Effects of Stimulation Frequency on Myocardial Extensibility

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ABSTRACT
Whether a change in myocardial contractility is accompanied by a change in diastolic extensibility was studied by altering stimulation frequency, including paired stimulation, as the inotropic intervention. Isometric and isotonic contractions of isolated cat papillary muscles were examined. Apparent changes in diastolic extensibility were observed with paired stimulation in isometric preparations. Rapid changes were seen only in muscles that had spontaneous aftercontractions between driven beats. These changes were believed not to reflect true extensibility changes but rather a change in timing between the relaxation of a beat driven by a paired stimulus and the onset of an aftercontraction. In continuous isotonic (not afterloaded) preparations, end-diastolic length depended on beat frequency. The muscle crept to a longer length when diastole was prolonged by decreasing the heart rate. In isotonic experiments without aftercontractions myocardial extensibility remained unchanged during paired electrical stimulation and changes in beat frequency.

ADDITIONAL KEY WORDS
- muscle mechanical properties
- aftercontractions
- papillary muscle
- paired stimulation
- cat

A characteristic property of cardiac muscle is that the contractile vigor is dependent on the muscle fiber length. This is the Frank-Starling law of the heart. Myocardial diastolic extensibility is important because it is one of the determinants of muscle fiber length. For a given diastolic pressure the myocardial fiber length is determined by the elastic properties of the heart and its geometry.

Extensibility has been defined as the reciprocal of the elastic modulus (1). It is used here in that sense to describe the passive elastic property of cardiac tissue in contrast to any change in muscle properties resulting from contractions or aftercontractions. The possibility that elastic properties of the resting heart change with inotropic effects has been investigated many times (2-22). Some investigators have found changes in the diastolic properties of the myocardium following administration of epinephrine or calcium, or stimulation of the stellate ganglion (6, 11, 17) and others have found no change (8, 10, 12-14, 16, 21). Recently, changes in diastolic compliance resulting from paired electrical stimulation have been reported by Bartleston et al. (18), Koch-Weser (19), and Scherlag et al. (20).

Properly timed paired electrical stimuli to cardiac muscle can produce a large positive inotropic effect. The mechanism may be related to the staircase phenomenon or interval-strength relationship (19, 23). Changing the stimulation frequency (including paired stimulation) has the advantages that it elicits a change in contractility that can be extinguished in only a few beats and that the inotropic changes are reproducible and can be elicited repetitively. This permits studies in short time intervals and minimizes problems of baseline shift.

Spontaneous aftercontractions are very important in the interpretation of the results;
they have been observed thus far only in isolated in-vitro myocardial preparations (24-28). They are distinct, small, slow contractions that occur in the absence of a propagated action potential and are most prominent at temperatures below 37°C. Experiments were done at 22°C to study the effects of aftercontractions.

This study was an examination of diastolic extensibility during inotropic changes elicited by changing the stimulation frequency. An in-vitro preparation of cat papillary muscle was used. Results similar to those of Scherlag et al. (20) were obtained but were related to the presence of aftercontractions, stress relaxation, or both, in an isometric preparation rather than to a change in passive extensibility.

Methods

Cats and kittens were anesthetized with pentobarbital, 25 mg/kg intraperitoneally. Papillary muscles were quickly excised from the right ventricle and placed in physiological salt solution. The composition of the solution in millimoles per liter was: sodium chloride, 90; potassium chloride, 5; calcium chloride, 2.5; sodium bicarbonate, 29; magnesium sulfate, 1; sodium phosphate, 1; sodium fumarate, 5; sodium pyruvate, 5; sodium glutamate, 5; dextrose, 10; disodium EDTA, 0.05; plus 5 units of insulin. The solution was gassed with 95% oxygen and 5% carbon dioxide, and a pH of 7.3 at 37°C resulted. The bath was kept at constant temperature in a water jacket with a circulating pump. Experiments were done at 22°C or 37°C. The muscle was stimulated through the bath with platinum electrodes that ran the length of the muscle but did not touch it. The stimulator (American Electronics Laboratory 104) provided rectangular pulses through an isolation unit. The intensity was adjusted to be just supramaximal, usually 10% to 50% greater than threshold. A second (paired) stimulus could be introduced with a variable delay without changing the basic rate.

The muscle was arranged so that muscle force and shortening could be measured simultaneously. The lower end of the muscle was held in a special spring clamp which is part of the force transducer. The force transducer (Honeywell) was entirely submerged in the bath and held by a sturdy post. The compliance of the force gauge was 0.30 mm/100 g.

The upper end of the muscle was attached to an isotonic lever (29) with a length of straightened, full, hard-temper stainless steel wire, which was tied to the chorda tendineae with 4-0 silk suture just long enough to make a knot. The knot was constantly below the bath surface. The

FIGURE 1

A prompt fall in diastolic isometric tension followed the first paired stimulation. This was an apparent increase in diastolic extensibility. Note the aftercontractions between driven contractions. The period between the onset of a stimulated contraction and the subsequent aftercontraction was 1.7 sec, which promptly increased to 2.2 sec with paired stimulation. Temperature, 22°C; diastolic muscle length, 8.3 mm; cross-sectional area, 205 mm². Stimulus rate was 23/min; duration, 5 msec; paired delay, 743 msec.
leverage was used to record instantaneous muscle length. The electronic circuit was modified to improve the stability and linearity. The equivalent mass of the lever was less than 80 mg. The lever was equipped with an adjustable micrometer stop. The length signal was differentiated by an operational amplifier (Philbrick P65) to obtain shortening velocity.

With this arrangement, the muscle could be studied under true isotonic, isotonic afterloaded, and isometric conditions by simply adjusting the load and the movable stop. Calibrations of all experimental variables were made after each experimental run. Length was calibrated with the micrometer stop on the lever, the force transducer with weights. Velocity was calibrated by a triangular wave generator (Hewlett Packard 3300A), which gave a trace on the calibrated length record, and the derivative on the velocity record. Recording was on an oscillograph (Brush 1707). At the end of the experiment, the muscle was weighed and cross-sectional area calculated on the basis of a cylinder of unit density. Muscle cross-sectional areas ranged from .40 to 2.6 mm². This paper reports the results from 15 technically satisfactory preparations; eight muscles were studied at 22°C and seven at 37°C.

**Results**

Isometric Contractions with Aftercontractions. When aftercontractions were present at 22°C, a decrease (Fig. 1), increase, or no change in isometric diastolic tension could accompany paired stimulation. Whether the isometric diastolic tension increased or decreased with paired stimulation was dependent on the timing of the extra stimulus. Figures 2 through 4 are records taken from the same muscle. In Figure 2, when the paired stimulus was delayed 550 msec, the diastolic tension increased, indicating an apparent decrease in diastolic extensibility. In Figure 3, the delay was increased to 650 msec, and there was no apparent change in resting extensibility. In Figure 4, a further increase
in delay to 740 msec gave a fall in resting tension and an apparent increase in extensibility.

The diastolic portions of the records in Figures 1 through 4 are not flat. In each case there is a small spontaneous aftercontraction between the larger contractions driven by the stimulator. Aftercontractions were also present during the period of paired stimulation. The aftercontractions are clearly seen in Figure 2, where the slower aftercontraction was on the down slope at the time the driven contraction began. Aftercontractions can be seen on close examination in Figures 1, 3, and 4; tension developed slowly and was interrupted by the rapid development of tension of the stimulated beat. The "shoulder" between the up slope of the aftercontraction and the rapid beginning of the driven contraction is readily apparent in Figure 3 and less apparent in Figures 1 and 4.

The time between the onset of a driven contraction and beginning of the subsequent aftercontraction was lengthened during paired stimulation. In Figure 1 the period between the beginning of the driven beat and the subsequent aftercontraction was 1.7 sec before and after paired stimulation. During paired stimulation the aftercontraction began 2.2 sec after the driven contraction. For the muscle shown in Figures 2, 3, and 4 the time between the onset of driven and aftercontraction was 1.3 sec during control periods. With paired stimulation, this increased to 1.5 sec in Figure 2, 1.8 sec in Figure 3, and 1.9 sec in Figure 4. This indicates that the second depolarization of paired stimulation in some way alters the onset of the spontaneous aftercontractions.

Eight muscles with aftercontractions were studied isometrically at 22°C. In three, diastolic isometric tension fell (an apparent increase in extensibility) with paired stimulation of the type seen in Figures 1 and 4. Aftercontractions disappear at low stimulation frequencies (24-28). It was not possible to obtain the right

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**FIGURE 3**

Unchanged diastolic isometric tension during paired stimulation. The period between the onset of a stimulated contraction and the subsequent aftercontraction was 1.3 sec, which promptly increased to 1.8 sec with paired stimulation. Stimulus rate was 25/min; duration, 4 msec; paired delay, 650 msec.
combination of aftercontraction, with basic beat frequency, or with proper paired delay interval to demonstrate a fall in diastolic isometric tension in all muscles. At 22°C an increase in diastolic isometric tension (an apparent decrease in extensibility) was observed in all eight muscles with aftercontractions.

**Isotonic Contractions with Aftercontractions.** Six muscles with aftercontractions were studied isotonically at 22°C. In all six, paired stimulation decreased diastolic length (an apparent decrease in extensibility). This effect was observed at high stimulation frequencies when aftercontractions followed each beat (three muscles) and also at low basic stimulation frequencies when aftercontractions were elicited by paired stimulation (six muscles). These effects are more completely described below in the section on aftercontractions. Decreased isotonic diastolic length resulting from paired stimulation in the presence of aftercontractions is illustrated in Figure 9. Increases in diastolic length accompanying paired stimulation were never observed in an isotonic preparation.

**Isometric Contractions without Aftercontractions.** Seven muscles were studied isometrically at 37°C without aftercontractions. None had rapid changes in diastolic tension with paired stimulation. When resting tension was kept low (below 0.3 g) little stress relaxation was observed (21). An experiment of this type is illustrated in Figure 5.

**Isotonic Contractions without Aftercontractions.** Seven muscles without aftercontractions were studied isotonically at 37°C. Under completely isotonic (not afterloaded) conditions, end-diastolic muscle length was dependent on the stimulation frequency—the longer the diastolic period, the longer the end-diastolic length the muscle would creep.

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**Figure 4**

Decrease in diastolic isometric tension during paired stimulation. The period between the onset of a stimulated contraction and the subsequent aftercontraction was 1.3 sec, which promptly increased to 1.9 sec with paired stimulation. There was an apparent increase in diastolic extensibility. Stimulus rate was 29/min; duration, 4 msec; paired delay, 740 msec.
FIGURE 5
Unchanged diastolic isometric tension during paired stimulation. Note the absence of after-contractions. Temperature, 37°C; diastolic length, 7.5 mm; cross-sectional area, 0.93 mm². Stimulus rate was 18/min; duration, 4 msec; paired delay, 212 msec.

FIGURE 6
Isotonic contractions. End-diastolic length was dependent on the duration of diastole. Increased stimulation frequency abbreviated diastole preventing the muscle from creeping to a longer length before the next contraction. Shortening downward; diastolic muscle length, 9.3 mm; cross-sectional area, 0.56 mm²; temperature, 37°C; duration, 4 msec. Load was 0.4 g.
Isotonic contractions. Essentially no change in diastolic length was observed with two periods of paired stimulation. The potentiation due to paired stimulation was manifest by increased shortening and shortening velocity. Shortening downward; diastolic muscle length, 7.5 mm; cross-sectional area, 0.93 mm²; temperature, 37°C. Rate was 30/min; duration, 4 msec; paired delay, 212 msec. Load was 0.3 g.

Isotonic contractions. After contractions which were not apparent at low stimulation frequency were elicited at higher stimulation frequency. Shortening downwards; diastolic muscle length, 6.2 mm; cross-sectional area, 2.42 mm²; temperature, 22°C. Load was 0.5 g.
Isotonic contractions. Aftercontractions were elicited by paired stimulation. From left to right, the stimulator was turned off for pauses after regular stimulation, after a single paired stimulus, after two paired stimuli, after three paired stimuli, and again without paired stimuli. Note that the diastolic length was shorter after the first paired stimulus in each case. Without a pause in stimulation the slow aftercontractions can be missed. Both the driven contractions and the aftercontractions were potentiated over several beats with paired stimulation. Shortening downward; diastolic muscle length, 7.3 mm; cross-sectional area, 1.64 mm²; temperature, 22°C. Rate was 15/min; duration, 4 msec; paired delay, 800 msec. Load was 0.4 g.

Conversely, the shorter the diastolic period, the shorter the end-diastolic length because of the viscous creep observed in diastolic muscle. These effects are illustrated in Figure 6.

Paired stimulation resulted in a very slight decrease in end-diastolic muscle length because of the shorter diastolic period when the muscle is stimulated twice. An increase in isotonic diastolic length (which would indicate an increased extensibility) with paired stimulation was never observed in these studies. The effects of paired electrical stimulation are shown in Figure 7.

Aftercontractions. Aftercontractions were a complication in this study. They were regularly present in some muscles following each stimulated beat as shown in Figures 1 through 4. However, they could be elicited by increases in the stimulation frequency, and if they had not been recognized, an erroneous conclusion of a decreased extensibility with increasing stimulation frequency might have been made. This is illustrated in Figure 8. At a stimulation of 3/min, the aftercontraction was not readily recognized—though the muscle did not show a smooth diastolic lengthening. When the stimulation frequency was increased to 6/min, the diastolic length decreased because of the aftercontraction. After the stimulation frequency was further increased to 12/min, a clear aftercontraction with distinct shortening was observed. Compare Figure 8 with aftercontractions to Figure 6 without aftercontractions.

In some muscles, paired stimulation would generate aftercontractions that were not previously present at the same beat frequency with single stimulation. Under isotonic conditions this could be erroneously interpreted as a decreased extensibility. Figure 9 illustrates the development of aftercontractions with paired stimulation; their presence might be overlooked if the stimulator had not been turned off for a pause. They were potentiated by paired stimulation over several beats similar to the potentiation of the stimulated...
beats. Potentiation of regularly occurring aftercontractions by paired stimulation can be seen in Figures 2 and 3.

Discussion

Four problems appear to be involved in the determination of myocardial diastolic compliance. These are: (1) incomplete relaxation at higher heart rates; (2) alterations in the shape or geometry of intact ventricles; (3) viscous effects in isolated or intact cardiac preparations; and (4) aftercontractions in isolated muscle preparations.

The importance of slow heart rates has been commented on by others (9, 22). If the diastolic properties of cardiac muscle are being studied it is apparent that heart rate must be slow enough to prevent mechanical fusion of beats. Partial mechanical fusion with a rate of 24/min at 22°C is shown in Figure 8.

During diastole, the relationship between pressure and volume of a ventricle is determined not only by its extensibility, but also by its geometry or shape. It has been pointed out that the left ventricle changes shape throughout the cardiac cycle (30, 31). Mitchell, Linden and Sarnoff (8) measured a segment length of the free left ventricular wall with intraventricular pressures during sympathetic and vagal stimulation. They found no change in the relationship between pressure and diastolic segment length with altered inotropism. Hefner and co-workers (11) measured left ventricular circumference with a mercury-in-rubber gauge with and without a norepinephrine infusion. They found a small change in the pressure-circumference relation with norepinephrine, an indication of increased extensibility. The possibility that the left ventricle changed shape with norepinephrine, and thus its pressure-circumference relation, cannot be ruled out in their experiments. It is possible that the relative pressures in the right and left ventricles change with norepinephrine and that a relative movement of the interventricular septum might alter the shape of the left ventricle. If this were the case, the segment length recorder on the free wall of the ventricle used by Mitchell, Linden and Sarnoff might be less affected by shape changes than a circumference gauge.

Viscous behavior has been reported in skeletal muscle (32, 33) and in frog (34) and cat (17, 21) myocardium. Sonnenblick and co-workers (21) have recently demonstrated viscous stress relaxation (decreasing tension at constant length) resulting from increase in contractile force in isometric papillary muscles and isochoric ventricles. Since isometric preparations show stress relaxation, an unequivocal demonstration of increased extensibility accompanying an increase in contractility cannot be made with an isometric preparation. However, the rapid fall in isometric diastolic tension after a single paired stimulus as reported by Scherlag et al. (20) and as shown in Figures 1 and 4 is very unlikely to result from viscous stress relaxation. In the present study, a rapid change in isometric diastolic tension following the first paired stimulus was observed only when aftercontractions were present. The rapid increase in diastolic tension with paired stimulation shown in Figure 2 cannot be attributed to stress relaxation, since the change is in the opposite direction from stress relaxation.

Rapid changes in diastolic isometric tension with paired stimulation when aftercontractions were present were believed to result from an alteration in the relative timing between driven contractions and aftercontractions. The extent to which the isometric tension fell was dependent on the duration of relaxation of the driven beat before the aftercontraction began. In effect this was a mechanical fusion between the end of the driven beat and the onset of the spontaneous aftercontraction. The observation that isometric diastolic tension could be made to increase, decrease, or remain constant as a function of the timing of the paired stimulus supports this interpretation. Also, the period between the beginning of a driven contraction and the subsequent aftercontraction lengthened and shortened in the expected manner. This resulted in delayed or earlier fusion of the driven contraction and aftercontraction and accounted for the change in diastolic tension.
Katzung (28) has studied aftercontractions in cardiac muscle from several species and found spontaneous increases and decreases in resting isometric tension following periods of stimulated contractions. Since the term aftercontraction did not seem appropriate for a fall in tension he named the phenomenon "diastolic oscillation." Katzung equated positive oscillation with aftercontractions described by others (24-26). A spontaneous fall in tension had not been reported previously. Katzung used an isometric preparation, and the decreases in tension he illustrated (Figs. 1B and 1C) may be due to stress relaxation. His Figure 1B shows a rather rapid fall in diastolic isometric tension at the onset of a period of stimulated beats. The fall is less abrupt than it appears because diastole following the first beat is mechanically fused with the second beat and is off scale. In any case, the rapid fall is in keeping with the rapid recovery at the end of the series of beats and indicates a short "time constant" for the viscous element. Katzung's Figure 1C shows a period of rest interrupted by a series of rapid contractions with mechanical fusion followed by a fall in resting tension with a slow recovery to the prestimulation resting tension. These two experiments show what would be expected in an isometric preparation showing stress relaxation.

The interpretation of a decreased isometric tension due to aftercontractions in the present paper is based on altered timing between driven contractions and aftercontractions. This differs from Katzung's negative diastolic oscillation or "hyper-relaxation." An isotonic preparation is not subject to stress relaxation. Muscles examined isotonically in the present study did not show "hyper-relaxation" when aftercontractions were present.

All observers agree that aftercontractions are more prominent at temperatures below 37° C and when Ca⁺⁺ is increased in the bath (24-28). The rapid diastolic changes associated with aftercontractions in this study were observed at 22° C. The rapid fall in isometric diastolic tension with paired stimulation reported by Scherlag et al. (20) was in a cat papillary muscle at 37° C. However, their Figure 2 shows unmistakable aftercontractions at 37° C. Figure 1 in their paper does not have sufficient time resolution to determine if aftercontractions are present. Katzung (28) reports aftercontractions at 35° C with increased Ca⁺⁺. In one of the isotonic preparations studied at 37° C, the Ca⁺⁺ was increased to 4 mM and aftercontractions were elicited by paired stimulation similar to the experiment shown in Figure 9.

The relation between aftercontractions and the strength of subsequent driven contractions has been investigated by Bravený, Sumbera and Kruta (27). They found that the greater the shortening due to an aftercontraction, the smaller was the next stimulated contraction. This was related to the Frank-Starling length-tension relationship. That is, if the muscle had shortened by an aftercontraction and then a stimulated contraction was superposed, the stimulated contraction was smaller because it began from an already shortened length. However, it would be misleading to describe the results in terms of a change in the passive extensibility of the muscle.

Figure 8 illustrates that aftercontractions are potentiated by increasing the stimulation frequency. This has been reported by several other workers (24-28). Eliciting aftercontractions by paired stimulation as shown in Figure 9 has not been previously reported. The difficulty of making interpretations concerning diastolic distensibility when aftercontractions are present is pointed up in Figures 8 and 9. Even when an isotonic preparation is used, slow aftercontractions may not be recognized unless the stimulator is turned off.

It is concluded that an unequivocal demonstration of a change in diastolic extensibility accompanying a positive inotropic effect should be free from: (1) mechanical fusion found at high heart rates, (2) shape changes found in intact ventricles, (3) viscous stress relaxation found in isometric and isochoric preparations, and (4) aftercontractions found with in vitro preparations. Within the limits
of the isotonic method used, a change in diastolic extensibility was not observed to accompany the inotropic effects of altered beat frequency or paired stimulation.

References

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