Force responses to rapid length changes in single intact cells from frog heart

F. Colomo, C. Poggesi and C. Tesi

Dipartimento di Scienze Fisiologiche, Università degli Studi di Firenze, Viale G. B. Morgagni 63, I-50134 Firenze, Italy

1. Force transients in response to step perturbations in length were recorded in intact atrial cells from frog heart at various temperatures (6–15 °C). Length changes of various sizes and in either direction, complete in 0·5 ms, were applied to single myocytes near slack length (initial sarcomere length 2·1–2·2 μm) just before the peak of an isometric twitch. The frequency response of the force transducers used was 2–4 kHz in Ringer solution.

2. An early quick force recovery phase was clearly observed after the elastic force response to the length step and before the start of much slower recovery processes. The quick recovery phase became progressively faster with larger shortening steps and was almost as fast as that originally described in intact frog skeletal muscle fibres (rate constants above 1000 s^{-1} in large releases at 10 °C).

3. The force–extension relation determined at the end of the length change (T_1 curve) indicates that an instantaneous shortening of 0·5–0·6 % of the initial cell length (L_0) brings the force to zero. The force–extension relation determined at the end of the quick recovery phase (T_2 curve) showed that the early recovery leads to an almost complete restoration of the original force with small stretches and releases (up to 0·3 % L_0) and that it becomes negligible in shortening steps of about 1·4 % L_0.

4. The results suggest that the mechanical properties of attached cross-bridges and the rate of transitions between attached cross-bridge states are approximately the same in frog atrial cells and fast skeletal muscle fibres.

5. The maximum velocity of shortening (V_0) of frog atrial cells, determined at the peak of isometric twitches by the slack test, was 0·5–1·0 L_0 s^{-1} at 6 °C and increased with temperature with a temperature coefficient (Q_{10}) higher than 3. The values of V_0 found are much lower than those reported for frog skeletal muscle fibres at the same temperature.

6. The findings provide evidence for a single mechanism of force generation in striated muscle in spite of large differences in the maximum velocity of shortening.

Studies of tension transients in response to sudden length changes applied to contracting fibres from frog skeletal muscle have provided much information about the processes contributing to force generation (Huxley & Simmons, 1971; Ford, Huxley & Simmons, 1977; Irving, Lombardi, Piazzesi & Ferenczi, 1992). According to an analysis by Huxley & Simmons (1971), the quick recovery of force following a fast length perturbation reflects the force-generating step in the cyclic interaction of myosin cross-bridges with actin.

The tension transients described in cardiac muscle (Steiger, 1977; Brady, 1979; Saeki, Sagawa & Suga, 1980; Ventura-Clapier, Saks, Vassort, Lauer & Elizarova, 1987; Berman, Peterson, Yue & Hunter, 1988), as well as in other slowly contracting muscles (Heinl, Kuhn & Rüegg, 1974; Warshaw & Fay, 1983), differ considerably from those of fast skeletal muscle. The rates of the earliest components of force recovery in slow muscle tissues are orders of magnitude slower than those found by Huxley & Simmons (1971) and exhibit the opposite dependence upon the amplitude and the direction of the applied length changes.

Single intact myocytes from frog atria have been used in the present study in an attempt to resolve the fast events of tension transients in heart muscle and investigate the properties of cardiac cross-bridges. Frog atrial cells are tendon-free striated fibres containing only one to four myofibrils 100–200 sarcomeres long (Hume & Giles, 1981).

METHODS

For the present experiments we improved the performance and time resolution of techniques that have been recently described to measure and control the force and length of myocytes from frog heart (Cecchi, Colomo, Poggesi & Tesi, 1992, 1993). Briefly, atrial cells were enzymatically isolated

from hearts of frogs (*Rana esculenta*), which had bee decapitated, the brain destroyed, and spinally pithed. Cells were mounted horizontally in a temperature-controlled chamber by sucking their tapered ends into the tips of two glass micropipettes. Experiments were performed on seven atrial myocytes at various temperatures between 6 and 15 °C. Force was measured by photoelectronically recording the elastic deflection of the tip of one suction micropipette, which doubled as a cantilever force probe of calibrated compliance. The other pipette was stiff and mounted on the lever arm of a length-control motor. With respect to the previously published technique (Cecchi *et al.* 1992, 1993), the force probes built for the present experiments had a lower compliance (2–7 nm nN^{-1}) and a higher frequency response (2–4 kHz in Ringer solution). In any given experiment, force probes were selected for compliance so as to limit cell shortening during isometric twitch contractions to 0·1–0·4 % of the initial cell length (L_0). Moreover, mounting of the stiff suction pipette in line with the motor lever arm improved the dynamic performance of the motor so that it could produce length steps of different size which were complete in 0·5 ms.

Force transients were elicited in atrial cells near slack length (sarcomere length 2·10–2·20 μm) by applying small length changes just before the peak of isometric twitches elicited by electrical stimuli at 0·05 Hz. Only the cells that exhibited negligible resting responses to the same length changes were selected for experiments.

RESULTS

In all the myocytes studied, the imposition of a rapid length change near the peak of an isometric twitch resulted in a multiphasic force response. Figure 1 shows the results of a typical experiment. It can be seen that force drops simultaneously with the shortening step and then recovers towards the original level before the step. The force recovery exhibits an early rapid phase, complete in a few milliseconds, followed, after a period when the recovery rate is greatly reduced, by a slower recovery. The early recovery phase was very fast and well evident in spite of the cell length creep caused by the compliance of the force probe. The rate of the early force recovery exhibited the same dependence on the size and direction of the length step as originally described in skeletal muscle fibres (Huxley & Simmons, 1971). As shown in Fig. 2, the rate of the early recovery is slowest in the response to the stretch and becomes progressively faster with larger shortening steps.

The extreme force reached at the end of the length change (T_1) and the force that is approached during the quick recovery phase (T_2) are plotted against the size and the direction of the length step in Fig. 3*A*. For both curves the amplitude of the applied length changes was corrected for the compliance of the force-probe pipette. The T_1 curve was linear for small stretches and releases and became slightly concave upwards for larger shortening steps. The linear part of the T_1 relation was the best approximation of the instantaneous elasticity of cardiac cells. The deviation from linearity observed with large releases was most likely to be the result of the early force recovery, which became progressively faster with increase in release amplitude (Fig. 3*B*) and truncated the instantaneous force change (see Fig. 1*C*). The minimum shortening required to drop isometric peak twitch force to zero, estimated by extrapolating the linear part of the T_1 curve to the length axis, was 0·54 % L_0 in the preparation of Fig. 3*A* and ranged from 0·50 to 0·60 % L_0 in seven cells tested. The T_2 curve of these cells was concave upwards on the stretch side and downwards on the release side. With small stretches and releases (up to 0·3 % L_0) the early recovery phase was almost complete. With larger releases the early recovery was progressively less complete and eventually became negligible in shortening steps of about 1·2–1·6 % L_0. In the preparation of Fig. 3 the early fast recovery became negligible with releases of about 1·4 % L_0. Unlike the T_1 curve, the T_2 curve was not sensitive to changes in temperature.

In Fig. 3*B*, an estimate of the rate of the quick recovery phase is plotted against the size of the length step applied to a cell at 10 °C. It can be seen that the rate constant for

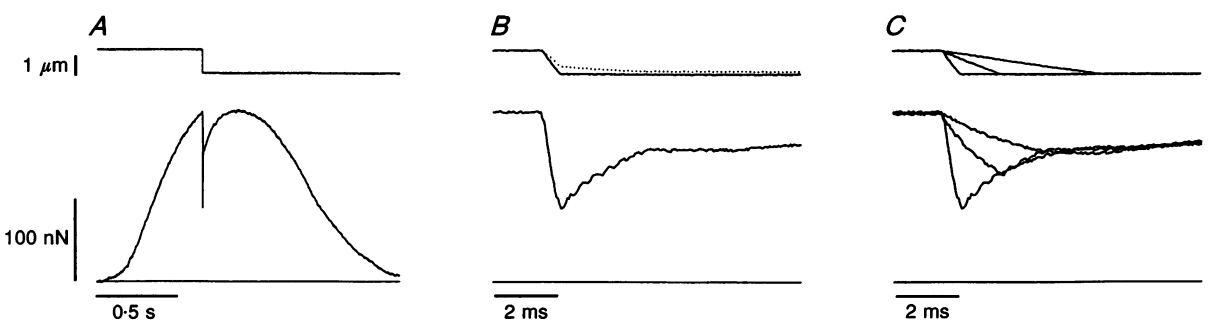

Figure 1. Force transient in a single frog atrial cell
A, force response to a step shortening complete in 0·5 ms, applied just before the peak of an isometric twitch. Upper trace: length step. Middle trace: force. Bottom trace: baseline for resting force. Temperature, 10 °C; resting cell length, 210 μm; force-probe compliance, 3·25 nm nN^{-1}. *B*, same records as in *A*, but on a faster time scale to resolve the early phases of the force transient. The dots superposed on the upper trace show the length change undergone by the cell during and following the applied step, corrected for the compliance of the force probe. A small length creep occurs as the force rises after the step. *C*, superposed records of force responses to step shortening of the same size as in *A*, but complete in 0·5, 2 and 5 ms.

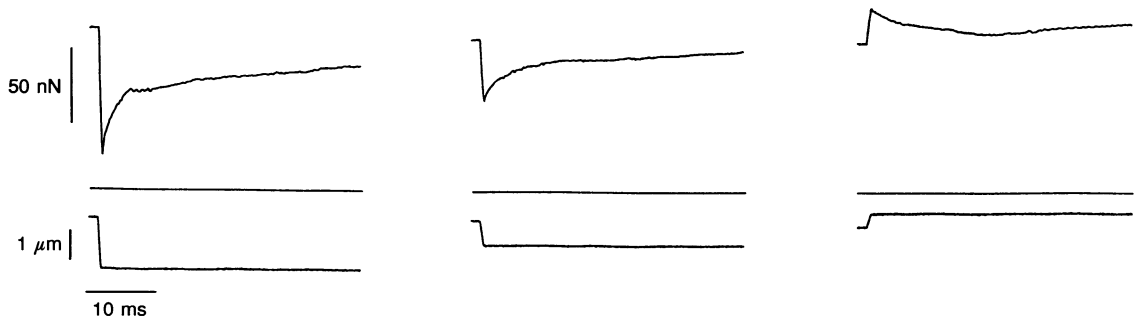

Figure 2. Early phases of force transient
Response to step changes in length of various size, complete in 0·5 ms, applied to a single frog atrial cell before the peak of an isometric twitch. Upper traces: force. Middle traces: baseline for resting force. Bottom traces: length change applied to the cell. Temperature, 8 °C; resting cell length, 200 μm; force-probe compliance, 7 nm nN^{-1}.

the rapid recovery of force (calculated from the reciprocal of the half-time) increases from about 300 s^{-1} in the region of the stretches to about 1000 s^{-1} in the region of the largest releases. Comparable results were also obtained in the other cells tested. The rate constant of the quick recovery phase in cardiac cells was highly sensitive to temperature (temperature coefficient (Q_{10}) around 2·5).

The maximum velocity of shortening (V_0) of frog atrial cells was determined by the slack test (Edman, 1979). Step releases of varying amplitude (range 1–4 % L_0) were applied to atrial cells just before the peak of isometric twitches, and the time required by the myocyte to take up the resultant slack was measured. V_0, measured from the slope of the relation between the size of the imposed shortening and the time required for the redevelopment of force, ranged between 0·5 and 1·0 L_0 s^{-1} in four cells at 6 °C and increased with temperature with a Q_{10} higher than 3.

DISCUSSION

The early fast events of the force response of cardiac muscle to step perturbations in length have been resolved for the first time in the present investigation. In the usual cardiac

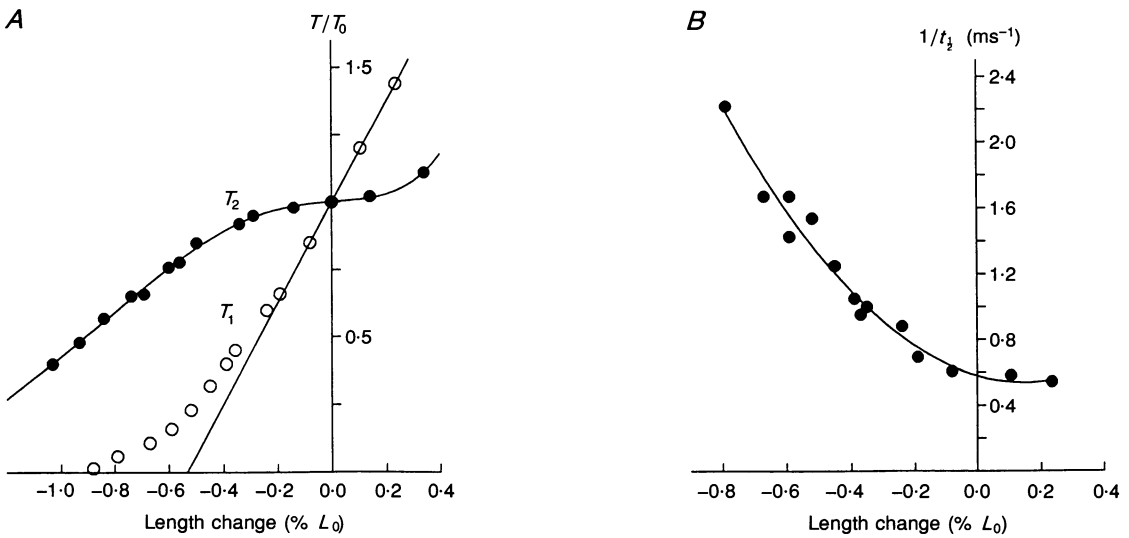

Figure 3. Forces and rate of force recovery *versus* length change in the early phases of force transients
A, extreme force reached at the end of the length change (T_1, ○) and force approached during the early recovery phase (T_2, ●) plotted against the amplitude and direction of the length step applied to a single atrial cell. Forces (T) are expressed as a fraction of the isometric force immediately before the step (T_0). For both curves the size of the length changes has been corrected for the compliance of the force-probe pipette. The continuous line in the T_1 curve is the linear fit to data points for stretches and small releases. The continuous line in the T_2 curve was drawn by eye. Temperature, 10 °C. Same cell as in Fig. 1. *B*, reciprocal of the half-time of early recovery phase plotted against amplitude and direction of the length change. Data points estimated from the same experiment as in *A* at 10 °C. The continuous line is a parabola fitted to the data points.

preparations an early force recovery process as fast as that decribed here has always been masked by a large end compliance (Delay, Vassallo, Iwazumi & Pollack, 1979). In experiments on single cardiac cells the only significant source of series passive compliance was the force-probe pipette. Reduction of force-probe compliance well below that of cardiac myocytes was an essential initial step to resolve the fast events of force transients.

In spite of some limits in the method used (compliance of the force probes and lack of direct control of sarcomere length) it appears that the force transients reported in this investigation reflect events at the sarcomere level. Moreover, force transients in atrial cells are almost indistinguishable from those of frog skeletal muscle fibres in which length changes were controlled at the sarcomere level, an indication that the underlying mechanisms are similar. In skeletal muscle, the earliest fast events of the force transient presumably reflect redistribution within the pools of attached cross-bridges (Huxley & Simmons, 1971). Analysis of such events in atrial cells can, therefore, provide information about the specific properties of cardiac cross-bridges. The T_1 and T_2 curves of cardiac cells are in reasonable agreement with those of fast skeletal muscle (Huxley & Simmons, 1971; Ford *et al.* 1977), indicating that the cross-bridge compliance and the range of filament sliding over which attached cross-bridges can exert force are the same in the two muscle types. The rates of the quick force recovery in atrial cells at 8–10 °C are very close to those reported by Huxley and co-workers at 0–4 °C and exhibit similar strain dependence. Due to the high temperature sensitivity, the rate of early recovery may be slower in cardiac than in fast skeletal muscle. The point cannot be firmly established because the creep of the cell length caused by the compliance of the force probes (Fig. 1*B*) can lead to underestimation of the real rate of the cardiac fast recovery process.

The values of V_0 found in single atrial cells are much higher than those previously reported for multicellular amphibian heart preparations (Sato & Mashima, 1981), but they are still several times lower than those typically found in frog skeletal muscle fibres (Cecchi, Colomo & Lombardi, 1978; Edman, 1979). The result demonstrates that frog atrial cells belong to a slow muscle tissue. On the other hand, the analysis of force transients suggests that the kinetic properties of the force-generating transition in the cardiac cross-bridge cycle are almost the same as in fast skeletal muscle. These findings provide evidence for a unique mechanism of force generation in muscle tissues in spite of different intrinsic speeds of shortening.

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