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Contraction Transients of Skinned Muscle Fibers

(force generation/shortening/cross-bridge/presteady motion)

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ABSTRACT **The contraction kinetics of calcium-activated skinned muscle fibers were studied after step decreases in load by means of a quickly responding recording system. The steady velocity at a given relative load was close to that found in electrically stimulated, intact muscle fibers. The presteady motion had the same shape as that of intact fibers, but the time scale of the transient was nearly two times slower. The duration of the initial phase of the motion, where the velocity was greater than the steady value, and the time at which the subsequent low velocity phase ended, were both stretched out to the same extent. Changing the temperature had the same effect on the length of these two phases of the transient. The results indicate that both phases of the transient are produced by the same underlying factors and can be taken as evidence that the entire transient originates in the cross-bridge mechanism. In this case the experimental technique described here provides a basis for distinguishing between chemical parameters that affect contractility by (*a*) controlling the number of sites at which cross-bridges can be formed, as opposed to (*b*) changing the kinetic properties of a given number of sites.**

There is now considerable evidence that the contractile force in muscle fibers is developed by cross-bridges that form between the myosin-containing and the actin-containing myofilaments (1). When the load on the fiber is less than the maximum force the cross-bridges can develop, the fiber shortens, the myosin and actin filaments slide past each other, and the cross-bridges turn over.

Since the behavior of intact muscle fibers should reflect the mechanochemical properties of the cross-bridges, attempts have been made to obtain information about the cross-bridges from physiological data. In the first quantitative analysis along these lines, A. F. Huxley (2) made use of the well-known relations between load and steady shortening velocity, on the one hand, and between load and energy production, on the other, to derive possible rate functions for the "making" and "breaking" of cross-bridges. Later, the presteady motions that follow step changes in load were also used for this purpose (3), since these contraction transients appeared to provide additional constraints on the cross-bridge parameters.

Methods have recently been worked out for making quantitative studies of the cross-bridges in skinned muscle fiber preparations (4, 5), that is, muscle fibers in which the outer membrane has been removed by microdissection (6). The principal advantage of this preparation is that the biochemical milieu surrounding the myofilaments can easily be controlled experimentally. This procedure has been used to study the influence of important chemical parameters (e.g., calcium ion concentration and ionic strength) on both force development and steady shortening velocity (5, 7, 8). Since the latter measurements were made with transducers that were too slow to detect transients in the motion, it seemed worthwhile to develop a technique for recording from skinned fibers in which the presteady motion could be seen. This was done in the present study. The data that were obtained provide evidence that the isotonic velocity transients arise entirely in the cross-bridge mechanism. This result implies that the characteristics of the transient at a given relative load can be used to distinguish between chemical parameters that affect the contraction mechanism solely through the activation process [as calcium ions are believed to do (5)] and those that modulate the kinetic properties of the cross-bridges [as has been suggested for ionic strength (8)].

Abbreviation: EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid.

METHODS

The displacement and force transducers were those described by Civan and Podolsky (9). They were adapted for use with skinned fiber preparations by cementing a short attachment wire to the end of each transducer. A fiber segment was isolated from the semitendinosus muscle of a *Rana pipiens pipiens* frog and the ends were tied to the attachment wires with square knots of 5–0 surgical silk. The segment length between the ties ranged from 1 to 4 mm; this was kept short to reduce the compliance of the moving system. The equivalent mass of the displacement transducer was about 3 mg and the natural period for moderate force steps was less than 5 msec.

The dissection and mounting of the fiber were carried out under cold mineral oil. The mounted segment was transferred to a relaxing solution consisting of 140 mM KCl, 5 mM ATP, 1 mM $MgCl_2$, 10 mM imidazole, and 3 mM ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid (EGTA). The sarcomere length was set close to 2.3 μm. In the contracting solution, 3 mM EGTA was replaced by 2.94 mM CaEGTA + 0.06 mM EGTA (pCa 5.0). The pH of the solutions was set at 7.0 at 0°. The solutions were contained in a temperature controlled solution changer (5) by means of which the preparation could be carried through as many as 25 cycles of contractile activity without much change in either the magnitude of the steady isometric force, P_0, or the time course of the motion at a given relative load.

RESULTS

Skinned muscle fiber segments were activated at pCa 5 and, after the isometric force became steady, the load was quickly changed to a value less than P_0. The panels in Fig. 1 show typical records of the force (middle trace) and displacement (upper trace) at 3°. After the load step the motion went through a nonsteady phase and then became steady. The general pattern of the motion is the same as that reported pre-

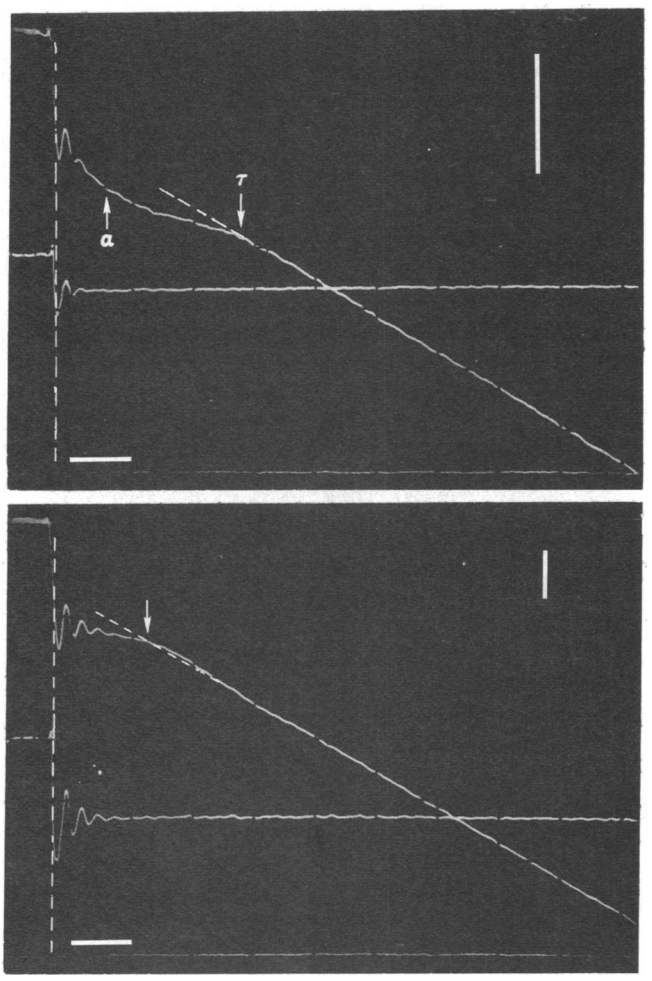

FIG. 1. Response of a skinned muscle fiber to step changes in load at 3°. Top trace, displacement; middle trace, force; bottom trace, zero of force. The steady isometric force is 72 mg weight; the relative load step is 0.17 in the upper panel and 0.37 in the lower panel. The back extrapolation of the steady motion is indicated by a fine *dashed line*; the upward *arrow* marks the point at which the instantaneous velocity in the transient is equal to the steady velocity; the downward *arrow* marks the null time. The steady velocities at the two relative loads are close to those found in intact fibers (see Fig. 3 in ref. 3). Segment length, 3.9 mm; *vertical scale bar*, 50 Å/half sarcomere; *horizontal scale bar*, 20 msec.

viously for electrically stimulated intact muscle fibers (9), but the time scale of the transient in the skinned fiber is considerably slower than that in the intact fiber.

In order to make quantitative comparisons of the presteady motion under various conditions, it is convenient to mark off times that correspond to certain well-defined phases of the motion. These are (*i*) the time origin t_0, when the falling force trace first reaches the value of the load; (*ii*) α, the time when the instantaneous velocity first reaches the value of the steady velocity; and (*iii*) the null time τ, when the actual motion beyond α first crosses the back extrapolation of the steady motion. In Figs. 1 and 3, the sloping *dashed line* is the back extrapolation of the steady motion and the three defined times are marked by (*i*) a vertical *dashed line*, (*ii*) an upward pointing *arrow*, and (*iii*) a downward pointing *arrow*, respectively. Since the motion is first faster than, and then slower than the steady motion, α measures the length of the initial phase of the motion during which the velocity is faster than the steady

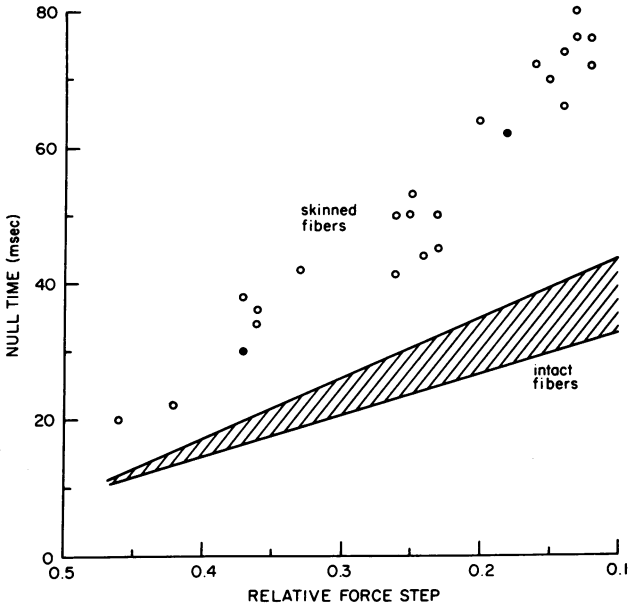

FIG. 2. Influence of force step on the null time. Abscissa is the force step expressed as a fraction of P_0; ordinate is the null time in msec. Data points are measurements taken from five skinned fiber preparations at 3°; *filled circles* are from the traces shown in Fig. 1. Hatched region represents values obtained from electrically stimulated intact muscle fibers at 3° (9).

value. The value of τ, on the other hand, marks the end of the relatively slow phase that follows the high velocity phase and the onset of motion at a speed close to steady value.

The experiments in Fig. 1, which were made at 3°, show that the null time τ is 63 msec for a load step of 0.17 P_0 (upper panel) and 30 msec for a load step of 0.37 P_0 (lower panel). These data and additional measurements from this and four other skinned fiber preparations are plotted as points in Fig. 2. Corresponding data from electrically stimulated intact muscle fibers (9) fall within the hatched area. At a given load step, the null time in the motion of skinned fibers bathed in contracting solution is about twice as long as that for intact fibers. The steady shortening velocity (in Å per half sarcomere/sec) at a given relative load is nearly the same in the two preparations [also see ref. (8)].

The value of α in the upper panel of Fig. 1 is 17 msec, which is about 0.3 τ. Since this relation between α and τ also holds for the corresponding transient in intact fibers (9), these characteristic times are both extended in the same proportion in skinned fibers. Unfortunately, α is obscured by the natural oscillation of the displacement transducer in the motion that follows the larger force step in Fig. 1.

The amount of shortening in the period between t_0 and τ is close to that found in intact muscle fibers. Although this quantity is difficult to measure directly because of the natural oscillation of the displacement transducer, the contribution of the natural oscillation to the displacement trace can be estimated from its reflection in the force trace (9). Using this procedure, the fiber shortening between t_0 and τ is found to be about 50 Å/half sarcomere for a force step of 0.17 P_0 (upper panel) and 30–40 Å/half sarcomere for a force step of 0.37 P_0 (lower panel), which is essentially the same as the values found for these force steps in intact fibers (3).

Fig. 3 shows the influence of temperature on the motion. In the upper panel, a force step of 0.23 P_0 was applied to a

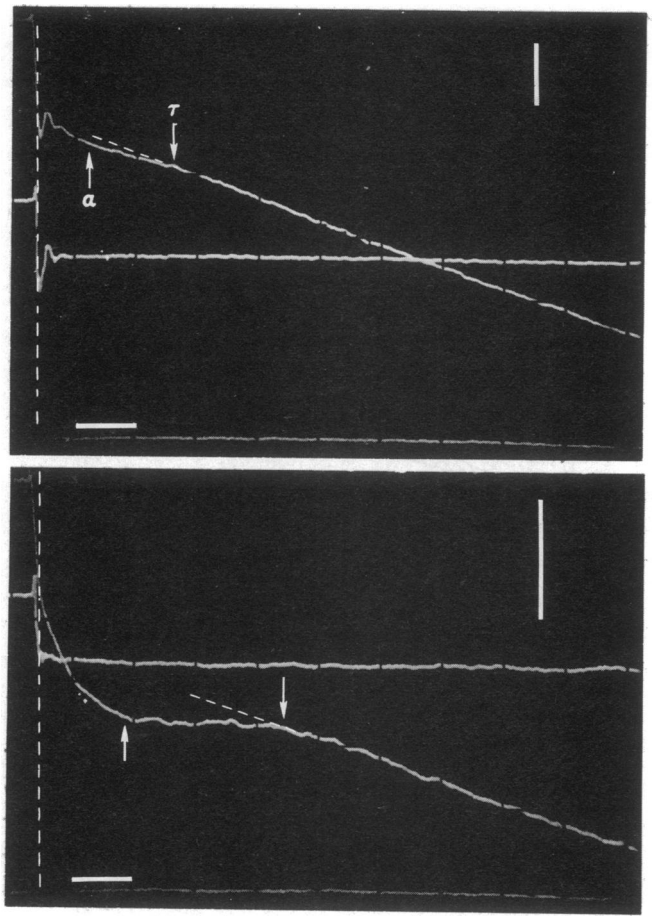

FIG. 3. Influence of temperature on the response of a skinned muscle fiber to a step change in load. Top trace, displacement; middle trace, force; bottom trace, zero of force. Upper panel, 4.5°; steady isometric force, 79 mg weight. Lower panel, 0.5°; steady isometric force, 49 mg weight. Relative load step, 0.23. The back extrapolation of the steady motion is indicated by a fine *interrupted line*; the upward *arrow* marks the point at which the instantaneous velocity in the transient is equal to the steady velocity; the downward *arrow* marks the null time. Segment length, 1.9 mm; *vertical scale bar*, 50 Å/half sarcomere; *horizontal bar*, 20 msec.

fiber at 4.5°; in the lower panel the temperature was lowered to 0° and the same relative force step was applied. At the higher temperature, α is 15–17 msec and τ is 44 msec (α/τ = 0.34–0.39). At the lower temperature, α is 27 msec and τ is 76 msec (α/τ = 0.35). Similar measurements from five other preparations are collected in Table 1. The data show that the temperature coefficients of α and τ are nearly the same. It should be noted that for this change in temperature the steady speed is halved. Since the null time is increased 2-fold, the product of the steady speed and the null time is independent of temperature.

DISCUSSION

The main finding in the present study is that the time scale of the transient in skinned fibers under our experimental conditions is about two times slower than that in intact fibers from the same species, even though the steady contraction speed is about the same. The cause of this slowing is not known. In skinned fibers the filament spacing is greater than in intact fibers (10), and, although a decrease in spacing does not affect

TABLE 1. *Influence of temperature on the presteady motion of skinned muscle fibers*

	0–1.5°			4–5°		
Experiment	α (msec)	τ (msec)	α/τ	α (msec)	τ (msec)	α/τ
18 iv 73	34	88	0.39			
	32	94	0.34			
8 vi 73a				15	31	0.48
8 vi 73b				15	43	0.35
13 vi 73	24	55	0.44			
	35	82	0.43			
	23	59	0.39			
14 vi 73				18	43	0.42
				12	36	0.33
13 vii 73	26	67	0.39	14	36	0.39
	26	71	0.36	16	41	0.39
				16	42	0.38
Mean	29	74	0.39	15	38	0.40
Standard error of the mean			0.01			0.02

α, the time when the instantaneous velocity first reached the value of the steady velocity, and τ, the null time, were measured after the load was quickly changed from P_0 to values between 0.75 and 0.80 P_0.

the transient (9), an increase in spacing above the maximum value found in intact fibers might have an effect on the motion. In addition, the level of ionized magnesium is probably lower (11), and the chloride concentration higher, for skinned fibers in the present bathing solution than in intact fibers, but the influence of these factors remains to be examined.

Both the duration of the initial high velocity phase (which is measured by α), and the end of the low velocity phase of the transient (which is measured by τ), appear to be stretched out to the same extent. Lowering the temperature also extends the two phases of the transient in the same proportion. These observations strongly suggest that the same underlying factors produce the motion during both phases of the transient.

Origin of the Velocity Transient. One of the conclusions that can be drawn from this result is that the entire isotonic transient originates in the cross-bridge mechanism. This is important because evidence relating the transient to the cross-bridge mechanism is quite limited. The original connection (12) was the fact that in intact muscle fibers the influence of temperature on the duration of the transient at a given force step, as measured by the change in the null time, is the same as its influence on the steady contraction velocity; the present data show that this relation also holds for skinned fibers. But since the null time marks only the end of the *slow* phase of the transient, it remained possible that the *initial* high velocity phase is produced by a different mechanism, such as the non-instantaneous relaxation of a passive structure (the Z disc or the M line, for example) in series with the cross-bridges. The present results argue against this, however, as the durations of both phases of the transient appear to be coupled to each other.

Relation to the Force Transient. The processes that produce the velocity transient after a step change in load would also be expected to give rise to a force transient after a step change

in length. Thus Huxley and Simmons (13) have shown that force redevelops very quickly in the first few milliseconds after a step decrease in length, which corresponds to the relatively high shortening velocity seen just after a step decrease in force.* These authors attributed the quick redevelopment of force to activity of the cross-bridge mechanism, and the present results, which link the initial phase of the presteady motion to the cross-bridge mechanism, provide additional evidence that this is the case.

Possible Application of the Experimental Technique. There are a number of chemical parameters that affect the steady isometric force of skinned muscle fibers but have no effect on the steady shortening speed at a given relative load. The influence of calcium ions in the concentration range between 10^{-7} and 10^{-6} M is a well known example. The explanation offered for this effect is that, when the calcium ion concentration is increased, the number of actin sites at which cross-bridges can be formed (active sites) increases, but that the kinetic properties of each active site remains the same (5). Therefore, one would expect the isotonic velocity transient at a given relative load to be the same at all calcium ion concentrations in this range.

Another example of a chemical parameter that changes force but not speed is ionic strength in the range between 190 and 330 mM (8). This effect may be due to a change of the kinetic properties of the sites at which cross-bridges are formed rather than, as in the case of calcium, a change in the number of active sites. In this event, the transients at a given relative load would be expected to be different at different ionic strengths.

The argument used here depends only on knowing that the presteady motion originates entirely in the cross-bridge mechanism and, therefore, it is model-independent. Thus, information about the mechanism of action of a chemical parameter on the contraction mechanism is potentially available from an analysis of its effect on the presteady motion.

Cross-Bridge Processes Underlying the Transient. The specific processes that produce the various phases of the presteady motion are not yet clear. However, it has been shown that the entire time course of the motion in intact fibers, over a wide range of load steps, can be closely reproduced by a model in which appropriate rate functions are selected for the making and breaking of cross-bridges, and the cross-bridge force is described by a single-valued function (3, 15). It is also possible to stretch out the time scale of the transients by modifying the three significant model parameters (unpublished calculations).

A different explanation of the presteady motion, containing additional parameters, has recently been put forward by Huxley and Simmons. These authors suggested (13, 16) that the initial high velocity response to a step change in load is due to a process with a fast rate constant (the rapid movement of attached cross-bridges down a local potential energy gradient) and that the subsequent low velocity phase ends when different processes with much slower rate constants (the making and breaking of cross-bridges) exert their effects. However, since the two phases of the motion are extended in proportion when the transient is stretched out, either by the changes associated with skinning or by a change in temperature, it seems unlikely that the two phases reflect the sequential involvement of different types of processes with markedly different rate constants. It is more probable that the same processes are involved in both phases of the motion, and that the time course of the presteady motion is determined by the continuous interaction of these processes, although further studies (15, 16) must be done to establish this point conclusively.

* For the moderate force steps used in the present study, the value of α provides a measure of the duration of the fast phase of tension redevelopment after the corresponding length step. This can be seen by comparing force step and length step data taken from the same preparation (compare Fig. 7 and Fig. 8 in ref. 14). It should be noted that, for a given force step and temperature, the values of α and τ in intact fibers are about twice as long in *Rana pipiens pipiens* as they are in the *Rana temporaria* used by Huxley and Simmons (compare Fig. 3 in ref. 9 with Fig. 7 in ref. 14), which may be correlated with the faster speed of steady shortening recorded by the latter authors.

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