Length-Dependent Activation
Its Effect on the Length-Tension Relation in Cat Ventricular Muscle

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SUMMARY In cat papillary muscles at 30°C, bathed with Tyrode's solution containing 2.25 mM Ca

The effect of various inotropic interventions (varying the stimulus frequency and continual paired stimulation) on the shape of the steady state length-tension relation was examined at lengths from Lmax, where tension production is maximal, to 0.87 Lmax. The relative steepness of the length-tension curves for peak tension developed (DT) and for maximum rate of tension development (dT/dt) varied inversely with the degree of potentiation. Thus, during paired pulse stimulation the relative decline in DT and dT/dt for a given change in muscle length was significantly less than the decline observed during simulation at 5 min⁻¹. When a muscle was stretched DT did not reach its final steady level for several minutes, and this slow increase in DT contributed significantly to the steepness of the steady state length-tension relation. The halftime of the slow increase in DT exhibited beat-dependency, and conditions that reduce the transsarcolemmal influx of calcium (reduction in bathing [Ca²⁺] or the presence of verapamil) significantly prolonged the time course of the slow increase and reduced its magnitude. These results support the hypothesis (1) that there is length-dependence of the excitation-contraction coupling process, such that an increase in muscle length is accompanied by greater activation of the contractile system; and (2) that this is due at least in part to an increased influx of calcium into the muscle cells. The implication of this hypothesis is that the influence of muscle length on myocardial performance (the Frank-Starling relation) should not be regarded as fundamentally different in character from other inotropic interventions.

A CHARACTERISTIC feature of all types of muscle is that active tension production increases when the muscle is stretched over a certain range of muscle lengths (the "ascending limb" of the length-tension relation). Tension development at a given muscle length depends on the degree to which the contractile system is activated. In cardiac muscle, this is often referred to as contractility or the level of inotropic state, and it can be varied by interventions that alter the completeness of the activation process (e.g., change in stimulus frequency or in bathing calcium concentration).

In both skeletal muscle and cardiac muscle there is now evidence that the activation process depends on resting muscle length. This is of considerable interest to cardiovascular physiologists, because length-dependent activation may be one of the mechanisms underlying the Frank-Starling relation in the intact heart. However, acceptance of this concept requires a radical departure from the traditional view that the inotropic state is independent of muscle length. In those studies, when a perturbation in the rate of stimulation (a single extra stimulus) constituted the inotropic intervention in rabbit papillary muscle, the shape of length-tension curve for maximal rate of tension development was not altered. It was proposed that the change in inotropic state produced by a perturbation in the rate of stimulation differs in this respect from that produced by varying the bathing Ca²⁺ concentration. The current study was undertaken partly to test this proposal under conditions in which the muscle performance was allowed to reach a new steady level after a change of stimulus frequency. In cat papillary muscles peak tension developed (DT) and maximum rate of tension development (dT/dt) have been measured at various frequencies of stimulation and during continual paired stimulation at different resting lengths. The steady state length-tension relations for DT and dT/dt during these interventions were compared for differences in shape of the kind observed in length-tension curves for DT in different bathing Ca²⁺ concentrations.

Other evidence, in addition to variation of the length-tension relation with the inotropic state of the muscle, also indicates that the activation process depends on muscle length. It has been demonstrated in papillary muscle that a change in resting muscle length, tension production not only changes immediately, but also manifests a significant additional change over a period of several minutes. The contribution of this slow change in tension...
production to the steepness of the steady state length-tension curve has been investigated in the present study and the slow change examined under conditions that alter the inotropic state by influencing the transsarcolemmal Ca²⁺ flux.

Methods

Papillary muscles were removed from the ventricles of young cats (0.5-1.5 kg) anesthetized with chloroform. One end of the muscle was fixed by a clamp and the other (tendinous) end was tied to a fine wire which was connected to a strain gauge force transducer. The characteristics of the apparatus and recording system have been described in detail in a previous report. The muscle was lowered into a chamber filled with a solution of the following millimolar composition: Na⁺, 135; K⁺, 5; Ca²⁺, 2.25; Cl⁻, 98.5; HCO₃⁻, 24; HPO₄²⁻, 1; SO₄²⁻, 1; acetate, 20; and glucose, 10; also, insulin, 5 U/liter. The bathing solution was bubbled with 95% O₂-5% CO₂ (pH 7.4) and maintained at a temperature of 30 ± 0.2°C. The stimuli were unidirectional square wave pulses, 2 msec in duration and of about 20% suprathreshold intensity (typically 0.4-0.8 V), which were delivered via punctate platinum electrodes. DT and dT/dt (obtained by electronic differentiation of the tension signal) were displayed on a strip chart recorder.

EXPERIMENTAL PROCEDURE

Each muscle was allowed to equilibrate under control conditions (2.25 mM Ca²⁺, stimulus frequency 20 min⁻¹) for 3-4 hours during which its length gradually was increased to Lmax, the length at which DT was maximal. The muscle lengths at which DT was approximately 75%, 45%, and 20% of its value at Lmax then were determined. Muscle performance continued to change for several minutes after a length change (Fig. 1A) and references to measurements of steady state DT or dT/dt indicate the final stable values reached after a change of length. When muscle performance had stabilized at a given muscle length the following interventions were made in random order: (1) continual paired stimulation at the shortest stimulus interval that would give a second response (typically 0.45 seconds); (2) regular stimulation at a frequency of 5 min⁻¹; (3) regular stimulations at a frequency of 80 min⁻¹. None of the muscles showed an increase in resting tension (incomplete relaxation) during stimulation at 80 min⁻¹. Each intervention was maintained until a steady level of performance was achieved (typically 0.5-3 minutes) and preparations failing to maintain a steady state level of performance were excluded from analysis of that intervention. Between interventions at a given length, the muscle was allowed to stabilize at 20 min⁻¹. In most muscles the entire sequence of interventions was repeated at four muscle lengths (designated Lmax, 0.96 Lmax, 0.92 Lmax, and 0.87 Lmax) (see legend of Table 1) with randomization of the order in which muscle lengths were studied. Length-tension curves during each intervention then were plotted. For the "control" curve at 20 min⁻¹ the mean was calculated for all the values available at a given muscle length (i.e., those obtained prior to, between, and subsequent to the interventions at the length—typically six to 10 values with a coefficient of variation of 4.4 ± 0.8%, mean ± SEM). In muscles in which two values of DT or dT/dt were available during an intervention at a given length, the mean of the two was used. Families of steady state length-tension curves for DT and dT/dt were thus obtained and the shapes of the curves were then compared.

In additional studies, several of the above preparations were used to examine the slow change in performance that occurs after a change in muscle length. Measurements of the magnitude and half-time of the slow increase DT after an increment in muscle length were made as illustrated in Figure 1B. In some experiments the increment in muscle length was varied; in others the length increment was constant, but was made after the muscle had stabilized at different frequencies of stimulation or in bathing solutions containing different Ca²⁺ concentrations. In other experiments the length increase was made before and after addition of dl-verapamil, an agent that reduces transsarcolemmal Ca²⁺ influx. The concentration of verapamil was titrated for each preparation to make DT approximately 5% of control (in 2.25 mM Ca²⁺ at 20 min⁻¹) and ranged from 5 x 10⁻⁷ to 5 x 10⁻⁶ M. Each muscle was allowed to equilibrate in verapamil for approximately 1 hour prior to study.

At the end of the experiments Lmax and wet weight of the muscle were determined. The cross-sectional area was calculated assuming a cylindrical shape, and DT and dT/dt were expressed as g mm⁻² and g mm⁻² sec⁻¹, respectively.

Numerical data quoted in the text are given as the mean ± SEM. Results were compared using Student’s t-test for paired observations.

Results

STUDIES OF THE LENGTH-TENSION RELATION UNDER STEADY STATE CONDITIONS

The steady state muscle performance was examined at four muscle lengths in a bathing solution containing 2.25 mM Ca²⁺ and at a stimulus frequency of 20 min⁻¹. The
procedure for collecting length-tension data involved adjusting the length of each muscle to obtain values of DT that were approximately 75%, 45%, and 20% of its value at L\(_{\text{max}}\). Pooled data from 12 preparations are given in Table 1, and the following points are worth noting: (1) The muscle lengths at which DT had the relative values given above varied very little from muscle to muscle. (2) The normalized values for DT and dT/dt at a given muscle length were remarkably similar. (3) Over a narrow range of muscle lengths (from L\(_{\text{max}}\) to 0.87 L\(_{\text{max}}\)), both DT and dT/dt fell to approximately 1/3 of their maximum values.

The length-tension data presented in Figures 2 and 3 show how DT (g mm\(^{-2}\)) and dT/dt (g mm\(^{-2}\) sec\(^{-1}\)) varied with muscle length under steady state conditions when the inotropic state of the muscle was changed by altering the frequency of stimulation and by continual paired stimulation. To compare the shapes of these length-tension curves, they have been normalized as shown in Figure 4 (i.e., DT and dT/dt are expressed as percentages of their maximum values). Only the curves obtained during paired stimulation and during stimulation at 5 min\(^{-1}\) are shown: the other curves in Figures 2 and 3 fall within this envelope. From Figure 4A it is readily apparent that the decline in DT for a given reduction of resting muscle length was significantly less when the muscle was in a more potentiated state (i.e., under conditions in which activation was more complete). Thus a reduction of muscle length from L\(_{\text{max}}\) to 0.92 L\(_{\text{max}}\) resulted in a decrease of DT of 70% during stimulation at 5 min\(^{-1}\), whereas during paired stimulation it fell by only 25%. Similar results were obtained when dT/dt was used to assess muscle performances, as shown in Figure 4B.

THE SLOW INCREASE IN TENSION FOLLOWING AN INCREASE IN RESTING MUSCLE LENGTH

When a change of length is imposed on the muscle between one beat and the next, as illustrated in Figure 1, mechanical performance is increased in the first beat and continues to increase for several minutes.

In this series of experiments we looked at the interactions of various factors that influence the slow increase in tension development. The magnitude of the slow increase varied with the length increment, as illustrated for six muscles in Figure 5. When muscles were stretched from 0.87 L\(_{\text{max}}\) to L\(_{\text{max}}\), the slow increase in DT was significantly greater than that following a stretch from 0.87 to 0.96 L\(_{\text{max}}\) (P < 0.01), which was in turn greater than that after a stretch from 0.87 to 0.92 L\(_{\text{max}}\) (P < 0.01). The steady state length-tension curve obtained by joining S, S', S'', in Figure 5 is significantly steeper than the "instantaneous" curve 1', 1'', 1'''. Although the magnitude of the slow increase in DT varied nearly 6-fold over the range of stretches examined (from 0.22 ± 0.05 to 1.23 ± 0.25 g mm\(^{-2}\)), the change in the corresponding half-time of the slow increase in DT (1.44 ± 0.13 to 1.79 ± 0.25 minutes) was not significant.

If an increase in transsarcolemmal Ca\(^{2+}\) flux contributes to the slow increase in tension after a stretch, then the slow change should be modified by interventions that reduce this flux. This prediction was tested by examining the effect of lowering the bathing Ca\(^{2+}\) concentration and of adding dl-verapamil to the bathing solution on the slow increase in DT after a stretch. Figure 6 shows the effect of these interventions on the magnitude and half-time of the slow increase after a stretch from 0.87 L\(_{\text{max}}\) to L\(_{\text{max}}\). Lowering the bathing Ca\(^{2+}\) concentration from 2.25 to 0.56 mm (Fig. 6A) and the presence of verapamil, 5 x 10\(^{-7}\) to 5 x 10\(^{-6}\) m (Fig. 6B), dramatically reduced the magnitude of the slow change in every muscle studied. The slow change, when expressed as a percentage of the corresponding steady state value for DT (see legend of Fig. 6),

### Table 1 Length-Tension Data Obtained under Control Conditions

<table>
<thead>
<tr>
<th>Muscle length (% L(_{\text{max}}))</th>
<th>100</th>
<th>96.0 ± 1.0</th>
<th>91.8 ± 1.4</th>
<th>87.4 ± 1.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak developed tension (g mm(^{-2}))</td>
<td>3.54 ± 0.40</td>
<td>2.62 ± 0.33</td>
<td>1.52 ± 0.19</td>
<td>0.65 ± 0.07</td>
</tr>
<tr>
<td>% maximum</td>
<td>100</td>
<td>72.9 ± 2.1</td>
<td>43.4 ± 1.9</td>
<td>18.8 ± 0.9</td>
</tr>
<tr>
<td>Maximum rate of tension development (g mm(^{-2}) sec(^{-1}))</td>
<td>15.5 ± 1.7</td>
<td>11.7 ± 1.3</td>
<td>7.0 ± 0.72</td>
<td>3.14 ± 0.32</td>
</tr>
<tr>
<td>% maximum</td>
<td>100</td>
<td>75.2 ± 1.7</td>
<td>45.5 ± 1.7</td>
<td>20.7 ± 0.9</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± ssm of data obtained from 12 preparations (L\(_{\text{max}}\) = 4.84 ± 0.39 mm; cross-sectional area = 0.47 ± 0.05 mm\(^2\)); 2.25 mm Ca\(^{2+}\) stimulus frequency = 20 min\(^{-1}\). Resting tension at L\(_{\text{max}}\) was 15.4 ± 2.0% of the total tension. These four muscle lengths are referred to in the text as L\(_{\text{max}}\), 0.96 L\(_{\text{max}}\), 0.92 L\(_{\text{max}}\), and 0.87 L\(_{\text{max}}\).
FIGURE 3 Length-tension curves for maximum rate of tension development (dT/dt, g mm⁻² sec⁻¹) under steady state conditions during stimulation at various frequencies and during paired pulse stimulation (PPS). Details otherwise as for Figure 2.

Increased from 35.9 ± 2.9% to 55.1 ± 2.8% (P < 0.01) when the bathing Ca²⁺ was lowered, but no significant change occurred when verapamil was added to the bathing solution. The reduction in the absolute magnitude of the slow increase was accompanied by a significant prolongation in the half-time of this increase (P < 0.03 for five muscles in Figure 6C and P < 0.01 for the four muscles in Figure 6D).

Because transsarcolemmal Ca²⁺ flux varies with the frequency of stimulation, we examined the beat-dependence of the slow increase in tension after a stretch. The results obtained in four preparations are shown in Figure 7. The muscles were stretched from 0.87 L_max to L_max in a bathing solution containing 2.25 mM Ca²⁺ after being stabilized at different stimulus frequencies. The solid line in each panel shows the hyperbolic relation that would be expected if the delayed increase were entirely beat-dependent (i.e., if the half-time of the increase depends only on the number of beats after the change of length); the dashed line indicates the relation expected if the delayed increase were completely independent of the number of beats after the increase in length. The curves have been fitted to the data point obtained at the control stimulus frequency (20 min⁻¹). In every muscle studied there was an inverse relation between half-time and frequency of stimulation. In panels A and B the values obtained for the half-time at the three other frequencies are fitted quite well by the hyperbolic curve (half-completion of the slow change required 22 beats in muscle A and 26 beats in muscle B). In the other two muscles (C and D) the data points are intermediate between the two theoretical curves indicating only partial beat-dependence of the slow increase in tension after a stretch.

Discussion

The changes in the length-tension curves for DT and dT/dt produced by varying the stimulus frequency and by continual paired stimulation show that the effectiveness of these interventions depends on resting muscle length. Anderson et al.²² and Manring et al.¹³ studied the force-frequency relation in rabbit papillary muscle by interpolating a single extra stimulus into an otherwise regular train and found that the ("instantaneous") force-frequency relation was independent of muscle length when dT/dt was used as an index of muscle performance. Figure 8 shows that this is certainly not the case for dT/dt or DT when the force-frequency relation is examined under steady state conditions, and it should be noted that length-dependence of the steady state force-frequency relation for both DT and dT/dt has also been demonstrated by an entirely independent experimental approach.²²

The observation that changes in the rate or pattern of stimulation alter the length-tension relation for both DT and dT/dt (Fig. 4) supports the hypothesis that resting muscle length regulates the completeness of activation.² ³

FIGURE 4 Normalized length-tension curves for peak tension developed (DT) (panel A) and maximum rate of tension development (dT/dt) (panel B) during continual paired pulse stimulation (PPS) and during stimulation at 5 min⁻¹. Pooled data from 10 preparations. The vertical bars show the SE of the mean difference in normalized tensions at each muscle length studied. The differences were significant at the P < 0.01 level at 0.96 L_max and at the P < 0.001 level at 0.92 L_max and 0.87 L_max.
Accordingly, as length is decreased from $L_{\text{max}}$, the concomitant decline in muscle performance is a result of decreased activation of the contractile system. Traditionally, the length-tension relation has been attributed to mechanical factors that could interfere with tension development—for example, double overlap of thin filaments, longitudinal compression of thick filaments, and sarcolemma restoring forces, all of which constitute "internal loads." However, recent studies of isolated papillary muscles have shown that at muscle lengths greater than 0.85 $L_{\text{max}}$, active sarcomere length is still well above that at which internal loading would be expected to become prominent. The fact that the tension production falls by less than 10% in maximally activated muscle when the muscle length is reduced from $L_{\text{max}}$ to 0.85 $L_{\text{max}}$ indicates that the change in myofilament overlap within each sarcomere is not an important factor regulating tension production over this range of muscle lengths.

Internal shortening during a contraction also has been implicated as a factor responsible for the steep ascending limb of the length-tension relationship. However, it recently has been shown that the steep length-tension relationship in cardiac muscle prevails even when internal shortening is prevented by the use of a length-clamp technique which allows tension production to be measured under truly isometric conditions.

When purely "mechanical" explanations for the steepness of the ascending limb of the length-tension relation have been ruled out, the hypothesis that it results from length-dependence of activation processes is the most plausible alternative. The hypothesis must account for the immediate change in tension production after an alteration of muscle length as well as the slow change that occurs subsequently (Figs. 1 and 5). It has been suggested that at longer length more Ca$^{2+}$ is released from the sarcoplasmic reticulum and this is supported by the observation that myoplasmic Ca$^{2+}$ concentration, as measured by the aequorin technique in skeletal muscle, increases as the muscle is stretched over the ascending limb of the length-tension relation. Other evidence in skeletal muscle suggests a length-dependence of the affinity of troponin for Ca$^{2+}$. These mechanisms could conceivably account for the immediate increase in tension that follows a stretch. However, DT continues to increase for several minutes after the stretch, and this slow increase makes a major contribution to the steady state DT at the new length (Fig. 5).

Current models of the excitation-contraction coupling process assume that tension development varies with the amount of Ca$^{2+}$ released from an intracellular storage site.
FIGURE 7 Variation of the half-time of the slow increase in peak tension developed (DT) after a stretch with the frequency of stimulation. Each panel shows the data obtained from a single muscle when it was stretched from 0.87 L max to L max after being stabilized at the stimulus frequency indicated. The horizontal dashed line passing through the point at 20 min⁻¹ (the "control" frequency) shows the relation expected if the half-time is independent of the stimulus frequency. The curve fitted to the same point is a rectangular hyperbole showing the relation expected if the slow increase in DT is purely beat-dependent (i.e., frequency × half-time = constant, the value of the latter being 22 beats in panel A, 26 in panel B, 36 in panel C, and 56 in panel D). The numbers above the graphs show the magnitude (g mm⁻²) of the slow increase in DT after a stretch at each frequency of stimulation.

when the muscle is excited and that the amount released varies with the degree to which this site is loaded with Ca²⁺ prior to excitation. A substantial body of evidence indicates that in most types of cardiac muscle transsarcolemmal Ca²⁺ flux determines the loading of the release sites. The fact that the magnitude of the slow increase in DT following a stretch is markedly decreased and the half-time is prolonged under conditions that reduce the transsarcolemmal influx of calcium (Fig. 6) makes it plausible to infer that this slow increase in DT reflects enhanced loading of the release sites. The beat-dependence of the slow increase in DT (Fig. 7) also supports the proposal that the slow change is partly due to an enhanced inward Ca²⁺ current. This might be correlated with the observed prolongation in the duration of the action potential that accompanies the slow increase in DT. It should be noted that earlier investigation on the effect of stretch on the action potential did not specifically examine the period during the slow increase in tension. Transsarcolemmal Ca²⁺ exchange in resting muscle also may vary with muscle length, and the increase in rate of heat production that follows a stretch in nonbeating cardiac muscle has been explained on the basis of an increased Ca²⁺ influx. However, the actual increase in transsarcolemmal flux following stretch may be too small to measure by conventional techniques. A basis for the proposed increase in Ca²⁺ influx in both resting and beating muscles might be provided by the observation that a stretch over the range used in the present study results in a significant increase in membrane surface area and caveolae neck diameters and a decrease in membrane resistance. The contribution of this instantaneous and slow increase to the final level of tension after a stretch is likely to be influenced by feedback within the chain of events of excitation-contraction coupling.

In conclusion, three types of investigation—(1) analysis of the length-tension relationship at different levels of potentiation, (2) observation of the slow increase in tension that follows a stretch, and (3) measurement of sarcomere length in living cardiac muscle—taken together provide a strong body of evidence that the level of activation or inotropic state depends on resting muscle length. This largely accounts for the length-tension relation over the range of lengths employed in the present study. Stretching the muscle over this range of lengths, then, is not fundamentally different from potentiation by interventions that enhance the inotropic state. The crucial question is whether this conclusion also is applicable to the intact heart. Some debate still exists as to what range of sarcomere lengths constitutes the "ascending limb" in the living heart, but measurements from fixed specimens suggest that diastolic sarcomere lengths are equivalent to those found in isolated muscle preparations. Ventricular function curves for peak left ventricular pressure and for maxi-
mum rate of pressure development during continual paired stimulation to do seem to vary in a way similar to those reported here, and slow changes in left ventricular pressure have been demonstrated after a change in preload. We therefore suggest that the Frank-Starling phenomenon, at least in part, results from a stretch-induced increase in activation, and that preload and inotropic state are therefore not independent regulators of cardiac output.

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