Norepinephrine Stores and Contractile Force of Papillary Muscle from the Failing Human Heart

By Charles A. Chidsey, M.D., Edmund H. Sonnenblick, M.D., Andrew G. Morrow, M.D., and Eugene Braunwald, M.D.

The observation of a diminished norepinephrine concentration in atrial biopsies obtained from some patients with congestive heart failure has led to the suggestion that depletion of the cardiac store of adrenergic neurotransmitter substance may occur in myocardial decompensation. More recently the concentration of the neurotransmitter in the ventricular myocardium has also been examined. Analyses of papillary muscles removed from the left ventricle during the course of mitral valve replacement revealed that a pronounced diminution of norepinephrine concentration occurred in some patients with heart failure, although considerable variation was found in the muscles that were studied.

The possibility that cardiac norepinephrine depletion occurs in heart failure has significant implications in view of the important influence that adrenergic nerves exert upon cardiac performance. The present investigation was conducted to determine whether a relation exists between the store of neurotransmitter and the functional state of the myocardium. The former was determined by measuring the norepinephrine content of the tissue whereas myocardial function was evaluated by determining the maximum isometric active tension that the excised left ventricular papillary muscle was capable of developing in an in vitro system as well as by measuring the inotropic response of the muscle to tyramine. Since the pharmacologic action of this substance is related to its ability to release endogenous norepinephrine from the sympathetic nerves, the response to tyramine provided a means of establishing the functional adequacy of the norepinephrine stores of the tissue.

Methods

Left ventricular papillary muscles were obtained at the time of mitral valve replacement in 17 patients. The patients ranged in age from 21 to 64 years; 12 were men and five were women. The major hemodynamic abnormality in 11 patients was mitral regurgitation, in two it was mitral stenosis, and in four combined stenosis and regurgitation were present. Four patients had associated aortic valve disease of sufficient severity to require simultaneous prosthetic replacement of the aortic valve. The valvular malformation resulted from rheumatic heart disease in 16 patients, and in one patient mitral regurgitation was caused by ruptured chordae tendineae to an otherwise normal valve. All of the patients were receiving maintenance digoxin therapy at the time of operation. Right and left heart catheterization was performed in all patients as a part of their preoperative evaluation.

The mitral valve was exposed during total cardiopulmonary bypass and, after the valve leaflets had been detached from the annulus, the papillary muscles were divided from the ventricular wall at their origins and the valve and muscle were removed en bloc. The patients' temperatures were usually 34 to 35°C, and bypass had been in progress for 10 to 15 minutes when the papillary muscles were transected. Immediately upon removal, the papillary muscles were placed in Krebs solution into which a 95 per cent O₂-5 per cent CO₂ gas mixture was bubbled. The thinnest discrete segment of papillary muscle was then selected and rapidly transferred to a myograph. If the papillary muscles were unduly thick, they were split longitudinally to provide a thin segment and to facilitate oxygenation. The lengths of the muscle segments, at the peak of their length-active tension curves, averaged 9.1 ± 3.6 (S.D.) mm. while the cross-sectional areas averaged 7.3 ± 3.9 mm². The remainder of the mus-
cle was then frozen in dry ice and later analyzed for norepinephrine concentration by the trihydroxindole method.8

As previously described,9 the papillary muscle was placed in a myograph and its lower nontendinous end was attached to the bottom of a temperature-controlled bath.7 The upper tendinous end of the muscle was attached by a thin suture to a Statham (G1-4-250) force transducer, which was mounted on a rigid Palmer stand. With this arrangement, the force of isometric contraction could be measured at any desired muscle length. The muscles were stimulated at a frequency of 12 per minute with supramaximal square wave impulses of 5 msec. duration,* delivered through large platinum plates placed parallel to the long axis of the muscle. Force and the stimulus artifact were recorded on a multichannel oscillograph.

The muscles were bathed in a volume of Krebs solution of exactly 10 ml. and were oxygenated with a gas mixture of 95 per cent oxygen and 5 per cent carbon dioxide at 30 C. The preparation was allowed to equilibrate in the myograph for 30 to 60 minutes before any measurements were made. Isometric length-tension curves were then determined in the papillary muscles by progressively increasing muscle length, and the maximum tension that the muscle could actively develop was thus established. The actively developed tension was calculated as the difference between the peak tension during a contraction (total tension) and the resting tension.

Following these measurements the responses of the muscles to the addition of tyramine were determined. The length of the muscle was maintained at the length required for the development of maximum active tension, and the force following the addition of this amine was recorded continuously. Tyramine was added in volumes of 0.1 ml. to achieve concentrations in the bath ranging from 10<sup>-4</sup> to 10<sup>-5</sup> M (free base), and the maximal response to each increment was measured. In six muscles in which a positive inotropic response to tyramine was not observed, norepinephrine (10<sup>-6</sup> to 10<sup>-5</sup> M) was added in the presence of the tyramine and the responses were recorded. To maintain optimal performance of the muscle preparations, a low frequency of contraction (12 per minute) and a low temperature (30 C.) were employed. Studies were terminated if mechanical performance deteriorated during the course of the study.

Results

Hemodynamic Data

The 17 patients from whom the muscles were obtained all had symptoms of markedly reduced cardiac reserve and were in functional classes III or IV (New York Heart Association). All but one patient had atrial fibrillation (table 1). The cardiac index was less than 2.50 L./min./M.², the lower limit of normal, in all but two of them and in 10 it was markedly reduced, to below 2.0 L./min./M.². Pulmonary hypertension, with pulmonary artery systolic pressures greater than 30 mm. Hg, was present in all but one patient, and elevation of the mean left atrial pressure above 12 mm. Hg was observed in 15 of 17 patients. The left ventricular end-diastolic pressure exceeded the upper limit of normal, 12 mm. Hg, in only two patients.

Norepinephrine Concentration of Papillary Muscle and Response to Tyramine

The concentration of norepinephrine in the samples of papillary muscle averaged 0.36 μg./Gm., and ranged from 0.09 to 0.80 μg./Gm. (table 2). The addition of tyramine resulted in significant increases of active tension in eight of 14 preparations (fig. 1). In these eight muscles, 10<sup>-5</sup>M tyramine produced an increase of active tension averaging 25 per cent and ranging from 13 to 46 per cent. In the other six preparations no increase of active tension was observed and, indeed, in two preparations small decreases, averaging 7 per cent, occurred (fig. 2). All six of these muscles however, were capable of responding to norepinephrine with significant increases in actively developed tension (figs. 2 and 3). The norepinephrine concentrations averaged 0.53 μg./Gm. in the eight muscles that demonstrated a positive inotropic response to tyramine and were significantly lower, averaging 0.16 μg./Gm. (<p < 0.01), in the six muscles in which no positive response was obtained.

Relation between Norepinephrine Concentration and Maximum Tension

The maximum actively developed tensions varied widely, ranging from 0.20 to 4.45 Gm./mm.² and averaging 1.78 Gm./mm.² (table 2). There was a statistically significant correlation between the ventricular norepinephrine concentration and the maximum active tension developed by the muscles (<p <
Table 1

Summary of the Clinical and Hemodynamic Data on 17 Patients from Whom Left Ventricular Papillary Muscles Were Removed and Studied

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, yr.</th>
<th>Diagnosis*</th>
<th>Cardiac index (L./mm./M.²)</th>
<th>Left atrial mean pressure (mm. Hg)</th>
<th>Left ventricular pressure s/d (mm. Hg)</th>
<th>Systemic arterial pressure s/d (mm. Hg)</th>
<th>Pulmonary artery pressure s/d (mm. Hg)</th>
<th>Right ventricular pressure s/d (mm. Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.P.</td>
<td>46</td>
<td>MS and MI</td>
<td>1.65</td>
<td>16</td>
<td>106/5</td>
<td>106/67</td>
<td>36/22</td>
<td>36/4</td>
</tr>
<tr>
<td>S.M.</td>
<td>21</td>
<td>MI</td>
<td>1.88</td>
<td>16</td>
<td>105/11</td>
<td>100/48</td>
<td>—</td>
<td>38/5</td>
</tr>
<tr>
<td>J.S.</td>
<td>38</td>
<td>MI and MS</td>
<td>2.26</td>
<td>18</td>
<td>113/6</td>
<td>118/82</td>
<td>40/22</td>
<td>40/5</td>
</tr>
<tr>
<td>W.M.</td>
<td>35</td>
<td>MS</td>
<td>2.08</td>
<td>14</td>
<td>105/5</td>
<td>108/60</td>
<td>30/14</td>
<td>31/2</td>
</tr>
<tr>
<td>M.P.</td>
<td>50</td>
<td>MS, MI, and TI</td>
<td>1.35</td>
<td>22</td>
<td>119/9</td>
<td>132/61</td>
<td>60/27</td>
<td>64/5</td>
</tr>
<tr>
<td>M.H.</td>
<td>42</td>
<td>MS and AI</td>
<td>2.48</td>
<td>21</td>
<td>108/8</td>
<td>108/62</td>
<td>42/22</td>
<td>42/5</td>
</tr>
<tr>
<td>T.G.</td>
<td>44</td>
<td>MI and MS</td>
<td>1.06</td>
<td>11</td>
<td>105/5</td>
<td>112/80</td>
<td>80/50</td>
<td>80/10</td>
</tr>
<tr>
<td>O.S.</td>
<td>64</td>
<td>MS</td>
<td>1.59</td>
<td>20</td>
<td>145/6</td>
<td>148/60</td>
<td>100/30</td>
<td>100/9</td>
</tr>
<tr>
<td>R.D.</td>
<td>44</td>
<td>AI and MS</td>
<td>1.22</td>
<td>27</td>
<td>110/10</td>
<td>120/32</td>
<td>80/40</td>
<td>80/6</td>
</tr>
<tr>
<td>E.E.</td>
<td>38</td>
<td>AI and MI</td>
<td>1.65</td>
<td>—</td>
<td>110/22</td>
<td>138/33</td>
<td>35/16</td>
<td>35/5</td>
</tr>
<tr>
<td>T.S.</td>
<td>36</td>
<td>MS and MI</td>
<td>1.40</td>
<td>27</td>
<td>118/17</td>
<td>115/53</td>
<td>65/33</td>
<td>65/15</td>
</tr>
<tr>
<td>G.P.</td>
<td>28</td>
<td>MI</td>
<td>2.07</td>
<td>24</td>
<td>112/5</td>
<td>110/60</td>
<td>85/35</td>
<td>85/10</td>
</tr>
<tr>
<td>E.W.</td>
<td>26</td>
<td>MI†</td>
<td>2.88</td>
<td>26</td>
<td>95/5</td>
<td>60/33</td>
<td>60/5</td>
<td>102/60</td>
</tr>
<tr>
<td>R.G.</td>
<td>39</td>
<td>MI</td>
<td>4.25</td>
<td>10</td>
<td>105/9</td>
<td>103/60</td>
<td>61/25</td>
<td>61/8</td>
</tr>
<tr>
<td>C.T.</td>
<td>47</td>
<td>AS, AI, and MI</td>
<td>2.17</td>
<td>10</td>
<td>106/10</td>
<td>84/53</td>
<td>33/15</td>
<td>33/10</td>
</tr>
<tr>
<td>M.W.</td>
<td>46</td>
<td>MI</td>
<td>2.42</td>
<td>25</td>
<td>150/6</td>
<td>110/60</td>
<td>60/30</td>
<td>60/3</td>
</tr>
<tr>
<td>L.L.</td>
<td>52</td>
<td>MI‡</td>
<td>0.93</td>
<td>22</td>
<td>81/9</td>
<td>80/60</td>
<td>61/25</td>
<td>61/8</td>
</tr>
</tbody>
</table>

*Diagnosis: MS, mitral stenosis; MI, mitral insufficiency; TI, tricuspid insufficiency; AI, aortic insufficiency; AS, aortic stenosis.
†Normal sinus rhythm present in this patient; all the others were in atrial fibrillation.
‡Mitr al insufficiency resulting from rupture of chordae tendineae.
Table 2
Summary of the Observations Made on Left Ventricular Papillary Muscles Removed from 17 Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Maximum active tension (Gm./mm.²)</th>
<th>Muscle cross-sectional area (mm.²)</th>
<th>Ventricular NE* (µg./Gm.)</th>
<th>Tyramine+ response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyramine responsive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.P.</td>
<td>2.45</td>
<td>10.6</td>
<td>0.31</td>
<td>24</td>
</tr>
<tr>
<td>S.M.</td>
<td>1.81</td>
<td>6.9</td>
<td>0.80</td>
<td>10</td>
</tr>
<tr>
<td>J.S.</td>
<td>4.45</td>
<td>3.6</td>
<td>0.60</td>
<td>26</td>
</tr>
<tr>
<td>W.M.</td>
<td>2.50</td>
<td>7.9</td>
<td>0.46</td>
<td>38</td>
</tr>
<tr>
<td>M.P.</td>
<td>1.60</td>
<td>7.3</td>
<td>0.49</td>
<td>25</td>
</tr>
<tr>
<td>M.H.</td>
<td>2.20</td>
<td>4.0</td>
<td>0.35</td>
<td>46</td>
</tr>
<tr>
<td>T.G.</td>
<td>2.10</td>
<td>4.0</td>
<td>0.50</td>
<td>16</td>
</tr>
<tr>
<td>O.S.</td>
<td>1.60</td>
<td>13.1</td>
<td>0.72</td>
<td>13</td>
</tr>
<tr>
<td>Mean</td>
<td>2.34</td>
<td>7.2</td>
<td>0.53</td>
<td>25</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.92</td>
<td>2.3</td>
<td>0.17</td>
<td>9</td>
</tr>
<tr>
<td>Tyramine unresponsive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.D.</td>
<td>0.50</td>
<td>8.0</td>
<td>0.24</td>
<td>0</td>
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<tr>
<td>E.E.</td>
<td>0.74</td>
<td>9.5</td>
<td>0.09</td>
<td>-7</td>
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<tr>
<td>T.S.</td>
<td>0.50</td>
<td>6.8</td>
<td>0.18</td>
<td>0</td>
</tr>
<tr>
<td>G.P.</td>
<td>1.40</td>
<td>4.2</td>
<td>0.09</td>
<td>-7</td>
</tr>
<tr>
<td>E.W.</td>
<td>0.50</td>
<td>7.7</td>
<td>0.22</td>
<td>-7</td>
</tr>
<tr>
<td>R.G.</td>
<td>0.20</td>
<td>9.8</td>
<td>0.11</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>0.64</td>
<td>7.7</td>
<td>0.16</td>
<td>-2</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.41</td>
<td>1.2</td>
<td>0.07</td>
<td>-</td>
</tr>
</tbody>
</table>

Response to tyramine—not tested

- C.T.: 2.41  3.5  0.36  -
- M.W.: 1.15  16.7  0.49  -
- L.L.: 2.00  1.1  0.57  -

*NE = norepinephrine.
†Tyramine response (%) represents the maximal increase in isometric active tension above the control tension that could be achieved with tyramine.

0.05, fig. 4). Two entire length-active tension curves are reproduced in figure 5: one was obtained from a muscle with a relatively high norepinephrine concentration and the other from a muscle with a low concentration. It is apparent that despite the marked difference in maximum tension the general shapes of the curves are similar, and maximum tension was achieved with approximately equal degrees of stretch above resting length (L₀).

In the eight muscles that exhibited a positive inotropic response to tyramine, the maximum tension developed prior to the addition of tyramine averaged 2.34 Gm./mm.². This was significantly greater than the tension developed by the six muscles not responding to tyramine, which averaged 0.64 Gm./mm.² (p < 0.01).

Although a correlation was observed between norepinephrine depletion and the contractile force that the papillary muscle could develop, no relationship was found between either the norepinephrine concentration or the maximum active tension and the hemodynamic variables determined preoperatively (table 1). Thus, no correlation was observed between the maximum tension actively developed by the papillary muscle or its norepinephrine concentration and the patient's cardiac index or mean left atrial pressure. Also, there was no relationship between the maximum tension corrected for the muscle's cross-
sectional area and the thickness of the muscle (table 2).

Discussion
Although the clinical and hemodynamic changes that occur in various forms of congestive heart failure have been well characterized, there is relatively little information concerning the underlying abnormality of the myocardium in this state. Thus, no definitive changes in the contractile properties of the muscle have been demonstrated, and there is no agreement concerning the basic biochemical abnormalities. In previous studies it has been shown that the cardiac stores of norepinephrine regularly undergo a pronounced depletion when heart failure is produced experimentally, and that similar changes occur in the atrial and ventricular tissues of many, but not all, patients with chronic heart failure. It does not seem likely that this biochemical abnormality is a primary one, but in view of the important role played by cardiac sympathetic nerves in the augmentation of the contractile state of the heart, the possibility must be considered that this depletion of the neurotransmitter store interferes with adrenergic transmission and thereby affects contractile function.

With these considerations in mind, it was considered of importance to compare the concentration of norepinephrine in the muscle with its capacity to develop tension, as well as its ability to augment tension when stimulated by the norepinephrine-releasing sympathomimetic amine, tyramine. None of the muscles was obtained from patients with normal hearts, and varying degrees of heart

Figure 1
Tracings of isometric force developed by a papillary muscle that responded with an increase in both active tension and its rate of development. In the upper panels a fast tracing is shown before and 5 minutes after the addition of tyramine (TA). In the bottom panel the slow tracing demonstrates the onset of the inotropic response to tyramine. Calibration of force: 5 Gm. = 12.5 mm. deflection.
failure were present in all patients. Both the norepinephrine concentration and the maximum active tensions, which the muscles developed prior to the addition of tyramine, exhibited considerable variation. A significant correlation was observed, however, between norepinephrine concentration and the maximum tension (fig. 5), and only the muscles with the greater concentrations of norepinephrine increased active tension further in response to tyramine. Even the muscles with the lowest norepinephrine concentrations were capable of increasing tension in response to norepinephrine itself. Thus, there is no evidence to suggest that the contractile process itself has been so deranged in the failing heart that its function cannot be increased by stimulation with catecholamines.

Although this correlation between norepinephrine concentration and contractile properties of the muscle was present, it is not clear whether these two variables are causally...

Figure 2
Tracings of isometric force developed by a papillary muscle that did not respond to the addition of tyramine. In the upper two panels the absence of response to $10^{-4}$ and $10^{-3}$ M tyramine (TA) is apparent. In the bottom two panels the inotropic response to norepinephrine (NE) is shown. In each panel, the left-hand portion is the control state and the right-hand portion is the state following addition of the drug. Calibration of force: 5 Gm. = 44.0 mm. deflection.
The dose-response curve obtained from one papillary muscle is reproduced in its entirety. The active tension is plotted on the ordinate and the concentration of the amines in the myograph are plotted on the abscissa. Although there is no response to tyramine (closed circles), there is a response to norepinephrine, which was 170 per cent of the control active tension at the largest dose (5 × 10⁻⁶ M).

Length-active tension curves of two papillary muscles are reproduced in their entirety. Active tension is plotted on the ordinate and the resting length, as per cent above the length at which no tension was developed by the muscle. One curve was obtained from a muscle with norepinephrine concentration of 0.60 µg/Gm., and the other from a muscle severely depleted of norepinephrine (0.09 µg/Gm.).

An important finding in this study was the severe depression of contractility, as assessed in the in vitro system, in the muscles obtained from a number of patients. Indeed, the maximum active tension that the muscles from five patients could develop was less than 1.0 Gm./mm.² Although no normal values for human myocardium are available for comparison, these five values were substantially lower than those reported for normal cat.
papillary muscle, 2 to 6 Gm./mm.\textsuperscript{7,18,19} Since human papillary muscles are thicker than those of the cat, it is necessary to consider the problem of the adequacy of the oxygenation of the muscle in the in vitro system used. In order to minimize the oxygen requirements of the muscle, the temperature was reduced to 30 C., and a slow frequency of contraction was employed. It is possible to recognize inadequate oxygenation of the muscle, since this condition leads to deterioration of mechanical performance. All of the maximum active tension measurements described in this report were made in muscles in which the mechanical performance was stable, suggesting that oxygen delivery was adequate. It therefore appears unlikely that inadequate oxygenation is the cause of the diminished contractility of some of these muscles. In this connection it is pertinent that no correlation was present between the cross-sectional area of the individual muscle and the maximum active tension per unit cross-sectional area (table 2).

Two basic mechanisms are involved in the regulation of myocardial function: (1) the Frank-Starling mechanism, through which changes in resting fiber length affect the strength of contraction; (2) the modulation of the contractile state of the myocardium at any given fiber length, through variations in the activity of the cardiac sympathetic nerves. The present investigation indicates how both of these compensatory mechanisms may fail in the course of chronic heart failure. First of all, the length-active tension curve may become depressed so that the heart muscle can develop only small increments in active tension when it is stretched (fig. 5). Secondly, the depletion of norepinephrine that can occur in this tissue undoubtedly interferes with the ability of the sympathetic nerves to release endogenous norepinephrine.

**Summary**

The relation between cardiac norepinephrine concentration and the functional state of the myocardium was studied in left ventricular papillary muscles removed from patients with congestive heart failure at the time of mitral valve replacement. Myocardial function was assessed by determining the maximum isometric active tension that the papillary muscle could develop in an in vitro system, and by measuring the increase in active tension in response to the norepinephrine-releasing sympathomimetic amine, tyramine. The norepinephrine concentration averaged 0.36 \( \mu \text{g.} / \text{Gm.} \) in 17 muscles. In eight of these a response to tyramine was observed with a 21-per cent increase in active tension, and the norepinephrine concentrations averaged 0.53 \( \mu \text{g.} / \text{Gm.} \); in the other six muscles tested with tyramine no positive inotropic response was observed, and the norepinephrine concentration was significantly lower, averaging 0.16 \( \mu \text{g.} / \text{Gm.} \). The maximum active tension in 17 muscles averaged 1.78 Gm./mm.\textsuperscript{2}, and a significant positive correlation between the maximum tension and the norepinephrine concentration of the individual muscles was observed. In eight muscles responding to tyramine, the maximum tension observed, 2.34 Gm./mm.\textsuperscript{2}, was significantly greater than that observed in the six muscles unresponsive to tyramine, 0.64 Gm./mm.\textsuperscript{2}. It is concluded that norepinephrine depletion appears to be associated with defective myocardial function although no causal relationship between these two abnormalities has been established.

**References**


6. CHIDSEY, C. A., HARRISON, D. C., and BRAUN-
NOREPINEPHRINE STORES OF PAPILLARY MUSCLE


Now the absence of the scientific habit of mind is a serious hindrance, because it favors belief in occult forces, rejects determinism in vital phenomena, and leads to the notion that the phenomena of living beings are governed by mysterious, vital forces which are continually invoked. When an obscure or inexplicable phenomenon presents itself, instead of saying "I do not know," as every scientific man should do, physicians are in the habit of saying, "This is life"; apparently without the least idea that they are explaining darkness by still greater darkness.—Claude Bernard: An Introduction to the Study of Experimental Medicine. New York, The Macmillan Company, 1927, p. 201. Centenary of First Publication, 1865.
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