Acute and specific collagen type I degradation increases diastolic and developed tension in perfused rat papillary muscle

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Lamberts, Regis R., Maurice J. J. M. F. Willemsen, Néstor G. Pérez, Pieter Sipkema, and Nico Westerhof. Acute and specific collagen type I degradation increases diastolic and developed tension in perfused rat papillary muscle. Am J Physiol Heart Circ Physiol 286: H889–H894, 2004. First published October 23, 2003; 10.1152/ajpheart.00967.2001.—Collagen degradation is suggested to be responsible for long-term contractile dysfunction in different cardiomyopathies, but the effects of acute and specific collagen type I removal (main type in the heart muscle) on tension have not been studied. We determined the diastolic and developed tension length relations in isometric contracting perfused rat papillary muscles (perfusion pressure 60 cmH2O) before and after acute and specific removal of small collagen struts with the use of purified collagenase type I. At 95% of the maximal length (95%Lmax), diastolic tension increased 20.4 ± 8.1% (P < 0.05, n = 6) and developed tension increased 15.0 ± 6.7% after collagenase treatment compared with time controls. Treatment increased the diastolic muscle diameter by 7.1 ± 3.4% at 95%Lmax, whereas the change in diameter due to contraction was not changed. Diastolic coronary flow and normalized coronary arterial flow impediment did not change after collagenase treatment. Electron microscopy revealed that the number of small collagen struts, interconnecting myocytes, and capillaries was reduced to ~32% after treatment. We conclude that removal of the small collagen struts by acute and specific collagenase type I degradation increases diastolic and developed tension in perfused papillary muscle. We suggest that diastolic tension is increased due to edema, whereas developed tension is increased because the removal of the struts poses a lower lateral load on the cardiac myocytes, allowing more myocyte thickening.

cardiac contraction; coronary flow; collagen struts; edema; flow impediment

THE CARDIAC COLLAGEN FIBERS, especially the struts, are generally thought to influence both active and passive properties of the heart (5, 13). An increased collagen content, like in cardiac hypertrophy or after aortic stenosis (2, 19), is thought to be responsible for increased diastolic stiffness. Collagen degradation is suggested to be responsible for contractile dysfunction, such as in stunned myocardium (7, 21) or in ischemia (16). However, the relation between collagen content and cardiac function is based only on indirect evidence because other mechanisms are activated during the pathological processes, such as increased cell size in hypertrophy.

Todaka et al. (18) studied the functional consequences of acute collagen degradation in beating rat hearts, but instead of a specific collagenase, they used a chemical compound that is thought to activate multiple endogenous collagenases. They reported a decrease in developed pressure, suggested to reflect a combined effect of edema and loss of collagen. Recently, Baicu et al. (3) reported a decrease in isometric developed tension in unperfused papillary muscles induced by collagen removal with the use of plasmin. However, plasmin is a broad-spectrum serine proteinase, which is presumed to cleave many extracellular proteins, including several types of collagen, by activating a cascade of matrix metalloproteinases, and thus not specific for collagen type I, the main type in the heart. MacKenna et al. (12) studied, in the isolated rat heart when perfusion was stopped, the effect of collagenase treatment on the passive properties only. They found a dilated heart, i.e., at similar diastolic pressure ventricular volume and sarcomere lengths were increased.

Because Todaka et al. (18) and Baicu et al. (3) did not use a specific type I collagenase, and MacKenna et al. (11) studied only diastole, and without cardiac perfusion, we decided to study the passive and active properties of perfused rat papillary muscle before and after acute and specific type I collagenase treatment.

Abovsky et al. (1) suggested that the tethering by the collagen struts would prevent total systolic collapse of blood capillaries in systole, thereby limiting the coronary arterial flow impediment. Therefore, we also measured the coronary inflow during contraction to test the role of struts on coronary flow impediment.

With the use of a purified specific type I collagenase we primarily affected the collagen struts that are thought to affect cardiac contractility and mechanics the most, leaving the larger bundles largely intact. We used the perfused papillary muscle because it has a simple, linear geometry allowing direct force measurements, whereas perfusion guarantees that ischemia/hypoxia does not occur (14).

METHODS

Preparations and setup. All animals were treated according to the guidelines of the Institutional Animal Care and Use Committee of the Vrije Universiteit of Amsterdam, The Netherlands. The hearts of male Wistar rats (n = 21) were used; six hearts were used to determine tension-length relations before and after collagenase treatment and six hearts were used for time-control experiments. For the quantification of the amount of collagen struts with electron microscopy, we used six hearts after collagenase treatment and three hearts as time controls.

Details of the preparation, setup, and measurements (force, diameter) for studying isolated perfused rat papillary muscles have been
presented earlier (10, 14). In brief, under ether anesthesia, the hearts were rapidly removed and perfused via the aorta with a crystalloid solution (for composition, see below). To prevent contraction of the heart 25 mM 2,3-butanedione monoxime was added. A papillary muscle with part of the septum and the septal artery was carefully removed from the right ventricle and superfused in the experimental bath. The septum was clamped on a Perspex plate and the muscle tendons were attached to a force transducer, which was attached to a micrometer for length adjustments. The septal artery was cannulated with the use of a glass cannula and connected to a pressurized reservoir through a pressure difference meter to determine instantaneous papillary muscle coronary inflow (14).

The bath (superfusion) and pressurized reservoir (perfusion) were filled with identical crystalloid solution containing (in mM) 140 NaCl, 5 KCl, 1 CaCl₂, 2 NaH₂PO₄, 1.2 MgSO₄, 10 glucose, and 5 HEPES, and 0.01 mM adenosine was added to maximally dilate the vasculature. Temperature was set at 27°C and the solution was gassed with 100% O₂ (pH 7.4).

Muscles were stimulated via a pair of platinum electrodes at 0.2 Hz to obtain muscle isometric contractions. Diastolic tension (the tension imposed on the resting muscle) and developed tension (Tdev, the difference between systolic tension and diastolic tension), the maximal rate of change of tension (dT/dt), and the time from stimulus to half relaxation (tHR) of isometric contractions were measured at 95% of the maximal length (95%Lmax) unless otherwise indicated, with Lmax being the muscle length at which maximal isometric force was developed. Muscle tension was expressed as force per unit cross-sectional area (mN/mm²).

The cross-sectional area was determined as previously described (20). Tension-length relations were determined by using a constant perfusion pressure of 60 cmH₂O (≈ 46 mmHg). The muscle diameter was measured continuously in the central segment of the muscle through a video analysis system (10, 14).

Diastolic and systolic flows were determined and their difference, normalized with respect to systolic tension (Δflow/systolic tension), was used to quantify the flow impediment. In this way, effects other than those of muscle contraction (e.g., strut removal) are brought forward.

**Mechanical data.** After a stabilization period of 1 h, Lmax was determined. Tension-length relationships were made with length steps of 1% in the range of 85–95%Lmax. This range was chosen because this is approximately the physiological range in the in vivo heart under normal conditions (15). Hereafter, we perfused the muscles with the purified collagenase type I (10 mg/l; C9891 crude filtered collagenase type IA, Sigma). From the results of pilot experiments, we decided to stop collagenase treatment when the diastolic tension (at 95%Lmax) had doubled. The time period needed to obtain this doubling was 32.8 ± 2.2 min of collagenase perfusion. Subsequently, collagenase was washed out with the standard crystalloid medium for 30 min, and during this period, diastolic tension substantially returned to control values (from doubling to 20.4 ± 8.1%). After collagenase washout, the measurement of the tension-length relationship was repeated and compared with its own control value and subsequently also compared with time-control values. The time-control experiments were carried out according to the same protocol except for the collagenase treatment.

**Quantification of collagen content.** For quantification of the number of collagen struts before and after collagenase treatment, electron microscopy was performed. We used crude bacterial collagenase (10 mg/l; C9891 crude filtered collagenase type IA, Sigma), the same collagenase and concentration as MacKenna et al. (11) used. This crude collagenase type I is not sterile and contains nonselective proteinases compared with the sterile collagenase type I used for the mechanical data. In pilot experiments, we found that all mechanical parameters were similar using the crude collagenase type I or sterile collagenase type I.

Collagen struts were determined in three time-control muscles and six collagenase-treated muscles. Each muscle was fixed via superfusion in the setup at 95%Lmax with crystalloid medium containing 4% glutaraldehyde. Thereafter, the muscles were stored at 4°C. To visualize the changes between treated and untreated muscles, scanning electron microscopy (model JSM 6400, JEOL) was performed. The muscles were divided in three longitudinal strips. Photos were taken of each section at ×1,500 magnification. Collagen content was quantified using the grid P2, as suggested by Howard and Reed (8). The grid sheet was placed over the scanning electron micrograph photograph. When a collagen fiber matched with a cross of the grid, it was marked as a count. From each muscle the three strips were counted and the mean of these counts was used as indication of the number of collagen struts for that muscle.

**Analysis and statistics.** All tension-length data were stored with a program developed in our department. Data were sampled with a frequency of 500 Hz. The effect of collagenase treatment was evaluated by ANOVA for comparison of collagenase-treated and time-control muscles. For within-muscle comparison (before and after collagenase treatment) repeated-measurements ANOVA was used. P values <0.05 obtained during post hoc test were taken to indicate statistically significant differences.

**RESULTS**

Figure 1, left, shows that the mean diastolic tension-length relation after collagenase treatment is increased (P < 0.05, n = 6). The values of the diastolic tension at 95%Lmax were 11.0 ± 1.7 before and 13.1 ± 2.1 mN/mm² after collagenase treatment, respectively; an increase of 20.4 ± 8.1% (P < 0.05). There was no change in diastolic tension over time in the time-control experiments (1.1 ± 6.9%, P > 0.05, n = 5).

![Fig. 1. Diastolic tension (left) and developed tension (right) before and after collagenase treatment. The diastolic tension-length relation of the time controls was virtually equal to the one before treatment, and therefore not shown. Lmax, maximum length. *P < 0.05, before vs. after collagenase treatment; #P < 0.05 after collagenase treatment vs. time controls (means ± SE, n = 6).](http://ajpheart.physiology.org/Content/Original/286/3/1874/sec2-15.jpg)
Figure 2. Twitch characteristics, developed tension ($T_{dev}$), maximal rate of change of tension ($dT/dt$), time from stimulus to peak tension ($tTPT$), and time from stimulus to half relaxation ($tHR$) at 95% maximum length ($95\%L_{max}$) in the time controls and collagenase-treated muscles. Data are normalized to those at the start of the experiment. NS, not significant. *$P < 0.05$, time controls vs. collagenase treatment (means ± SE, $n = 6$).

Figure 1, right, shows the developed tension-length relation before and after collagenase and for the time controls. Compared with the time controls, the tension-length relation of the collagenase-treated muscles was higher at all muscle lengths ($P < 0.05$, $n = 6$). The $L_{max}$ did not shift due to the collagenase treatment or in the time controls. At 95% $L_{max}$, the tension generated after collagenase treatment was 6.5 ± 3.2 mN/mm² higher than the tension of the time controls. The developed tension after collagenase treatment was lower than before collagenase treatment, at 95% $L_{max}$ 57.0 ± 4.2 and 53.2 ± 4.1 mN/mm², respectively, whereas developed tension for the time controls at 95% $L_{max}$ was 46.8 ± 3.4 mN/mm².

Figure 2 shows the relative change of the twitch characteristics ($T_{dev}$, $+dT/dt$, $tTPT$, and $tHR$) at 95% $L_{max}$ of the collagenase-treated muscles and their time controls. The decrease of developed tension over time is larger in the control muscles than in the collagenase-treated muscles (as in Fig. 1). The greater tension in collagen-treated muscles compared with the time controls is accompanied by a larger relative decrease in $tHR$, with no difference in the parameter $+dT/dt$ and $tTPT$ between both groups. Thus after collagenase treatment the tension development is increased and relaxation is accelerated compared with the time controls.

Figure 3 shows the diastolic muscle diameters (left) and the muscle diameter changes from diastole to systole (right) before and after collagenase treatment. The values of the diastolic muscle diameter at 95% $L_{max}$ were 0.815 ± 0.037 and 0.873 ± 0.049 mm for control and collagenase-treated muscles, respectively ($P < 0.05$, $n = 6$); an increase of 7.1 ± 2.4% ($P < 0.05$). The absolute systolic diameter also was larger after collagenase treatment. However, the change in muscle diameter between the diastole and systole during contraction was not statistically different before and after collagenase treatment (Fig. 3, right). There was no change of diastolic muscle diameter at 95% $L_{max}$ in the time controls (2.1 ± 1.1%, $P > 0.05$, $n = 5$).

The increase in muscle diameter with an increase in muscle length is a measurement artifact. The muscle diameter is measured at the same distance from the base of the papillary muscle. Because the muscle is triangular, the diameter is larger at the base. Thus when the muscle is stretched to a larger muscle length, the diameter is measured at a place closer to the base of the muscle and is thus larger.

There is no difference in the diastolic coronary flow before and after collagen treatment at 95% $L_{max}$, as shown in Fig. 4, left. Figure 4, right, shows that the flow impediment ($\Delta$flow/systolic tension) is not affected by collagenase treatment, indicating that the small collagen struts do not affect coronary flow impediment.

Figure 5 shows examples of the effect of acute and specific collagenase type I treatment on rat right ventricular papillary muscle. Figure 5, top, shows a control muscle ($\times$1,500); collagen fibers can clearly be seen. Figure 5, middle, shows a collagenase-treated muscle. Only large collagen bundles remained, whereas the struts (thin fibers) have almost completely disappeared. Figure 5, bottom, shows a muscle that was treated too long with collagenase. The cell membrane was damaged and this muscle lost contractility almost completely. The counts of the small collagen fibers in time-control and collagenase-treated muscles were 125 ± 10 ($n = 3$) and 40 ± 5 ($n = 6$), respectively; i.e., collagenase treatment results in a decrease in fiber number to ~32% of control ($P < 0.05$).
DISCUSSION

This study was designed to provide insight into the functional relation between the collagen struts and cardiac muscle mechanics and flow impediment. We perfused rat papillary muscle with collagenase type I to acutely and specifically remove the collagen struts, leaving the larger collagen bundles mainly intact. After treatment, we saw an increase in diastolic tension accompanied by an increase in diastolic muscle diameter. When compared with time controls, collagenase treatment significantly increased developed tension and accelerated relaxation, but the diameter change during contraction, the diastolic coronary flow and the coronary flow impediment were not different after collagenase treatment.

Cardiac muscle mechanics in diastole. Todaka et al. (18) studied collagenase treatment in the perfused beating heart, thus under conditions comparable with our perfused muscle, and suggested that two counteracting phenomena take place. Collagen disruption makes the diastolic pressure-volume (force length) relation more compliant, whereas edema formation causes the ventricle to become stiffer, as also reported by Kresh (9). Thus, during perfusion, with significant edema, the net effect is a stiffer diastolic muscle. McKenna et al. (11) reported that edema formation before and after collagenase treatment is similar, and in their nonperfused hearts, arrested in diastole, the effect of treatment is a more compliant ventricle. After collagenase treatment in our perfused papillary muscle the diastolic tension-length relation is shifted to higher diastolic tensions (Fig. 1, left) and diastolic diameter increased (Fig. 3, left). The increase in diastolic diameter after collagenase treatment was equal for all muscle lengths. The question arises whether the increase in diameter is a result of vascular filling, edema formation, or both. From the data of Fig. 3, it can be calculated that the increase in diastolic muscle diameter is ~7.1 ± 3.4%. This means that the volume increase is ~15%. We assume that with constant perfusion, constant diastolic coronary flow (Fig. 4) and adenosine-induced maximal dilation vascular volume does not increase so that the increase in diastolic muscle diameter results from edema. Thus edema formation that increases muscle stiffness has probably a more pronounced effect than the disruption of the struts that decrease muscle stiffness.

In the papillary muscle, the collagen struts connect myocytes and vessels mainly in the radial direction (5), and thus removal of struts in this preparation has limited influence on mechanics in the length direction. The unperfused total heart, as studied by MacKenna et al. (11), with its three-dimensional and “curved” structure and lack of increased edema, probably tips the balance the other way.

The $L_{\text{max}}$ did not change as result of the collagen removal (or in the time controls), which implies that at least sarcomere length has not changed due to collagenase treatment.

Diastolic coronary flow is not changed (Fig. 4) after collagenase treatment, indicating that edema has little effect on coronary resistance and thus on vessel diameters, which is in agreement with Charan et al. (6).

Cardiac muscle mechanics in systole. Todaka et al. (18), studied whole (perfused) hearts and reported no change in developed pressure 1 h after the start of treatment with DTNB, which releases various endogenous collagenases. Recently, Baicu et al. (3) reported a decrease in isometric developed tension in the unperfused rat papillary muscle after plasmin treatment by activating a cascade of matrix metalloproteinases. Both authors did not specifically remove collagen type I.

We found a significant increase in developed tension and an accelerated relaxation after acute and specific removal of collagen type I compared with time controls. Removal of the collagen struts resulted in an increase in diastolic muscle diameter (Fig. 3), but the contraction-induced change in diameter (Fig. 3) and flow impediment (Fig. 4) were not altered. The latter indicates that although edema is present, during the contraction, the coronary vasculature is emptied to a similar extent.

Recently, Willemsen et al. (20) showed, in the rat papillary muscle, that developed tension was reduced when the myocyte diameter increase when contraction was hindered. Because the emptying of the coronary vasculature appears not to be affected by strut removal, a change in hindrance through the coronary vasculature seems unlikely. We therefore hypothesize that the...

Fig. 4. Left, diastole coronary flow at 95% $L_{\text{max}}$, flow before treatment is set at 100%. Right, flow impediment normalized to the tension (means ± SE, $n=5$).
removal of the collagen struts itself or the resulting increase in interstitial fluid poses a lower lateral load on the contracting myocytes than neighboring myocytes, thereby decreasing the hindrance, allowing more myocyte thickening and increasing developed tension.

Because flow impediment is not increased after collagenase treatment (Fig. 4), but even shows a tendency to decrease, the collagen struts are unlikely to play an important role in limiting coronary flow impediment.

These results are not in line with the results obtained from clinical studies (2, 16, 17, 21). From the results of these studies, it was concluded that there is a direct relation between the reduction collagen and contractile dysfunction. However, these studies were all long-term (follow-up) studies, where the effect of changes in the contractile machinery and changes in (the effect of) cardiovascular active hormones also could lead to contractile dysfunction. Furthermore, edema formation in clinical pathologies could be responsible for decreased cardiac performance by increased diffusion distances.

Collagen degradation. The objective of this study was to remove collagen struts as selectively as possible. MacKenna et al. (11) perfused their rat hearts for up to 60 min. Unlike MacKenna et al. (11), we found myocyte damage within 60 min (Fig. 5, bottom). Therefore, we decided, after several pilot experiments, to stop collagenase treatment when diastolic tension at 95% $L_{\text{max}}$ was almost doubled. Our electromicrographs (Fig. 5) showed that no myocyte damage occurred with this standardized procedure. Myocyte damage is also unlikely because we found an increase in developed tension, not a decrease as observed with myocyte damage.

The number of collagen struts after collagenase treatment was reduced to $\sim 32\%$ of control. The thicker coiled perimysial fibers were still present. This is in agreement with MacKenna et al. (11) and Todaka et al. (18), who also saw a reduction in the number of the smaller endomysial fibers but only a small reduction in number of in the larger collagen fibers. According to Matsubara et al. (12), the number of struts is a more direct determinant of cardiac function than the total collagen content because the struts are the collagen fibers connecting myocytes and capillaries. Because we are interested in the functional effect of collagen struts and not in the collagen content we used electromicroscopy to determine the collagen struts.

In conclusion, collagenase type I treatment to remove acutely and specifically the small collagen struts in perfused rat papillary muscle increases diastolic and developed tension and causes edema. We suggest that the increase in diastolic tension is due to edema formation resulting from removal of the struts. The increase in developed tension results from the direct removal of the struts, which poses a lower lateral load on the cardiac myocytes, thereby decreasing the hindrance, allowing more myocyte thickening and increasing developed tension.

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