The Diastolic Viscous Properties of Cat Papillary Muscle

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SUMMARY The measurement of ventricular diastolic compliance is complicated by the presence of viscous properties. To approach this problem, cat papillary muscles contracting isometrically at 15/min at 26°C were stretched at different velocities during diastole. At a given length there was no excess of force above that defined by the static force-length curve until velocity of stretch exceeded 1 muscle length/sec. At greater velocities of stretch there was an increase of force with increasing velocity of stretch denoting viscous resistance to stretch. The viscous characteristic was alinear and increased with muscle length. At a given muscle length potentiation of contractile state by paired pulse stimulation caused a change in the diastolic force-length curve but there was no apparent relationship between viscous resistance to stretch and contractile state or the length of the “series viscous element.” These results lead one to expect viscous resistance to stretch during rapid filling of the intact left ventricle.

THE PRESENCE of viscous resistance to stretch in relaxed myocardium has been recognized. In a previous study of intact dogst it was concluded that viscous properties might well be causing a deviation of left ventricular diastolic pressure from the elastic pressure-volume relationship at times of rapid ventricular filling (early diastolic filling and atrial systole). To study this effect in more detail it is necessary to record changes in the pressure-volume curve at different velocities of stretch, an impractical experiment to carry out in an intact animal. In the present study, isolated strips of cardiac muscle (cat papillary muscles) have been stretched during diastole at different velocities.

Methods

Kittens weighing 2 kg or less were anesthetized with sodium pentobarbital, 30 mg/kg intraperitoneally, and their hearts were rapidly excised and placed in an oxygenated solution of the following millimolar composition: Na+, 145; K+, 4.2; Ca2+, 2.5; Mg2+, 1.2; Cl−, 125.5; SO42−, 1.2; H2PO4−, 1.2; HCO3−, 27; and glucose, 5.6.

SST02 measured forces up to 1 kg. When a 50-g weight was hung on the end of the transducer, a 0.05-mm deflection of the tip was measured. The force transducer was calibrated and checked for linearity with weights. The frequency response was flat to over 1,000 Hz. The muscle lever consisted of a hollow cylinder of acrylic plastic with transverse holes drilled through it to reduce its mass. When a 200-g weight was hung on the end, a 0.03-mm deflection of the tip occurred; the deflection with a 10-g weight was too small to measure accurately with the vernier scale. The forces measured in the present series of experiments never exceeded 10 g. For each experiment, the force transducer was calibrated and checked for linearity with weights. The apparatus is illustrated diagrammatically in Figure 1. The force transducer (Devices Instruments, model 2ST02) measured forces up to 1 kg. When a 50-g weight was hung on the end of the transducer, a 0.05-mm deflection of the tip was measured with a microscope mounted on a micromanipulator; with a 10-g weight the deflection was visible but too small to measure accurately with the vernier scale. The forces measured in the present series of experiments never exceeded 10 g. For each experiment, the force transducer was calibrated and checked for linearity with weights. The frequency response was flat to over 1,000 Hz. The muscle lever consisted of a hollow cylinder of acrylic plastic with transverse holes drilled through it to reduce its mass. When a 200-g weight was hung on the end, a 0.03-mm deflection of the tip occurred; the deflection with a 10-g weight was too small to measure. The lever was attached to the axle of a Brush 86475 pen motor, the angle of which was sensed by a Metrisite differential transformer. The demodulated angle signal was linearly related to displacement of the lever tip over a range of 0.6 mm. The largest stretch used in the experiments was 0.8 mm, over which range an alinearity of up to 5% occurred. In such cases the velocity of stretch was...
calculated from the change of angle signal over the linear part of its range. This signal was recorded, and it was used as a negative feedback into the driver amplifier of the pen motor. The gain of the feedback loop and the damping were adjusted to give a rise time of 2–3 msec in response to a square wave input. The timing of events was achieved with a Digitimer 3290 set to recycle with a period of 4-400-msec delay (adjusted according to response) into an OR circuit. The resulting paired trigger pulses were then fed into the stimulator.

Another Digitimer pulse was used to trigger during diastole a waveform generator which was set to put out a single positive triangular wave. A diode removed any negative output. The resulting signal was fed to the driver amplifier of the lever motor and produced a constant velocity stretch and relaxation. The velocity of stretch was set by adjusting the frequency setting of the waveform generator. The amplitude of the stretch was controlled by a 10-turn potentiometer with a linearly calibrated dial. Initial muscle length was only known as a deviation from an arbitrary length corresponding to a fixed reading on this dial. The actual muscle length corresponding to this reading was obtained at the end of the experiment by measuring the muscle between the metal crimps with a traveling microscope.

**RECORDING**

Force and length (lever angle) signals were fed through Analog Devices 602-J-10 amplifiers and displayed on a Devices Instruments model 3120 oscilloscope. The output of this oscilloscope (1 V/cm deflection) was fed into a Biomac 500 analog to digital converter averaging oscilloscope system (Data Laboratories) for automatic analysis. Eight cycles were averaged to avoid error due to individual variation between beats. The beginning of analysis for each stretch was triggered by a pulse (just before the start of stretch) from the Digitimer. The time base was adjusted to spread as many as possible of the 250 addresses or "bins" across the period of stretch. The averaged data were punched on paper tape for permanent storage and analysis. Visual records were obtained by recording the output of the store onto a Cambridge multichannel physiological recorder or by photographing the oscilloscope screen with a Cossor camera.

**PROTOCOL**

Initial muscle length was first set at a point at which resting force was always less than 5% of developed force. It is at this length that resting force is just detectable by feeding the Digitimer pulse with 1-msec delay together with a pulse of approximately 400-msec delay (adjusted according to response) into an OR circuit. The resulting paired trigger pulses were then fed into the stimulator.
FIGURE 2 Changes in force and length during contraction at a very short length followed by an isovelocity stretch in diastole. Continuous lines = slow stretch; dashed lines = faster stretch. Analysis was carried out at given lengths (horizontal dotted isolength lines). Forces at each length were compared for different velocities of stretch (vertical dotted lines to arrows). Initial muscle length = 5.0 mm. Amplitude of stretch = 0.8 mm. Amplitude of force during stretch = 46 mN.

ascending limb of the length-tension curve. The relationship between diastolic force and length was first determined by slowly stretching the muscle over the range to be subsequently explored. This was done during continuous stretching over a period of 31 seconds, at the minimum stretch velocity indicated in Table 1. During this time the muscle was stimulated to contract at 15/min or 20/min, the isometric contractions being superimposed on the stretching ramp. This method is identical to that of Hoffman et al.5 Step changes of length were not imposed, and all measurements of diastolic force were made during stretching. This was done to avoid making measurements during stretch relaxation which occurs as soon as the stretching stops or muscle shortening is allowed. A series of stretches of varying speed were then imposed during diastole. This protocol and the method of analysis are illustrated in Figure 2. A stretch at moderately slow rate (continuous lines) is compared with a faster stretch (dashed line). At any given length the force at the two speeds of stretch was measured (illustrated by dotted lines and arrows for two different lengths). The velocity of stretch was given by the slope of the length signal during stretch. With more rapid diastolic stretches it was possible to impose them immediately after relaxation was complete and after delays of 1 and 2 seconds. Such changes of timing were not accompanied by detectable differences in the force curves. Force was plotted against velocity of stretch for a given length (Fig. 3). In three muscles, this protocol was repeated during paired pulse stimulation which produced an approximate doubling of systolic force. The protocol was then repeated with the stimulus switched off so that there were no systolic twitches.

Results

Stretching of papillary muscles at velocities below 1 muscle length/sec caused an approximately exponential rise of force along the static diastolic force-length curve (Fig. 2). Relaxation of the muscle after stretch was always accompanied by hysteresis, force falling below the force-length relationship defined during stretch. At a given length there was no excess of force over and above that defined by the "static" force-length curve (i.e., at minimum stretch velocity, Table 1) until velocity of stretch exceeded 1 muscle length/sec (Figs. 3 and 4). At velocities of stretch above 1 muscle length/sec there was an increase of force with increasing velocity of stretch (Figs. 3 and 4). The force at the highest velocity of stretch was compared with the force at the lowest velocity of stretch at a given length in each muscle by the paired sign test. In this test a chi square is calculated with a correction for continuity because of small sample size. It was found that the probability of the higher force at higher velocity of stretch occurring by chance was less than 0.005. (There was no
can be observed. The oscillatory forcing function technique may also be regarded as artificial since there are no cycle because systole intervenes before stress relaxation filling. Stress relaxation is of little relevance in the cardiac stiffness. An increase in dynamic stiffness with increasing frequency of oscillation indicates viscous properties.

This phenomenon is responsible for the hysteresis observed during shortening back to the original length after the step change accompanying a diastolic quick release.1, 10 The approach in the present study has been oriented toward the situation in the intact heart during diastolic filling. Stress relaxation is of little relevance in the cardiac cycle because systole intervenes before stress relaxation can be observed. The oscillatory forcing function technique may also be regarded as artificial since there are no statistically significant fall in force with increasing velocity of stretch at velocities less than the maximum.) However, this effect did not continue above velocities of 4 muscle lengths/sec. At higher velocities, the force-velocity relation tended to plateau (Fig. 4). The increase in force with velocity in the range 1–4 muscle lengths/sec was greater at longer lengths.

Paired pulse stimulation caused a decrease in diastolic force for a given length. Asystole caused an increase in diastolic force for a given length. The results show that below velocities of stretch of 1 muscle length/sec, force remains unchanged, i.e., there is no viscous resistance to stretch. Above velocities of stretch of 1 muscle length/sec, force rises, indicating viscous resistance to stretch. However, the force tends not to rise progressively with velocity but to flatten out (Fig. 4); thus the relationship is not linear. The viscosity over the rising phase between velocities of stretch of 1–3 muscle lengths/sec is approximately 1 mN sec mm⁻¹, which is the same order of magnitude as that found by Loeffler and Sagawa8 (their Fig. 5). They assumed two linear parallel viscous elements so that the alinearity (my Fig. 4) was obscured. However, the dependence of viscosity on muscle length was found in both studies (compare my Fig. 4 with Fig. 5 of Loeffler and Sagawa8) and was recognized previously by Lundin.1

Is the viscous resistance to stretch related in any way to contractile state during systole? In the present study a fall in force at any given length was observed during potentiation of isometric force by paired pulse stimulation (Fig. 5). Conversely, a rise in the force-length curve was seen when the muscle was not stimulated (Fig. 5). The magnitude of these changes was very variable from muscle to muscle and was most marked in that shown in Figure 5. There is no agreement in the literature as to whether these changes in diastolic force-length relation result from the change in contractile state itself5 or from stretch of a series viscous element (in the case of increased contractility) by the increased contractile element force.5, 12 If stretch of a series viscous element is the mechanism, one would expect a change in viscous resistance to stretch; this was not found. This finding is compatible with the results of Templeton et al.12 who found that the increase in dynamic stiffness with increased forcing frequency was independent of inotropic state, i.e., viscous properties were not affected. They are also compatible with the conclusion of Loeffler and Sagawa8 in that they regard the diastolic viscous elements as being in parallel with the “active branch” of their model; i.e., they cannot be stretched by a higher contractile element force. These results do not rule out the possibility of a series element with long-term stress relaxationb, 14 nor do they throw light on other possible mechanisms for the change in the length-force curve.

Are the present findings relevant to diastolic filling in the intact heart? I have made the following speculation using numerous assumptions. To attempt an answer, one requires an approximation of velocity of stretch in that situation. Diastolic filling occurs principally in the circumferential dimension.18 If one assumes (1) an ejection fraction of 50%, (2) equal minor semiaxes, (3) a truncated ellipsoid model,14 and (4) dimensions taken from the liter-
for dog left ventricle, the stretch during diastole will be 0.42 muscle length for endocardial fibers and 0.26 muscle length for epicardial fibers. About half of filling occurs in the first 50 msec of diastole.4 17 18 This gives estimates of early diastolic velocities of stretch of 4.2 muscle lengths/sec for endocardial and 2.6 muscle lengths/sec for epicardial fibers; these values are in the range in which viscous resistance to stretch begins to become evident in the present study.

A number of factors are likely to cause differences between the viscous properties of papillary muscle and those of intact heart, e.g., higher temperature of intact heart lowering viscosity, frictional forces between bundles layers and within interstitial fluid raising viscosity in the intact heart; there is also considerable thinning of the wall.16 19 Quite apart from this, cat and dog myocardium may have different viscosity. Nevertheless, if one ignores these problems one might ask: Is the excess force due to velocity of stretch at 2 muscle lengths/sec and short muscle length (such as would be encountered in early diastole) large enough to give important excess pressures in the left ventricle? The answer appears to be yes, because the cross-sectional area of the left ventricular muscle in the meridian (from Van der Meer et al.16) is about 1,008 mm², giving an excess pressure in the ventricle of 0.8 kPa (6 mm Hg) for 1 mN mm⁻² excess force due to viscosity, a likely value at 2-5 muscle lengths/second. (In the estimations of velocity of stretch and excess pressure in the ventricle, the effect of angulation of the fibers86 is ignored because the effects on force and velocity are inverse and the conclusions are unaffected.) Thus one would expect increases of pressure of the order of 6 mm Hg quite soon after the velocity of stretch in diastole exceeds the critical value. While there is little variation from the elastic pressure-volume curve under relatively quiescent conditions,3 4 increases of pressure of this order of magnitude are found with more rapid filling.9 17 The present findings give support to the previous conclusion that viscous resistance to stretch may distort the diastolic pressure-volume curve when filling is more rapid. This effect can lead to an erroneous conclusion that diastolic compliance has fallen when the only change has been an increased velocity of stretch, i.e., increased rate of filling.

References

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