Myocardial mechanics in papillary muscles of the rat and cat


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HENDERSON, A. H., D. L. BRUTSAERT, W. W. PARMLEY, AND E. H. SONNENBLICK. Myocardial mechanics in papillary muscles of the rat and cat. Am. J. Physiol. 217(5): 1273–1279. 1969.—Species differences in myocardial mechanics were studied in rat and cat papillary muscles. The intrinsic velocity of contractile element shortening (V_max) was 3 times higher in the rat at comparable frequencies. Resting and developed tension at the top of the length-tension curves was similar, but time to peak tension was 3 times shorter in the rat while peak rate of tension development (dT/dt) was correspondingly higher. Increased rates of contraction in the rat were associated with decreases in V_max and dT/dt as well as in time to peak tension. In the rat at low frequencies contractility was not further augmented by paired stimulation, whereas at higher frequencies this increased contractility but, in contrast with the cat, did not shorten time to peak tension. Iso- proterenol significantly shortened time to peak tension in both species and increased contractility but only slightly at low frequencies in the rat. These species differences imply basic differences in the control of intracellular calcium movement or in rates of the contractile process.

Although the ultrastructure of various mammalian hearts appears similar, several distinct differences between the physiological responses of the rat heart and the hearts of other mammalian species have been noted. Thus, the "negative staircase," wherein an increase in frequency of contraction leads to a decrease in contractile force, is a characteristic of the rat myocardium (3, 6, 9, 14–16, 24, 26). Further, in the intact animal, the velocity of contraction of the rat ventricle has been shown to be higher, and the time to peak tension shorter, than in larger mammals such as the dog (7). In addition, the atrium of the rat is 100-fold less sensitive to the effects of digitals glycosides than that of the cat (13). However, the differences between the mechanical characteristics of rat and cat myocardium have not previously been analyzed and compared in detail and under similar experimental conditions. Accordingly, the mechanics of cat and rat papillary muscles have been studied and their relations to inotropic influences have been explored. The results suggest that there are important differences in the intrinsic mechanisms whereby contractility is controlled in the two species.

METHODS

Posterior left ventricular papillary muscles were rapidly dissected from 250- to 300-g male Sprague-Dawley rats, narcotized with a gas mixture of 50% CO₂ and 50% O₂. Papillary muscles from the right ventricle of the cat were prepared, as previously described (18), and similarly suspended in a muscle bath containing bicarbonate buffer equilibrated with 95% O₂ and 5% CO₂. The buffer was made up with 2.5 mm calcium, 143 mm sodium, 5.9 mm potassium, 1.19 mm magnesium, 126 mm chloride, 1.19 mm monobasic phosphate, and 5 mm dextrose. The isotonic lever, isometric tension transducer, and the air-jet apparatus for quick-release experiments have also been described previously (17). Measurements of force of contraction and changes in muscle length, along with their derivatives relative to time, were recorded on a multichannel oscillograph (Hewlett-Packard 7858) at a paper speed of 100 mm/sec. Signals were also displayed simultaneously on a memory oscilloscope (Tektronix model 564) and photographs taken when indicated. Experiments were carried out over a 2-hr period following initial stabilization for approximately 45 min.

The papillary muscles were assumed to be cylindrical. Mean cross-sectional area was calculated by dividing muscle mass by specific gravity (1.051) and by length. The mean weight of the rat papillary muscles was 3.9 ± 0.34 mg (mean ± se), the mean length at the apex of the length tension curves was 1.6 ± 0.18 mm, and the mean cross-sectional area was 1.2 ± 0.12 mm² (N = 28). The weight of the cat muscles was 10.1 ± 1.0 mg, the length 7.2 ± 0.6 mm, and the cross-sectional area 1.3 ± 0.11 mm² (N = 14).

Tension and the maximum rate of tension development (dT/dt) were measured in isometric contractions and were normalized for muscle cross-sectional area. The time to peak tension (TPT) was measured from the beginning of tension development and thus excludes the latent period following electrical stimulation.

Force-velocity relations. In order to evaluate force-velocity relations, initial muscle length was established at three preloads, i.e., 0.1, 0.35, and 0.6 g. At each preload, initial length was maintained constant and the peak velocity of shortening was measured as a function of total load as increasing loads (afterloads) were added. Velocity of shortening was expressed in muscle lengths/sec (ML/sec). The maximum velocity of shortening (V_max) at zero load was calculated from these force velocity relations by assuming the first portion of the curve to be hyperbolic and solving the three simultaneous equations provided by velocity of shortening and load at the three earliest points of this curve. Force-velocity relations in cat papillary muscle have been shown to be hyperbolic at low loads, and V_max obtained
FIG. 1. Maximum velocity of shortening at zero load (V_{max}) in muscle lengths per second, maximum isometric developed (D), and associated resting (R) tensions in g/mm², time to peak tension (TPT) in msec, and peak rate of isometric tension development (dP/dt) in g/mm² per sec at resting tensions of 0.6-0.8 g/mm² in rat and cat papillary muscles. Measurements were made at 29 C and at a rate of 6 beats/min (except those of maximum isometric tension in the cat by extrapolation from this portion of the curve has been shown not to vary with changing preload (4, 18, 21). The same appears to be true in rat papillary muscle (Fig. 2). V_{max} for each muscle was therefore taken as the average of the calculated values at preloads 0.1, 0.35, and 0.6 g. Small preloads were used and accordingly the parallel elastic element was ignored.

Series elasticity. Series elasticity was studied by quick releases using an air-jet solenoid system (18). In these studies, muscles were attached by wire connections to minimize equipment compliance, for which appropriate correction was then made. The load-extension curve of the series elastic component for the papillary muscle was plotted and the modulus of elasticity (dT/dl) derived from the curve as previously described (18).

Isotropie influences. Isoproterenol was added to the bath to give a concentration of 10^{-9} M. Paired stimulation, or sustained postextrasystolic potentiation, was produced by adding a second stimulus to the basic rhythm, placed beyond the absolute refractory period.

RESULTS

Isotonic and isometric contractions at 29 C and at a frequency of 6/min were compared in rat and cat papillary muscles (Fig. 1). V_{max} (Fig. 1A) was 3 times greater in the rat than in the cat, averaging 4.6 ± 0.39 muscle lengths/sec (mean ± se, N = 10) and 1.7 ± 0.15 (N = 13) muscle lengths/sec, respectively (P < 0.001). At preloads of 0.6 g/mm², the peak recorded velocity of shortening of the non-afterloaded papillary muscles averaged 2.9 ± 0.25 (N = 6) muscle lengths/sec in the rat, and 0.98 ± 0.06 (N = 13) muscle lengths/sec in the cat (P < 0.001). At preloads of 0.1 g/mm², the maximum recorded velocity of muscle shortening in the rat was 3.7 ± 0.32 (N = 9), or 80% of the extrapolated value for V_{max}. Resting and developed isometric tension, measured at the apex of the length-active tension curves, were similar in the two species (Fig. 1B).
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#### FIG. 3. A: load-extension curves of series elastic component of rat and cat papillary muscles at 29 C at 2 comparable preloads. Changes of muscle length occurring during quick releases are corrected approximately for contractile element shortening during these short periods in order to give length changes due to series elastic component: amount of contractile element shortening is taken as product of the duration of quick release and velocity of muscle shortening measured immediately following quick release. Since intrinsic velocity of contractile element shortening in cat is approximately one-third that of rat, this correction in cat amounted to one-third of correction made in rat. Afterload is expressed as g/mm² and extension as percentage of initial muscle length. Mean values ± se are given from 5 rat muscles, together with previously published data from this laboratory from 13 and 32 cat muscles, respectively, at 2 different preloads (20). Differences between percentage extension at 4 g/mm² afterload in rat and in cat are significant (P < 0.05) at both preloads. B: modulus of elasticity (dT/dl) plotted against total load in single representative rat and cat muscles, showing derivation of values for slope k (% ML), from the linear part of the slope, and for intercept e (g/mm² per % ML).

However the time to peak tension (Fig. 1C) was 3 times shorter in the rat than in the cat, averaging 128 ± 2.3 (N = 12) msec and 386 ± 14 (N = 10) msec, respectively (P < 0.001), whereas dT/dt at comparable preloads (Fig. 1D) was correspondingly higher, averaging 54.4 ± 3.5 (N = 5) g/mm² per sec in the rat and 28.3 ± 3.6 (N = 10) g/mm² per sec in the cat (P < 0.005). Thus, despite similar tension development in both these species, the intrinsic speed of contraction was substantially greater and the duration of contraction correspondingly shorter in the papillary muscle of the rat than in that of the cat.

In Fig. 2 are shown the force-velocity curves for a rat papillary muscle obtained with increasing preloads and initial muscle lengths. The characteristic force-velocity curve for the rat was inverse but was hyperbolic only at low loads. When the initial muscle length was increased, the force-velocity curve was shifted to the right with an increase in the maximum force of contraction. Nevertheless, Vₘₐₓ, which was calculated by simultaneous equations from the velocity points at low loads, was generally unchanged by altering preload from 0.1 to 0.6 g/mm².

The load-extension curves for the series elastic component of the rat and the cat at 29 C are shown in Fig. 3A. Curves were determined with two preloads as described. The series elastic component in the papillary muscle of the rat was found to be slightly but significantly more compliant than that of the cat. Since these curves were generally exponential except at higher loads, the modulus of elasticity of the series elastic component (dT/dl) could be plotted as a linear function of load (P) with a slope k and an intercept e when load was zero (18) (Fig. 3B). The mean value for k in the rat was 0.29 ± 0.02 % muscle length (N = 5) at 29 C, e being 0.10 g/mm² per % muscle length. Previous studies in this laboratory show that in the cat k is 0.43 ± 0.02 % muscle length (N = 8, P < 0.001) and e is 0.10 g/mm² per % muscle length (27).

In order to evaluate the differences in intrinsic speed of contraction between the rat and the cat at physiological temperature, the effects of temperature on Vₘₐₓ and on the components of isometric contractions were compared (Fig. 4). The differences between the cat and the rat noted at 29 C (Fig. 1) persisted over the temperature range of 24–37 C. Since with rising temperature the time to peak tension decreased to a relatively greater extent than the dT/dt rose, the peak developed tension generally fell (Fig. 5). The latent intervals between electrical stimulation and the onset of tension development in the rat and cat at different temperatures are shown in Table 1.

The effects of altering frequency of contraction on isometric and isotonic contractions of the papillary muscle are compared during the steady state for the rat and cat in Figs. 6 and 7. In the rat (Figs. 6A and 7A) increasing frequency, from rested beats (rested-state contraction after 10 min without stimulation) to 60 contractions/min, always resulted in a decrease in developed tension. This decline can be attributed to a fall in both the dT/dt and the time to peak tension. Vₘₐₓ derived from force-velocity curves at 6–36/min also diminished with increasing frequency. By contrast, increasing frequency of contraction in the cat (Figs. 6B and 7B) induced a moderate increase in peak developed tension, accompanied by a substantial increase in Vₘₐₓ and in dT/dt and associated with a concomitant decrease in the time to peak tension.

Paired stimulation of the rat papillary muscle resulted in an increase in developed tension except at the lowest frequencies where tension developed with single stimulation was near maximal (Figs. 6A and 8). This increase in tension was accompanied by a similar increase in dT/dt, without any apparent shortening of the time to peak tension. Preliminary experiments at frequencies of 6–60/min showed that the effect of paired stimulation in the cat was similar at all frequencies; at 12 beats/min paired stimulation caused an increase in developed tension from 7.3 ± 0.07 to 11.0 ± 0.06 g/mm² (N = 10; P < 0.005) and in dT/dt from 32.6 ± 4.7 to 78.2 ± 8.6 g/mm²/sec (N = 10; P < 0.001); however, in contrast with its effect in the rat, paired stimulation in the cat led to a decrease in time to peak tension from 361 ± 11 to 272 ± 7 msec (N = 10; P < 0.001). Thus time to peak tension was significantly shortened by paired stimulation in the cat but did not appear to be altered in the rat at similar frequencies and in association with comparable increases in dT/dt.

Isoproterenol (10⁻⁴ M) produced an increase in developed tension in the rat but only at higher frequencies of contraction (Figs. 9 and 10). Nevertheless, at all frequencies, isoproterenol induced some increase in dT/dt and a significant decrease in the time to peak tension. The effects of isoproterenol were similar in the cat; at a contraction rate of 12/min, developed tension was increased from 8.6 ± 1.1 to 10.2 ± 0.3 g/mm² on June 15, 2017.
**FIG. 4** Effect of temperature on A: $V_{\text{max}}$ in isotonic contractions, and B: time to peak tension (TPT), in isometric contractions for rat and cat papillary muscles. Absolute values for $V_{\text{max}}$ and TPT at 29°C are given to scale in muscle lengths/sec and msec, respectively. Values at 24, 34, and 37°C are given as percentage changes relative to the values obtained in same muscles at 29°C. All values are given as mean ± se, and number of muscles used in each group is indicated in bars.

**Table 1. Latent interval between electrical stimulation and onset of pressure development**

<table>
<thead>
<tr>
<th>Latent Interval, msec</th>
<th>Rat</th>
<th>Cat</th>
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<tbody>
<tr>
<td></td>
<td>24°C</td>
<td>29°C</td>
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<tr>
<td>Mean</td>
<td>25.2</td>
<td>10.3</td>
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<tr>
<td>SE</td>
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<td>0.8</td>
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<tr>
<td>N</td>
<td>4</td>
<td>12</td>
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<tr>
<td>$P^* &lt;$</td>
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<td>0.001</td>
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<td>$P^+ &lt;$</td>
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* Significance of differences with temperature, t test. † Significance of differences between species, t test.

**DISCUSSION**

In the present study, it has been shown that the intrinsic velocity of contractile element shortening, or $V_{\text{max}}$, is substantially greater in the rat than in the cat papillary muscle at comparable low frequencies of contraction. Nevertheless, maximum developed tension was similar in muscles from both species. The tension development depends both on the duration and on the intensity of active state, as represented by time to peak tension and $dT/dt$, respectively (10, 99, 93).
In the rat the time to peak tension was shorter than in the cat, while the \(\frac{dT}{dt}\) was correspondingly greater, in accord with the higher \(V_{\text{max}}\) found in the rat. The data show however that, whereas \(V_{\text{max}}\) was 3 times greater in the rat than in the cat muscles, \(\frac{dT}{dt}\) was only twice as high. This discrepancy may be attributed to the lower modulus of elasticity of the series elastic component in the rat papillary muscles, since \(\frac{dT}{dt}\) is the product of the contractile element velocity (\(\frac{dl}{dt}\)) and the series elastic modulus (\(\frac{dT}{dl}\)), i.e.,

\[
\frac{dT}{dt} = \frac{dl}{dt} \cdot \frac{dT}{dl}.
\]

The negative frequency staircase found in the rat heart preparations has generally been described only in terms of tension development or degree of shortening (3, 9, 14-16, 24); it has recently been noted that \(\frac{dT}{dt}\) and the time to peak tension both decrease with increasing frequency of contraction (26). The present study confirms that the decrease in tension with increasing frequency can be attributed to a decrease in contractility, as evidenced by \(V_{\text{max}}\) and in the isometric contractions by \(\frac{dT}{dt}\), as well as to a shortening of time to peak tension. The positive staircase in the cat, on the other hand, is associated with an increase in contractility sufficient to outweigh the effect of shortening time to peak tension.

The difference in time to peak tension between the two species persisted at all frequencies and temperatures studied. Its short duration in the rat may be related to the short action potential peculiar to the rat (12). Moreover, it has been noted that the time to peak tension of the twitch in various skeletal muscles is also inversely related to \(V_{\text{max}}\) (5).

The demonstrated species difference in \(V_{\text{max}}\) at low frequencies might be regarded from these studies as reflecting only opposite ends of inverse contractility-frequency relationships. The present results cannot quantitatively be extrapolated to physiological temperature and frequency since the responses to these variables are not linear; moreover no
significant difference is apparent between what may be considered as approximately maximal values for $V_{\text{max}}$ at 29°C in the two species, as provided by rat muscles at 6/min (4.6 ML/sec) and by cat muscles on paired stimulation at 12/min (3.9 ML/sec). Nevertheless, in vivo studies have shown that the difference in $V_{\text{max}}$ between the two species is found also in the intact animals (6.5 ML/sec in the rat at a frequency of 440/min, and 3.9 ML/sec in the cat at 180/min) commensurate with the observed differences in the time to peak tension of single isovolumic beats in vivo, 50 msec in the rat and 130 msec in the cat (7).

It may be noted that the values for $V_{\text{max}}$ obtained from the present study of rat papillary muscles greatly exceed previously published values for $V_{\text{max}}$ in rat trabeculae carneae (25). In this prior study (25) force-velocity relations were obtained from muscles at high resting tensions and allowance was then made for the parallel elastic element; $V_{\text{max}}$ was calculated from the Hill equation (8), $(P + a)(V + b) = (P_0 + a)b$, by inserting an empirically derived value for the constant $a$. This method was therefore not comparable with that used in the present study. It may be noted that in the present study, the actual measured shortening velocity of the muscles at low preload (3.7 ML/sec) greatly exceeded the calculated value of $V_{\text{max}}$ (1.1 ML/sec) in the former study (25). It has been shown that correction of force-velocity curves for series elastic extension in cardiac muscle converts the experimental curves into hyperbolic form throughout their length, so giving a higher value for $P_0$ and providing a more direct method for obtaining the Hill constants (4). This method of analysis of force-velocity relations was found to be similarly applicable to the results obtained from two rat papillary muscles, and yields the same value for $V_{\text{max}}$ as that obtained by the methods used in this study. It thus provides further support for the validity of these methods (4, 17, 21).

The series elastic element is shown to be slightly more compliant in rat papillary muscles at low loads than in those of the cat. However the relationship of the modulus of elasticity ($dT/dl$) to total load departed somewhat more at high loads from linearity than in the cat. The significance of these observations is difficult to assess. It is as possible that they are attributable to structural differences peculiar to the papillary muscles as that they are due to intrinsic differences in the series elasticity of the myocardium in the two species.

The negative staircase found at all frequencies in the rat is shown to be the result of decrements of contractility with increasing rate, as well as to the shortening of time to peak tension found in other species. Thus in the rat, maximal contractility is reached in the “rested beat.” Moreover, paired stimulation, which in the cat is the most potent of inotropic interventions, effects no further increase of contractility in the rat at these very low frequencies. Whether in the cat paired stimulation is equally ineffective at the highest frequencies which represent maximal contractility in this species remains unknown, since at such high frequencies inadequate oxygenation would limit the validity of studies on nonperfused papillary muscle preparations. On the other hand isoproterenol, the mechanism of whose inotropic effect is probably different from that of paired stimulation (11, 19), did in the rat cause some increase in $dT/dt$ of the rested beat. Isoproterenol also significantly shortened the time to peak tension at all frequencies in the rat, whereas paired stimulation had no significant effect upon time to peak tension in this species.

Quantitative and qualitative differences in the mechanical behavior of isolated preparations of rat and cat myocardium, studied under identical conditions, have therefore been demonstrated. The underlying mechanism for these differences is unknown. Although $V_{\text{max}}$, or the intrinsic speed of contraction, reflects the maximum rate of turnover at contractile sites, the controlling factors have not been defined. $V_{\text{max}}$ is however related to the actomyosin ATPase activity of the contractile proteins (1, 2) which provides for a limit to the fully activated system. Whether the ATPase of the actomyosin interaction is different in the myocardia of different species is unknown and warrants further investigation.
study. The rate of reaction at contractile sites also depends on the rate and extent of calcium delivery to the contractile sites. The demonstrated species differences in intensity and duration of active state, and the different responses to changes in frequency and to the inotropic influences of paired stimulation and isoproterenol, may therefore involve basic differences in the control of calcium movements in the cell.

It has been postulated (6) that a negative staircase is more dependent upon changes in the availability of calcium from intracellular sources, whereas small increments of intracellular calcium derived from outside the sarcolemma are of prime importance in the development of a positive staircase. Although changes in contractility with changes in frequency are likely to be related to the amount of calcium made available to the actin and myosin filaments and the rate at which it is delivered with each beat, the fundamental mechanism of the negative staircase remains unknown.

From the findings described, it is clear that species differences must be considered when inotropic influences are being assessed or when control mechanisms of contractility are being studied. Indeed these basic differences may help to shed light on the underlying mechanisms involved.

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REFERENCES


