

# THE VARIATION IN TENSION OUTPUT WITH ACTIVE SHORTENING IN RAT MYOCARDIUM

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## INTRODUCTION

It has long been discussed whether the end-systolic pressure-volume relationship of the ventricle is the same in isovolumic and ejecting beats. An exhaustive discussion on these topics is reported in the papers by Suga and Yamakoshi (29) and by Weber *et al.* (32). Similar problems have been encountered in isolated cardiac muscle. Downing and Sonnenblick (9) and Sonnenblick (26) noted that, in cat papillary muscle, tension output depends only on instantaneous length. Later investigations (5, 8, 30, 31) have shown that active shortening lowers the capability to bear tension.

The uniqueness of the tension-length relationship has been still recently examined by Strobeck *et al.* (28).

The aim of the present investigation was to study the deactivation phenomenon by active shortening in rat papillary muscle, which has electrical and mechanical properties and  $\text{Ca}^{2+}$  kinetics different from those of other mammalian hearts (4, 12, 22). In order to pursue this goal, isometric responses, stopped isotonic and afterloaded contractions have been studied. As the matter is connected with the activation process, the influence of two different inotropic interventions (high calcium concentration and caffeine) has been explored.

## METHODS

*A. Preparation and apparatus.* Twenty papillary muscles were obtained from left ventricles of adult male Wistar rats (ca. two months old, 200 g body weight) under light ether anaesthesia.

After careful dissection, each muscle was placed in a small thermoregu-

lated bath, filled with Krebs-bicarbonate solution, bubbled with 95%-5%  $O_2$ - $CO_2$  mixture (pH 7.45). The solution, kept at a constant temperature of 20°C, had the following millimolar composition:  $Na^+$  145.2,  $K^+$  3.6,  $Ca^{++}$  2.5,  $Mg^{++}$  1.2,  $Cl^-$  128.4,  $H_2PO_4^-$  1.2,  $HCO_3^-$  24.8,  $SO_4^{--}$  1.2, glucose 5.6.

The papillary muscle was tied by means of silk threads to a force transducer (Statham G1-1,5-300) and to a light isotonic lever (equivalent mass 60 mg). Displacement of the lever was detected by means of a linear photoelectric transducer, made up of a germanium photodiode. The compliance of the whole setup (with the silk threads) was about  $10^{-3}$  mm/mN.

The papillary muscle was stimulated at a frequency of 2/min, through platinum multifile electrodes, by square wave pulses of 3 msec duration and supramaximal voltage.

A synchronous motor moving the isometric transducer could change muscle length, when the isotonic lever was blocked. When the lever was free to move, loading of the lever (preload and/or preload plus afterload) was achieved by stretching a stainless steel coil spring (spring compliance: 3 mm/mN), soldered at a point very close to the lever fulcrum. The amount of muscle shortening could be controlled by using a manual stop device, placed in front of the isotonic lever. This apparatus allowed us to obtain isometric, stopped isotonic and after loaded contractions. Quick releases were imposed to preparations both at rest and during isometric contractions by means of an electronically controlled stop device.

*B. Experimental procedure.* At the beginning of each experiment papillary muscles were stretched to 1.15-1.2  $L_0$  ( $L_0$  is the muscle length at zero load) and then were stimulated and allowed to contract isometrically during two hours.

Afterwards the muscle length at which developed tension is maximal ( $L_{max}$ ) was found. The length was then set to 0.95  $L_{max}$  (initial length or  $L_i$ ) and the muscle was allowed to equilibrate till resting and developed tensions became stable.

The effect of shortening on tension output was examined by comparing the isometric responses with the responses during which an isotonic phase was imposed.

The distance between the isotonic lever and the manual stop device determined the extent of shortening ( $\Delta L$ ) which was varied between 1 and 10%  $L_i$ . The following procedure was used: the muscle was shortened just before stimulation (A-I in Fig. 1) and an isometric twitch at any given length  $L_i - \Delta L$  was obtained (I-E in Fig. 1); after that the muscle was stretched back to  $L_i$ . As the shortening was interrupted by the stop device, the displacement ( $\Delta L$ ) was strictly equal to that imposed at rest, before the corresponding isometric twitch. In this way, when only a preload was applied to the preparations, stopped isotonic contractions (A-H-F in Fig. 1) were obtained, whereas, when an afterload was added to the preparations, stopped isotonic contractions with afterload (A-B-G-F) or afterloaded contractions (A-C-F) could be obtained.

The sequence of twitches was then reversed. This procedure and the low frequency of stimulation allowed us to avoid the influence of previous mechanical events on subsequent twitches (16), though this phenomenon seems to be scarcely present in rat myocardium (25).

In some experiments the above procedure was repeated after treatment of the preparation with inotropic agents. The response to an increase in the external  $Ca^{2+}$  concentration (from 2.5 to 10 mM) was explored in five experiments. The effects of caffeine (10 mM) with high  $Ca^{2+}$  concentration were studied in five experiments.

*C. Determination of muscle length and cross-sectional area.* Muscle length was evaluated by means of a micrometer connected to the force transducer.

At the end of each experiment the muscle was weighed and its equivalent cross-sectional area was calculated by the weight/length ratio, on the

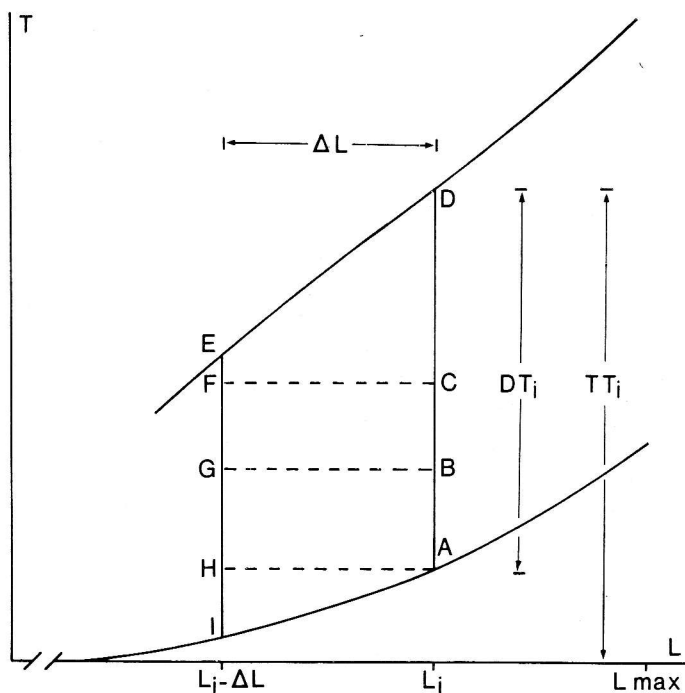


Fig. 1. - Tension ( $T$ )-length ( $L$ ) diagram.

Schematic drawing which represents the experimental procedure used in the present investigation.  $DT_i$  and  $TT_i$  correspond to developed and total tension, in the isometric twitch performed at the initial muscle length ( $L_i$ ). Controlled muscle shortening ( $\Delta L$ ) is achieved at rest (A-I) or during activity in afterloaded contractions (A-C-F) and in stopped isotonic contractions with (A-B-G-F) or without afterload (A-H-F).

assumption that the specimen was a cylinder of length  $L_i$  (ca.  $0.95 L_{\max}$ ) with a relative density of 1.0. A direct evaluation of the diameter, performed in some preparations, by means of a microphotographic method, evidenced that the calculated value of the cross-sectional area was overestimated by a factor of 1.2-1.3. This discrepancy might be due to the fact that the muscles were weighed without blotting to remove excess fluid from the surface.

*D. Recording and measurement of responses.* The signals from the force and displacement transducers were displayed on a storage oscilloscope (Hp 1.201 A) and recorded with a tape recorder (Tandberg 115) for further analysis. Data processing was carried out by means of a two channel memory unit (Kemo 1.024 AM) which fed both an XY/t recorder (Bryans 29.000 A3) and an alphanumeric printer device (Facit 4.554).

For each contraction resting tension, peak tension (developed and total) and amount of shortening were measured; the time to the peak of the twitch and the time at which the displacement took place were measured from the onset of contraction.

In the present investigation the peak tension of different responses

was taken as an indirect index of the degree of activation. This is a simple mean of assessing contractile activity provided that the time to the peak of the responses is stable (15). Actually, in the present experiments isotonic shortening little delayed the peak of the responses.

## RESULTS

Table 1 summarizes dimensions and tensions (developed and resting) of the twenty papillary muscles selected for the present investigation. The criteria for selection of the specimens were: *a*) cross sectional area (at  $L_i$ ) less than  $1.5 \text{ mm}^2$  and *b*) resting-developed tension ratio (RT/DT) at  $L_i$  less than 0.25 (13, 17, 27). The values of resting and developed tensions (Table 1) are lower than those usually reported for Wistar rat papillary muscle (*e.g.* 4). However, it must be noted that tensions are evaluated at  $L_i$  (ca.  $0.95 L_{\max}$ ) and that the cross sectional area is probably overestimated (see Methods).

1. *Effects of the amount of active shortening.* — In Fig. 2 muscle length ( $L$ ) is plotted versus total (TT) tension in isometric and in different kinds of isotonic contractions. It is evident that the reduction in total tension, in contractions with an isotonic phase, cannot be predicted on the basis of the isometric length-tension relationship and that this deficit in tension increases with the extent of active shortening. A better evidence of this phenomenon can be obtained if total tension in isotonic contractions ( $\tilde{T}$ ) is expressed as percent of the isometric tension at the same instantaneous length (TT) (Fig. 3). All these results agree with those already reported by Brady (5), Brutsaert and Housmans (8) and Suga *et al.* (30).

TABLE 1. — Mean values and standard errors for dimensions and isometric tensions (resting and developed) of the twenty papillary muscles used.

Muscle weight (mg)	$5.50 \pm 0.54$	Developed tension at $L_i$ (mN/mm <sup>2</sup> )	$23.57 \pm 1.63$
Muscle length at $L_i$ (mm)	$4.71 \pm 0.23$	Time to peak tension (msec)	$240 \pm 14$
Cross sectional area at $L_i$ (mm <sup>2</sup> )	$1.14 \pm 0.07$	Resting tension at $L_i$ (mN/mm <sup>2</sup> )	$4.13 \pm 0.34$

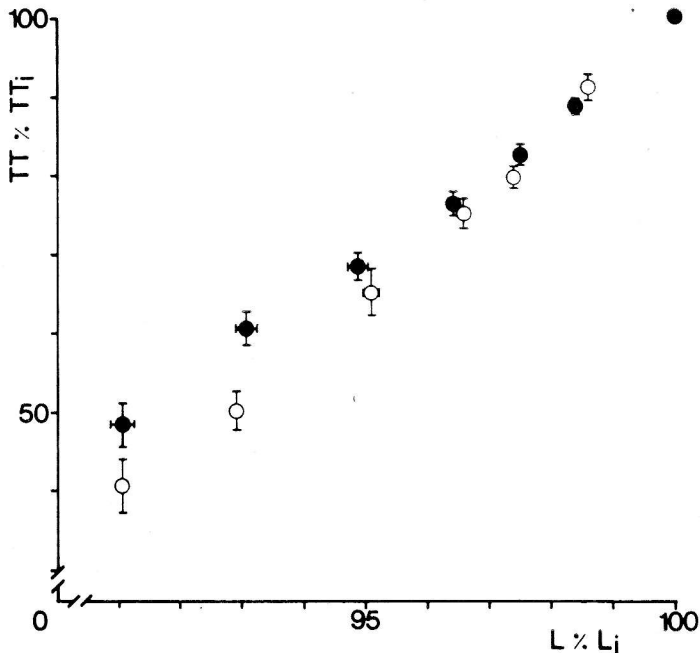


Fig. 2. - *Tension-length relationships.*

Total tension (TT) obtained from isometric twitches (solid symbol) and contractions with an isotonic phase (open symbols) are plotted versus final lengths (L). TT and L are expressed as % of tension ( $TT_i$ ) and length at  $L_i$  ( $L_i$  is equal to  $0.95 L_{max}$ ). Data from 20 experiments.

2. *Effect of the time at which shortening begins.* — The possible dependence of the shortening effect on the mechanical history of the twitch, was analyzed by comparing the tension output in contractions in which the same extent of active shortening took place at different times during the rising phase of the twitch. This was achieved by increasing the amount of afterload in stopped isotonic contractions: this procedure did not allow us to surely attribute the observed effects to the time rather than to load or shortening velocity. In all the experiments it was observed that the later the displacement took place, the less was the tension output (Fig. 4). This effect was quite small: total tension deficit increased by 3-5% when

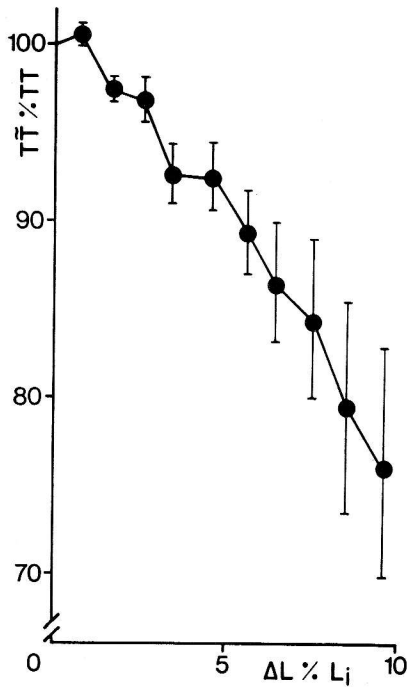


Fig. 3. - Relationships between tension, in contractions with an isotonic phase, and the extent of muscle shortening.

Total tension measured ( $\tilde{T}$ ) in isotonic contractions is expressed as % of tension obtained ( $T$ ) in isometric contractions at the same final length and is plotted against the extent of displacement ( $\Delta L$ ), expressed as % of the initial length ( $L_i$ ). Data from 20 experiments.

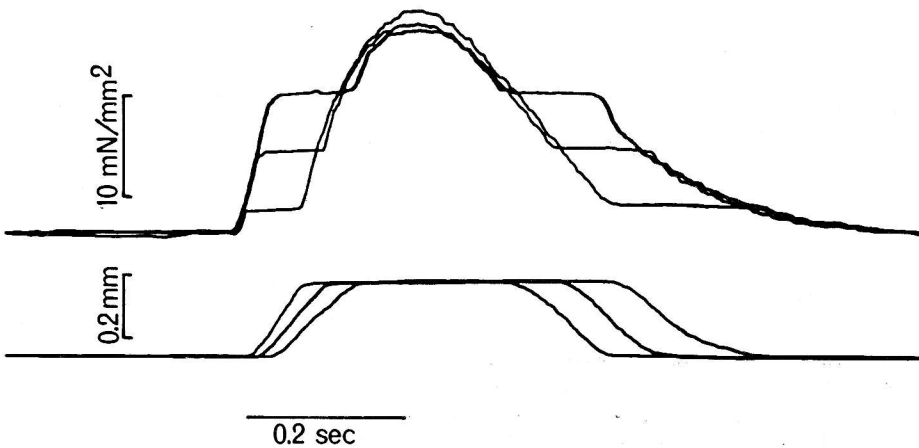


Fig. 4. - Series of three afterloaded isotonic contractions in which the amount of shortening remained constant, whereas the amount of afterload increased.

the time at which shortening began varied from zero to 50% of the time to peak tension. Moreover, peak tension reduction was accompanied by a slight increase in the time to peak.

TABLE 2. — Effect of the increase in  $[Ca^{2+}]_o$  on total tension ( $T\tilde{T}$ ) (means and S. E. of 5 experiments) in contractions with an isotonic phase.

	$\Delta L\%L_i$	$T\tilde{T}\%TT$
$[Ca^{2+}]_o = 2.5 \text{ mM}$	$5.20 \pm 0.17$	$85.27 \pm 1.41$
$[Ca^{2+}]_o = 10 \text{ mM}$	$5.35 \pm 0.22$	$86.61 \pm 0.57$

3. Effect of inotropic interventions. — The increase of  $Ca^{2+}$  concentration in the bathing solution slightly reduced ( $-10.66\% \pm 2.27$ ) the time to peak tension, but did not influence either developed tension ( $-4.59 \pm 2.40$ ) or tension deficit due to active shortening (see Table 2).

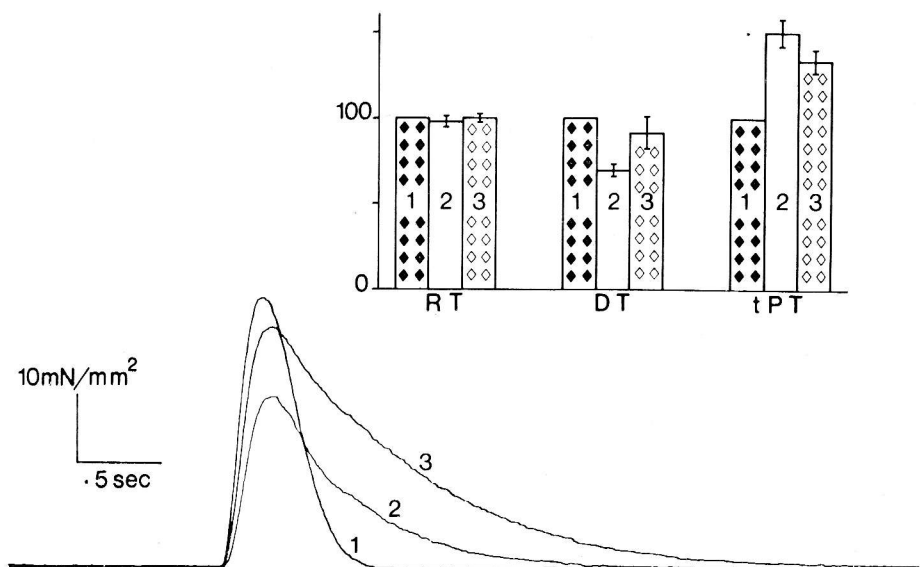


Fig. 5. — Effects of caffeine (10 mM) and of caffeine (10 mM) plus calcium (10 mM) on the characteristics of isometric contraction in a papillary muscle.

1, control; 2, in the presence of caffeine and 3, after addition of caffeine in high Calcium solution. In the inset is shown the behaviour of resting tension (RT), developed tension (DT) and time to peak tension (tPT). Data (means and S.E.) from 5 experiments.

Caffeine added to the perfusing medium prolonged the time to peak tension and decreased peak twitch tension as observed also by Bodem and Sonnenblick (4) and Lecarpentier *et al.* (29). This reduction in tension output was in part prevented by increasing also  $[Ca^{2+}]_0$  (Fig. 5).

The effect of caffeine on tension development of rat papillary muscle is opposite to that observed in other species and is probably attributable to the peculiar E-C coupling mechanisms of rat myocardium, as suggested by Bodem and Sonnenblick (4).

Fig. 6 shows that the depressant effect of shortening on total tension almost disappeared when caffeine plus calcium were added to the bathing solution; this means that under these conditions isometric and isotonic length-tension relationship coincided.

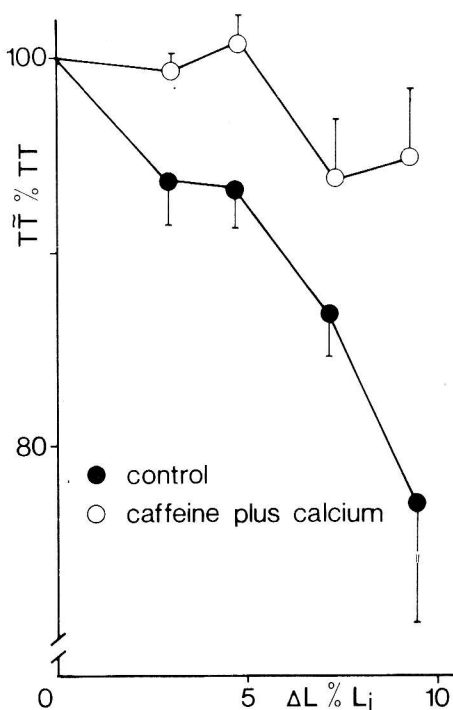


Fig. 6. — Effect of caffeine (10 mM) plus calcium (10 mM) on the relationship between total tension and the amount of displacement in contractions with an isotonic phase.

Total tension ( $\tilde{TT}$ ) in isotonic contractions is expressed as % of total tension (TT) in isometric contractions at the same final length and is plotted versus the extent of displacement ( $\Delta L$ ), expressed as % of initial muscle length ( $L_i$ ). Each point represents Mean  $\pm$  S.E. of 5 experiments.



## DISCUSSION

In rat papillary muscle active shortening reduces peak tension below the value predicted by the isometric length-tension relationship. This reduction is directly related to the amount of shortening and is scarcely affected by the history of contraction. These results support the view that the length-tension relation is not unique in isolated cardiac muscle.

This phenomenon has been often referred to the "shortening deactivation" shown with more appropriate experimental approaches in both skeletal and cardiac muscle. Active shortening might increase the disappearance of calcium from the sarcoplasm (and therefore from troponin) and its uptake by the sarcoplasmic reticulum (3, 19). Actually a fall of intracellular calcium concentration, indicated by the light emission of aequorin, has been shown to occur during and after a quick release imposed on tetanized skeletal muscle fibres (1). Other observations on skeletal muscle (10) suggest that active shortening might alter the calcium affinity of regulatory proteins.

In any case, if the shortening effect on tension output involves activation mechanisms, it is likely due to a decrease of calcium available for activation.

In line with this hypothesis is the finding that caffeine make unique the length-tension relationship obtained from isometric and isotonic contractions; in fact this drug increases activator calcium availability by inhibiting its uptake and increasing the transsarcolemmal calcium flux (2). Similar results have been obtained in studies on "shortening deactivation" in skeletal (11) and cardiac muscle (3).

The observation that the shortening effect is unaffected by changes of extracellular calcium concentration (in contrast with the finding of Kauffman *et al.* (19), that "shortening deactivation" is reduced by high calcium solution in cat papillary muscle) is not surprising and does not weaken the validity of the above exposed explanation. In fact studies on the species variation in cardiac properties (4, 14) suggest a little importance in rat heart of the entry of calcium during action potential and consequently a small sensitivity to external calcium concentration (12, 22). Therefore an increase of  $[Ca^{2+}]_0$  little modifies the intrasarcomeric calcium availability in rat papillary muscle.

Alternatively the difference observed between the length-tension

relationships obtained from isometric and isotonic twitches, might be explained by the time constraints due to the interplay between the quick decay activation and the properties of cross bridges, while the intensity and time course of activation might be unaffected by active shortening. In fact the displacement during a twitch determines the breakage and shift in compression of a number of attached cross bridges. When the muscle is returned to isometric conditions, cross bridges are reformed with a delay depending on the attachment rate constant; however the fast decay of activation limits the number of cross bridges which can be reattached, so diminishing the tension development ability (18).

When the peak of the twitch takes place during the isotonic phase (afterloaded contractions) the trend of the velocity-length plot (7), which shows the decline of shortening velocity as muscle length decreases, might account for the fact that muscle fails to reach the length values predicted by the isometric tension-length relation, as a consequence of time constraints, imposed by the rapid fall of active state (28).

However, present observations do not seem to support this explanation; in fact the difference between isotonic and isometric tension output is abolished by caffeine, while the small prolongation of the time to peak activity induced by the drug is probably not enough to suppress the time constraints due to the fast decay of activation. We find a small dependence of the effect of shortening on the history of contraction. This dependence is more likely correlated to the time at which the isotonic phase begins than to the velocity of shortening. In fact with the same amount of movement the variation of the shortening rate in the different isotonic contractions is not very large (Fig. 4); moreover contrasting results have been obtained by other Authors about the effect on the "shortening deactivation" of wide range variations in the displacement velocity (6, 10, 19, 20, 23, 24).

Anyhow an explanation of this last finding still remains unclear and needs further investigation.

#### SUMMARY

The effect of active shortening on tension development has been studied in rat papillary muscle preparations at 20 °C.

Total tension at the peak of a contraction with a shortening

phase is reduced below the value predicted by the isometric length-tension relationship. At the same instantaneous length, total tension is less in contractions with an isotonic phase than in isometric responses and the amount of the tension deficit increases with the extent of shortening.

The influence of the time at which shortening takes place, on the amount of tension deficit, has been explored.

The increase in  $[Ca^{2+}]_o$  does not affect the tension deficit. Conversely an increase in  $[Ca^{2+}]_o$  associated to addition of caffeine to the bathing solution almost abolished the effect of active shortening on tension development. Several possible explanations of the reduction of tension development by shortening are discussed.

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