Storage and metabolism of norepinephrine after experimental myocardial infarction

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Mathes, Peter, Charles Cowan, and Sigmundur Gudbjarnason. Storage and metabolism of norepinephrine after experimental myocardial infarction. Am. J. Physiol. 220(1): 27-32. 1971.—Serial determinations of the norepinephrine (NE) content in all four chambers of the canine heart were carried out following experimental myocardial infarction. Myocardial NE retention and subcellular distribution were determined after injection of tracer doses of dl-norepinephrine-7-14C. Normal left ventricular muscle contained 0.96 µg NE/g tissue; following coronary artery occlusion, the infarcted tissue lost its norepinephrine content completely by the 4th day and the noninfarcted tissue showed a marked decline during the first 10 days, reaching a level as low as 0.35 µg in the basilar and 0.14 µg NE/g in the apical portion of the left ventricle. The decrease in myocardial norepinephrine content extended to the right ventricle and both atria as well. Norepinephrine levels rose again 2 weeks after infarction and reached normal values 6 weeks after coronary artery occlusion. Despite these changes in endogenous norepinephrine levels, the myocardial retention of labeled NE, as well as the subcellular distribution of endogenous and exogenous NE, remained unaltered. Upon restoration of myocardial function, the cardiac norepinephrine content returns to normal.

METHODS

Experiments were performed on 57 mongrel dogs, weighing 14-21 kg. Myocardial infarction was produced experimentally during anesthesia with sodium pentobarbital, 25 mg/kg given intravenously. The trachea was intubated and artificial respiration was instituted with a Harvard respirator pump. Thoracotomy was performed through the left fifth intercostal space, and the pericardium was opened anterior to the left phrenic nerve. Several branches of the left anterior descending and circumflex coronary arteries were ligated to produce an anterolateral infarction of approximately uniform size. The pericardium and chest were closed, the pneumothorax was evacuated, and the animal was allowed to recover. On sham-operated animals, the same procedure was carried out, except for ligation of the coronary artery branches.

At varying intervals from 1 to 42 days following myocardial infarction the animals were restudied. They were anesthetized with a combination of Sublimaze (0.04 mg/kg body wt), Inapsine (2 mg/kg), and sodium pentobarbital (8 mg/kg) to avoid depression of the respiratory activity.

Catheters were placed in the left ventricle and descending aorta and directly connected to P23Db Statham strain gauges. Pressures were recorded on an Electronics for Medicine recorder; the zero level was set at the left ventricular apex. Left ventricular end-diastolic pressure was recorded at the end-expiratory phase of the respiratory cycle, using higher sensitivity. The first derivative of the left ventricular pulse (dp/dt) was obtained by means of an RC differentiating circuit.

Upon completion of the hemodynamic measurements, the catheters were withdrawn and the animal was sacrificed by an intravenous injection of saturated potassium chloride solution. The heart was removed and dissected on crushed ice. Samples were frozen in liquid nitrogen and kept frozen until homogenized. The entire heart and its individual chambers were weighed. The infarcted area was excised and weighed separately.

The endogenous norepinephrine content was determined in both atria, right ventricle, and noninfarcted left ventricle base and apex and in the infarcted tissue. The infarcted and noninfarcted areas of the left ventricle were differentiated from each other by means of the changes in color and appearance. The infarcted area appeared dull brownish blue in color and showed signs of fatty infiltration or fibrosis on dissection, while the non-
infarcted area showed the normal shine and was pink. The determination of norepinephrine was done in 2-g tissue samples which were grossly freed from blood, fat, and connective tissue, (1 g for atria, respectively) according to the method of Anton and Sayre (1) as modified by de Champlain et al. (7). Alumina and tissues were prepared according to the method of Anton and Sayre (1). The norepinephrine was oxidized with potassium ferrocyanide and converted to trihydroxy-indole derivatives by adding alkaline ascorbate solution according to the method of von Euler and Lishajko (21). Norepinephrine was determined fluorometrically in 3.0-ml aliquots, transferred into matched cuvettes. The samples were activated at 405 nm, and the resulting fluorescence was read at 525 nm on an Aminco-Bowmann fluoromicrophotometer.

Recoveries were determined in duplicate with each assay, and the results were corrected accordingly. The average recovery was 77.4 ± 7.7 % (SD). All determinations for the left ventricular tissue content were done in duplicate. The standard deviation for these duplicates was ±5.4 %. Results were expressed in micrograms norepinephrine per gram of wet tissue.

The myocardial uptake and accumulation of labeled NE were determined in three groups of animals, a) control group (n = 6), b) 10 days after infarction (n = 6), and c) 6 weeks after infarction (n = 6).

The accumulation of intact NE and degradation products of NE were determined as well as the subcellular distribution of endogenous and exogenous, labeled NE. dl-Norepinephrine-1-14C (SA = 45.7 mc/mM), 1 μg/kg body wt, was injected intravenously, and the animal was sacrificed 60 min later with an intravenous injection of saturated potassium chloride solution. The endogenous norepinephrine content was determined as mentioned above, and the radioactivity of 1.0-ml aliquots of the neutralized perchloric acid extract and the acetic acid eluate was determined in the liquid scintillation spectrometer after addition of 15 ml dioxane scintillation mixture. In this system, the counting efficiency for 14C was 82 %, and external radioactive standards were used to correct for individual variations. Sufficient counts were obtained to maintain a standard deviation of counting below 1 %. An estimate of the total NE-14C metabolites was obtained by calculating the difference between the radioactivity of the perchloric acid extract and the radioactivity of the acetic acid eluate from the alumina.

Studies of the subcellular distribution of norepinephrine were done by differential centrifugation of a homogenate of noninfarcted left ventricular muscle using the technique described by Campos and Shideman (3). The homogenate was centrifuged at 2,000 × g for 5 min and the supernatant fluid containing the contents of the cells ruptured by homogenization was separated from the pellet (coarse fraction). The initial supernatant fluid was then fractionated by centrifugation at 100,000 × g for 1 hr into a pellet of subcellular particles (particulate fraction) and a second supernatant portion containing the cell fluid (soluble fraction). The endogenous norepinephrine content as well as the radioactivity were determined in all three fractions as described above.

**TABLE 1. Compensatory cardiac hypertrophy induced by coronary artery ligation**

<table>
<thead>
<tr>
<th></th>
<th>TH/BW</th>
<th>LV/BW</th>
<th>RV/BW</th>
<th>S/BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 11)</td>
<td>6.24±0.16</td>
<td>3.32±0.11</td>
<td>1.55±0.06</td>
<td>1.52±0.06</td>
</tr>
<tr>
<td>10 days after infarct (n = 11)</td>
<td>6.97±0.39</td>
<td>3.74±0.16</td>
<td>1.76±0.07</td>
<td>1.71±0.09</td>
</tr>
<tr>
<td>% Change</td>
<td>+11.6</td>
<td>+12.7</td>
<td>+13.5</td>
<td>+12.5</td>
</tr>
<tr>
<td></td>
<td>P &lt; .05</td>
<td>P &lt; .05</td>
<td>P &lt; .05</td>
<td></td>
</tr>
<tr>
<td>6 Weeks after infarct (n = 9)</td>
<td>6.57±0.22</td>
<td>3.15±0.09</td>
<td>1.76±0.08</td>
<td>1.81±0.10</td>
</tr>
<tr>
<td>% Change from control</td>
<td>13.3</td>
<td>5.1</td>
<td>18.5</td>
<td>+19.1</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>P &lt; .05</td>
<td>P &lt; .025</td>
</tr>
<tr>
<td>% Change from 10 days after infarct</td>
<td>-17.7</td>
<td>-15.7</td>
<td>0.0</td>
<td>+5.8</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>P &lt; .01</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

TH = Total heart weight (LV + RV + S), g. BW = body weight, kg. LV = left ventricular weight, g. RV = right ventricular weight, g. S = septal weight, g.

**RESULTS**

1) Changes in ventricular weight. Table 1 illustrates the myocardial hypertrophy ensuing after infarction, expressed in heart and chamber to body weight ratio. The heart body weight ratio increased by 11.6 % (P < .05) 10 days after infarction, and the left ventricle to body weight ratio increased by 12.7 % (P < .05). Six weeks after infarction, the left ventricle showed a decline in weight, indicating that the previously necrotic area had been replaced by a relatively smaller amount of scar tissue; the heart-to-body weight ratio did not show a significant change at this time.

2) Cardiac norepinephrine content. The infarcted heart muscle showed a rapid decline in norepinephrine content from the control level of 0.96 ± 0.04 to 0.35 ± 0.06 μg NE on the 1st day after infarction. On the 2nd day, the
NOREPINEPHRINE STORES IN MYOCARDIAL INFARCTION

TABLE 2. Cardiac norepinephrine content after myocardial infarction

<table>
<thead>
<tr>
<th>Days After Infarct</th>
<th>Control (n = 11)</th>
<th>Sham operated (n = 5)</th>
<th>1 Day (n = 3)</th>
<th>2 Days (n = 3)</th>
<th>4 Days (n = 3)</th>
<th>6 Days (n = 3)</th>
<th>10 Days (n = 11)</th>
<th>14 Days (n = 3)</th>
<th>21 Days (n = 3)</th>
<th>28 Days (n = 3)</th>
<th>42 Days (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricle basilar part</td>
<td>0.96±0.03</td>
<td>0.56±0.02</td>
<td>0.81±0.01</td>
<td>0.66±0.01</td>
<td>0.54±0.01</td>
<td>0.48±0.02</td>
<td>0.35±0.01</td>
<td>0.36±0.02</td>
<td>0.55±0.03</td>
<td>0.78±0.07</td>
<td>0.93±0.04</td>
</tr>
<tr>
<td>Apex</td>
<td>0.56±0.02</td>
<td>0.54±0.02</td>
<td>0.44±0.01</td>
<td>0.34±0.02</td>
<td>0.21±0.03</td>
<td>0.16±0.04</td>
<td>0.14±0.05</td>
<td>0.16±0.02</td>
<td>0.27±0.07</td>
<td>0.42±0.05</td>
<td>0.55±0.08</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>0.95±0.04</td>
<td>0.54±0.05</td>
<td>0.54±0.01</td>
<td>0.49±0.04</td>
<td>0.35±0.07</td>
<td>0.32±0.03</td>
<td>0.33±0.05</td>
<td>0.35±0.04</td>
<td>0.50±0.07</td>
<td>0.70±0.02</td>
<td>0.87±0.02</td>
</tr>
<tr>
<td>Left atrium</td>
<td>1.81±0.15</td>
<td>1.80±0.04</td>
<td>1.82±0.05</td>
<td>1.84±0.20</td>
<td>1.91±0.18</td>
<td>1.30±0.16</td>
<td>1.39±0.10</td>
<td>1.21±0.18</td>
<td>1.31±0.16</td>
<td>1.69±0.03</td>
<td>1.87±0.04</td>
</tr>
<tr>
<td>Right atrium</td>
<td>1.80±0.07</td>
<td>2.01±0.19</td>
<td>1.86±0.07</td>
<td>1.85±0.02</td>
<td>1.90±0.18</td>
<td>1.71±0.04</td>
<td>1.54±0.21</td>
<td>1.33±0.20</td>
<td>1.62±0.08</td>
<td>1.69±0.06</td>
<td>1.94±0.13</td>
</tr>
</tbody>
</table>

Values are means ± se. Cardiac norepinephrine content given in micrograms per gram wet tissue. *P < .001 in comparison to control. †P < .001 in comparison to control 10 days after infarction.

After infarction, did not show a decline in myocardial NE content; the values for the left ventricle were 0.96 ± 0.07/μg in contrast to 0.35 ± 0.01 for animals with myocardial infarction. For the right ventricle the values were 0.94 ± 0.05 and 0.32 ± 0.03 μg, respectively.

Both atria showed a similar but less extensive decline in their norepinephrine content. The lowest level in the left atrium was reached after 2 weeks decreasing from 1.81 ± 0.13 to 1.21 ± 0.18 μg NE. The lowest level for the right atrium was reached 3 weeks after infarction, diminishing from 1.88 ± 0.07 to 1.23 ± 0.08 μg NE/g tissue. Thereafter, the NE levels rose gradually and reached the control level by the 6th week following the infarct.

III) Hemodynamic changes. The maximal rate of left ventricular pressure rise fell from a control value of 4,777 ± 265 to 2,676 ± 303 mm Hg/sec on the 1st day following infarction. Subsequently, a gradual increase was observed until control levels were reached by the 4th week following myocardial infarction. No correlation between left ventricular dp/dt and NE content was found. The decrease and subsequent increase in LV function after infarction preceded the changes in NE content (Fig. 2).

IV) Myocardial retention of dl-norepinephrine-7-14C. The term "retention" here refers to the balance between myocardial uptake and release of dl-norepinephrine-7-14C 1 hr after intravenous injection of 1 μg/kg body wt. At this time, the heart of control animals contained 118 ± 5.20 × 10-12 moles of labeled norepinephrine per gram tissue (Fig. 3), and the amount of radioactive metabolites present was 14.2 ± 1.54 × 10-12 moles/g tissue, accounting for approximately 12% of the intact norepinephrine retained. Despite the marked decline in endogenous norepinephrine levels 10 days after infarction, the retention did not change; 115.3 ± 7.09 × 10-12 moles of norepinephrine-14C per gram tissue accumulated during the 1st hr after injection, and the metabolite level was not significantly altered, as demonstrated in the amount of metabolites present, 14.3 ± 3.25 × 10-12 moles/g tissue. Six weeks after infarction, when normal endogenous levels of norepinephrine were reached again, the retention of the labeled material injected was close to control values; no significant changes were observed (Fig. 3). It should be pointed out that the d form of norepinephrine is not actively taken up into the neuron, and the uptake is limited to the l form. 14C-Labeled norepinephrine was, however, only available as a mixture of both the d and l forms.
V) Subcellular distribution of norepinephrine. The tissue levels of norepinephrine determined in homogenates of heart muscle prepared in phosphate buffer did not differ significantly from those determined in homogenates prepared in 0.4 N perchloric acid. Normal left ventricular muscle contained 0.95 ± 0.04 μg NE/g wet tissue, and 10 days after infarction, the noninfarcted heart muscle contained 0.36 ± 0.03 μg NE/g tissue. The subcellular distribution is illustrated in Fig. 4. In normal left ventricular muscle, 58.9% of the total norepinephrine content was found in the particulate fraction, and the soluble fraction contained 13.7%. Ten days after infarction, the absolute amount in both fractions was markedly lower; however, the distribution was similar. The particulate fraction contained 61.1% and the soluble fraction contained 13.8% of the total norepinephrine present. Six weeks after infarction, no significant change was observed. It is thus apparent that the relative subcellular distribution of norepinephrine remained unaltered after infarction, despite the marked variation in total content.

VI) Retention of dl-norepinephrine-7-14C in subcellular fractions. There is a close correlation between the amount of endogenous norepinephrine present in subcellular fractions and the amount of radioactivity found in these fractions 1 hr after the injection of 1 μc dl-norepinephrine-7-14C/kg (Table 3). In control animals, 58.9% of the endogenous and 60.2% of the radioactive norepinephrine were found in the particulate fraction, and the figures of the soluble fraction are equally close. Ten days and 6 weeks after infarction the retention in the subcellular fractions was also similar to control values.

DISCUSSION

Previous studies (12, 16, 17, 20) have demonstrated a significant elevation in plasma levels of norepinephrine...
following myocardial infarction accompanied by increased urinary excretion of this compound. These alterations in norepinephrine metabolism are attributed to an increased activity of the sympathetic nervous system, leading to a gradual reduction in cardiac norepinephrine stores. A similar increase in activity of the sympathetic nervous system is present in congestive heart failure and hemorrhagic shock and is known to result in reduction of norepinephrine stores in heart muscle (4-6, 10).

The rapid irreversible depletion of norepinephrine stores in infarcted heart muscle is the result of the death of the ischemic muscle cells (2) and leads ultimately to complete release of norepinephrine (Fig. 1).

In the noninfarcted, functioning myocardium, a continuous decline in norepinephrine content was observed during the first 10 days following infarction (Fig. 1). These low levels, approximately one-third of the normal values, persisted until the 14th day following infarction; thereafter, a gradual increase in norepinephrine levels was observed, and normal levels were reached again 6 weeks after infarction. The reduction in norepinephrine content is generalized and affects both ventricles, and to a lesser extent, the atria as well (Table 2). Both ventricles and the total heart as well showed significant hypertrophy following infarction (Table 1). The left ventricle body weight ratio increased by 19.7% 10 days after infarction. It must be taken into consideration that the infarcted tissue has a higher water content (83.8% ± 0.7%) at this time than the noninfarcted muscle (78.5 ± 0.4%), making the increase in total muscle mass appear too large. Even if this is not taken into consideration, the amount of hypertrophy observed could account for only a fraction of the observed decline in norepinephrine content.

Examination of the myocardial water content revealed that no appreciable amount of edema did develop in non-

<table>
<thead>
<tr>
<th>Control (n = 6)</th>
<th>10 Days After Infarction (n = 6)</th>
<th>6 Weeks After Infarction (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coarse</td>
<td>Particulate</td>
</tr>
<tr>
<td>Endogenous norepinephrine, µg/g tissue</td>
<td>0.26±0.01</td>
<td>0.56±0.02</td>
</tr>
<tr>
<td>% of Total dl-Norepinephrine-7-14C, 10^-12 moles/g tissue</td>
<td>30.7±1.42</td>
<td>68.6±2.42</td>
</tr>
<tr>
<td>% of Total</td>
<td>27.1</td>
<td>60.2</td>
</tr>
</tbody>
</table>

Values are means ± se.

infarcted muscle. Determination of the proline/hydroxyproline ratio in noninfarcted heart muscle showed no significant change following myocardial infarction, excluding increased fibrosis as a cause for the decrease in norepinephrine content. Changes in tissue perfusion might have altered the delivery of norepinephrine-14C to the noninfarcted portion of the myocardium, but the fractional distribution of cardiac output as measured by the 82Rb technique showed no difference in perfusion between normal hearts and noninfarcted myocardium 10 days and 2 months after infarction (15).

Changes in myocardial retention or subcellular distribution of labeled norepinephrine were not observed and could not be related to the changes in norepinephrine content of noninfarcted muscle.

The reasons for the decline in cardiac norepinephrine content are not understood, but two possibilities appear probable: a decrease in de novo synthesis of norepinephrine or an increased release of norepinephrine due to increased sympathetic activity. Further studies are required to provide the answer.

It is important to note that the decrease in norepinephrine content is reversible. Upon completion of compensatory processes and restoration of normal myocardial function (Fig. 2), the cardiac norepinephrine stores return gradually to normal.

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