereafter.

Lowering the external Ca$^{2+}$ concentration to 0.5 mM reduced the magnitude of the K-contraction to 0.3–0.6 $P_o$. The $V_s$ was always smaller at the peak of the K-contraction at 0.5 than at 2.5 mM Ca when compared for the same preparations (Fig. 5). In two out of four preparations, the $V_s$ was as low as less than 50% of that at the maximum activation with the peak force level of greater than 50% $P_o$. Such a low $V_s$ may not be interpreted only by a relative load increase due to the decrease in the force level. It is possible that lowering the activation level might reduce the crossbridge cycling rate; since the $V_s$ was greater during the rise in force than at the peak in the K-contraction at 0.5 mM Ca as at 2.5 mM Ca, despite the lower level of force.

In three preparations, the $V_s$ was measured in the contraction induced by 10 mM caffeine, a dose level which gives maximal force development. The peak force level and the $V_s$ in the caffeine contraction were similar to those in the K-contraction at 0.5 mM Ca.

DISCUSSION

This study demonstrated that when an isotonically contracting muscle was stretched transiently and released again, the rapid shortening phase disappeared in the shortening after stretching. Since the muscle strip of the guinea pig tenea coli appeared to be lengthened uniformly upon stretching to its original
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length, it is thought that the individual muscle cells also returned to their original lengths. The time course of the rapid shortening phase was quite reproducible when a quick release was applied in successive contractions of the same preparation even if the force level varied to some extent (Fig. 1). Therefore, it is unlikely that the rapid phase disappeared as the result of different means of elastic recoil in the 1st and 2nd shortenings. Edman [10] reported, with a skeletal muscle fiber, that the elastic recoil of a prestretched, unstimulated fiber is slowed greatly at the time of quick release by activating the fiber. This indicates that the presence of active cross-bridges slows down or interrupts rapid myofilament sliding due to the recoil of the parallel elastic component responsible for the resting force. As clearly shown in Fig. 3A, the amount of instantaneous, elastic recoil is greater in the 2nd shortening than in the 1st shortening. Taking into account that the rapid phase tended to be restored when the period of stretching was prolonged (Fig. 3), our observations suggest that the shortening phase which appeared in the 1st shortening but not in the 2nd one is related to some activity of the cross-bridges accompanied by the change from isometric to isotonic contraction state. When a skeletal muscle fiber is suddenly altered from an isometric contraction state to an isotonic state, the fiber experiences transiently an oscillatory shortening by a half- or one-cycle before establishing steady shortening [2]. In some invertebrate striated muscles [12, 13] and vertebrate skeletal muscles not fully activated [14, 15], the velocity of shortening after a quick release decreases monotonically before reaching steady shortening. The time course of shortening in smooth muscles resembles such type of shortening. It is possible that the rapid shortening phase in the guinea pig tenia coli has the same origin as that of shortening with a monotonically decreasing velocity in striated muscles. Consequently, the rapid shortening phase in the 1st shortening seems to reflect the adjustment of the cross-bridges to the change from isometric to isotonic contraction, analogous to the velocity transient in the skeletal muscles.

The $V'_{\text{max}}$ in fully activated guinea pig tenia coli has been reported to be about 0.3L/s at 37°C and 0.2L/s at 22°C [16, 17]. These values are greater than the $V'_c$ (about 0.08L/s) at 25°C reported in this study. The discrepancy may reside partly in the difference in the phase at which the velocity was measured; it appears that the previous authors measured the velocity at a phase earlier than ours. Another possibility is the difference in the level of activation. The isotonic K solution used in this study did not seem to activate the muscle fully, since the application of a large field stimulation capable of causing irreversible damage to the muscle resulted in the development of a force much greater than the K-contraction (unpublished observation). Since the $V'_{\text{max}}$ decreased as the activation level was lowered in the guinea pig tenia coli [9], it is possible that the activation level was higher in previous studies than in this one.

The results reported here show that the $V'_c$ was higher when the release was made during the rise of force than at the peak, despite the lower force level in the former than the latter. This indicates, regardless of whether an internal load is present or not, that the cross-bridge cycling rate decreases greatly during a prolonged contraction known as the “catch state” in molluscan smooth muscles [18] and the “latch state” in some types of mammalian smooth muscles [19, 20]. The decrease in cross-bridge turnover rate in the early phase of contraction of the guinea pig tenia coli may be due to the development of latch-bridges, which are dephosphorylated cross-bridges and detach slowly from the actin filaments, as in the molluscan catch muscle [21]. In coincidence with this theory, the phosphorylation of a light chain of myosin becomes maximal before the peak force is reached and declines substantially thereafter [22].

The velocity in the slow shortening phase decreases gradually with time. Warshaw et al. [6] observed a similar phenomenon in a single smooth muscle cell isolated from a toad stomach. In their case, the shortening was interrupted for a short period by holding the muscle at a length attained by the shortening, not by stretching it to the original length as in this study. Since the time course of shortening was not affected by the short holding, they concluded that the deceleration in shortening velocity resulted from increased resistance to shortening by the compression of some intracellular materials. If so, the time course of the slow shortening phase should be reset by stretching to the original length. But this was not the case in this study. Therefore, the main cause of the gradual decrease in the shortening velocity of the slow phase in our preparation is not the compression of inert materials acting as the parallel elasticity. As discussed previously, the decrease in $V'_c$ in the early phase of contraction suggests the development of latch-bridges. The latch-bridge hypothesis would interpret the decrease in shortening velocity, if the population of the latch-bridges increases relative to the active cross-bridges during shortening. Another possibility is shortening deactivation as originally reported with skeletal mus-
cle fibers [23]. However, since the regulation of acti-
vation resides in the thin filament in the skeletal mus-
cle [24] while in the myosin side in smooth muscle
[25], and since deactivation may be related to the
mechanism of thin filament activation [26], the slow-
ing of shortening in smooth muscles is not due to the
shortening deactivation as observed in the skeletal
muscle. A decrease in the number of active cross-
brides during shortening, if any, would interpret the
observed slowing of shortening without assuming
latch-bridges. The stiffness measurement during the
slow shortening phase, such as that done by Hell-
strand and Nordstrom [17] during the rapid shortening
phase, will provide some insight about this problem.

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