Specific Avidity of the Heart Muscle to Absorb and Store Epinephrine and Norepinephrine

By W. Raab, M.D. and W. Gigee, A.B.

Circulating epinephrine and norepinephrine are avidly absorbed and stored by the heart muscle but not by skeletal muscle of dogs. Stimulation of the cardiac sympathetic nerves augments myocardial norepinephrine but not epinephrine. Sympatholytic drugs do not diminish, some even increase active catecholamines in heart muscle. The results explain a number of pathophysiologic conditions briefly enumerated.

The important role, played by the oxygen-wasting, potentially hypoxiating sympathomimetic catecholamines, epinephrine and norepinephrine, in various myocardial disorders suggested the following study of their concentration in the heart muscle of the dog. Normal and certain experimental conditions were investigated and the concentrations of these substances in cardiac and skeletal muscle were compared.

METHODS

Dogs, anesthetized with Nembutal, were killed by quickly excising the heart. Pericardium, fat and large vessels were removed, the chambers were cut open and the surfaces were dried with filter paper. The coronary vascular system was not flushed, in order to avoid falsification of the results by washing out catecholamines from the tissue. Besides, the catechol content of the coronary blood, even after injection of large doses of epinephrine and norepinephrine, proved too small to constitute a significant source of error. Muscles of the thigh were dissected and, likewise, dried on the surface with filter paper.

The tissues were extracted and prepared for (1) colorimetric assay of total catechols with the method of Shaw, as modified by one of us,6 and (2) bioassay of norepinephrine and epinephrine separately, according to von Euler's method, as described in detail by Goodall7 and ourselves; the blood pressure of Nembutal-anesthetized, atropinized and cocaineized cats with the adrenals tied off and the hen's isolated rectal coecum were used.

The stellate ganglia and/or postganglionic sympathetic fibers of the heart were stimulated unilaterally or bilaterally for 5 to 36 (average 20) minutes, and the crural nerves unilaterally (10 minutes), with attached shielded electrodes, using AC current from a "variable voltage—constant output" transformer (Varian). The lowest voltages which produced definite cardiac acceleration or vigorous muscular contractions respectively were applied. They varied between 2 and 25 volts. Stimulation of the crural nerves was done rhythmically to elicit 120 contractions per minute.

Norepinephrine (10 mg. per Kg.) and epinephrine (10 mg. per Kg.) or both combined (3.5 mg. per Kg. epinephrine plus 7.5 mg. per Kg. norepinephrine) were injected intraperitoneally. Except in a few cases of spontaneous death from epinephrine toxicity, the dogs were killed 10 minutes after injection.

Regitine (3 mg. per Kg.) and benzodioxane (5 mg. per Kg.) were injected intraperitoneally, Dibenamine (20-30 mg. per Kg.) was given intravenously. The dogs were killed 15 to 30, 45 to 70 and 60 to 120 minutes respectively after injections.

In one group, bilateral stimulation of the cardiac sympathetic nerves was carried out for 15 minutes, beginning 10 minutes after intravenous injection of Regitine (3 mg. per Kg.). Stimulation of the crural nerves was begun at the time of intraperitoneal injection of norepinephrine and epinephrine and continued for 10 minutes until the dogs were killed, except in cases of earlier death from epinephrine toxicity. In some of the experiments, the heart rate was registered electrocardiographically by Dr. E. Lepeschkin.

RESULTS

In the control hearts, norepinephrine constituted, on an average, 84 per cent of the pharmacodynamically active catecholamines, the rest being epinephrine. The colorimetric readings for total catecholamines, expressed
in color units (each unit equaling the color intensity of 0.001 μg of epinephrine) were several times higher than bioassay-recovered norepinephrine and epinephrine combined (table 1). This is probably due to the participation of some other pharmacodynamically inactive chromogenic catechols in colorimetry, and to partial inactivation of norepinephrine and epinephrine during the extraction process for bioassay, while the chromogenic and adsorbable catechol ring remains intact at pH 8.5.

Electric stimulation of the cardiac sympathetic nerves augmented the myocardial norepinephrine content (+93 per cent) but left the epinephrine concentration practically unchanged. Injection of norepinephrine was followed by a marked increase of the myocardial norepinephrine alone. Injection of epinephrine resulted in an enormous accumulation of epinephrine in the heart. Norepinephrine was also increased. Combined injection, norepinephrine and epinephrine in a 1:2 proportion, led to the greatest accumulation of both in approximate proportion.

The injections of norepinephrine and epinephrine produced also large proportionate increases of the colorimetrically demonstrable total myocardial catechols. In these instances, the colorimetric values corresponded rather closely to the bioassay results, indicating that practically the entire mass of catecholamines absorbed by the myocardium was recovered in an active form.

Administration of sympatholytic drugs (benzodioxane, Dibenamine, Regitine) (table 2) not only failed to reduce the amounts of pharmacodynamically active norepinephrine and epinephrine in the heart muscle, but Dibenamine and Regitine produced even an augmentation of myocardial norepinephrine. After administration of Regitine, combined with electric stimulation of the cardiac sympa-
thetic nerves, the norepinephrine augmentation was not greater than that produced by either one of these two procedures per se (tables 1, 2). The discrepancies between colorimetric and bioassay values were smaller in the animals treated with sympatholytic drugs than in the controls.

In order to evaluate a possible error, caused by the amounts of catecholamines, present in the coronary blood of the non-flushed hearts at the time of the animals' death, 2 hearts of epinephrine-injected dogs and 2 hearts of norepinephrine-injected dogs were thoroughly flushed through the coronary bed with 1200 cc. of Ringer solution before extraction, and besides, 40 cc. arterial blood samples were withdrawn before injection and again 10 minutes after injection, immediately before the animals were sacrificed.

In table 3, a comparison between the average results, obtained from the nonflushed and flushed hearts, shows an apparently considerable loss of excess myocardial epinephrine, due to washing out of the heart muscle, yet abnormally large excess quantities remained, while all the absorbed norepinephrine seemed to be firmly anchored on the myocardial cells and non-removable by washing.

The maximal amount of coronary blood, present in the heart muscle after epinephrine injection, was 7 per cent of the myocardial volume (W. van B. Robertson, personal communication). On this basis, 7 per cent of the catechols contained in 1 cc. of blood have to be correlated with the catechol content of 1 gram of heart muscle tissue in order to evaluate the degree to which blood catechols interfered in the myocardial readings.

Table 4 gives the blood catechol values before and 10 minutes after intraperitoneal

<table>
<thead>
<tr>
<th>Type of Experiments</th>
<th>Controls</th>
<th>Benzodioxane</th>
<th>Dibenamine</th>
<th>Regitine</th>
<th>Electric stimulation of cardiac sympathetic nerves plus Regitine</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Specimens</td>
<td>27</td>
<td>5</td>
<td>9</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Norepinephrine ng/Gm. (average) (SDm ±)</td>
<td>0.202 (±0.035)</td>
<td>0.340 (±0.06)</td>
<td>0.90* (±0.17)</td>
<td>0.43* (±0.05)</td>
<td>0.43 (±0.104)</td>
</tr>
<tr>
<td>Epinephrine ng/Gm. (average) (SDm ±)</td>
<td>0.025 (±0.0032)</td>
<td>0.020 (±0.003)</td>
<td>0.011 (±0.004)</td>
<td>0.059 (±0.017)</td>
<td>0.030 (±0.015)</td>
</tr>
<tr>
<td>Norepinephrine + Epinephrine ng/Gm. (average) (SDm ±)</td>
<td>0.225 (±0.032)</td>
<td>0.360 (±0.030)</td>
<td>0.511* (±0.114)</td>
<td>0.489* (±0.086)</td>
<td>0.116 (±0.115)</td>
</tr>
<tr>
<td>Total Catechols (Colorimetric) color units/Gm. (average) (SDm ±)</td>
<td>1567 (±86)</td>
<td>1255 (±27)</td>
<td>841* (±17)</td>
<td>300 (±21)</td>
<td>243 (±24)</td>
</tr>
</tbody>
</table>

* These figures differ significantly at the 1 per cent probability level (t-test, Fisher) from the corresponding mean figures of the control group, except for the last group (stimulation plus Regitine) in which the significance was calculated in relation to the preceding (Regitine) group.

Table 3.—Comparison of the Catecholamine Content of Hearts Whose Coronary Vascular System Had Not Been Flushed and of Others Which Were Worked Up After Flushing

<table>
<thead>
<tr>
<th>Substance Injected</th>
<th>Treatment of Coronary Vascular Bed</th>
<th>No. of Specimens</th>
<th>Norepinephrine ng/Gm. (average)</th>
<th>Epinephrine ng/Gm. (average)</th>
<th>Norepinephrine + Epinephrine ng/Gm. (average)</th>
<th>Total Catechols (Colorimetric) color units/Gm. (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine (i.p.) 10 mg/Kg.</td>
<td>Not flushed</td>
<td>5</td>
<td>1.39</td>
<td>2.86</td>
<td>4.25</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Flushed</td>
<td>2</td>
<td>0.73</td>
<td>0.30</td>
<td>1.03</td>
<td>70</td>
</tr>
<tr>
<td>Norepinephrine (i.p.) 10 mg/Kg.</td>
<td>Not flushed</td>
<td>10</td>
<td>1.16</td>
<td>0.03</td>
<td>1.19</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>Flushed</td>
<td>2</td>
<td>3.45</td>
<td>0.05</td>
<td>3.50</td>
<td>99</td>
</tr>
<tr>
<td>None (Controls)</td>
<td>Not flushed</td>
<td>27</td>
<td>0.20</td>
<td>0.02</td>
<td>0.22</td>
<td>84</td>
</tr>
</tbody>
</table>
TABLE 4.—Catecholamine Content of Arterial Blood Before and 10 Minutes After Intraperitoneal Injection of 10 mg. per Kg. of Epinephrine and Norepinephrine Respectively

<table>
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</thead>
<tbody>
<tr>
<td>Epinephrine (i.p.) 10 mg./Kg.</td>
<td>134</td>
<td>—</td>
<td>0.047 0.062 0.100 0.06 0.10 0.0100 43 57</td>
<td>225</td>
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</tr>
<tr>
<td>Norepinephrine (i.p.) 10 mg./Kg.</td>
<td>136</td>
<td>0.05 0.08 0.13 0.0100 43 57</td>
<td>185</td>
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<tr>
<td></td>
<td>135</td>
<td>0.062 0.266 0.328 19 81</td>
<td>580</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>137</td>
<td>0.212 0.047 0.250 82 18</td>
<td>350</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>0.018 0.013 0.031 58 42</td>
<td>185</td>
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<tr>
<td></td>
<td></td>
<td>0.740 0.086 0.820 90 10</td>
<td>1000</td>
<td></td>
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</tbody>
</table>

TABLE 5.—Catecholamines in Skeletal Muscle

<table>
<thead>
<tr>
<th>Type of Experiments</th>
<th>No. of Specimens</th>
<th>Norepinephrine γ/cc (average) (SDm ±)</th>
<th>Epinephrine γ/cc (average) (SDm ±)</th>
<th>Norepinephrine plus Epinephrine γ/cc (average) (SDm ±)</th>
<th>Total Catechols (Colorimetric) color units/Gm. (average) (SDm ±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>11</td>
<td>0.039 (±0.0075)</td>
<td>0.021 (±0.0039)</td>
<td>0.060 (±0.0084)</td>
<td>573 (±123)</td>
</tr>
<tr>
<td>Electrical stimulation of crural nerves</td>
<td>6</td>
<td>0.034 (±0.007)</td>
<td>0.030 (±0.009)</td>
<td>0.064 (±0.010)</td>
<td>766 (±51)</td>
</tr>
<tr>
<td>Norepinephrine (i.p.) 10 mg./Kg.</td>
<td>10</td>
<td>0.025 (±0.0067)</td>
<td>0.021 (±0.0065)</td>
<td>0.046 (±0.010)</td>
<td>1154 (±158)</td>
</tr>
<tr>
<td>Epinephrine (i.p.) 10 mg./Kg.</td>
<td>7</td>
<td>0.033 (±0.012)</td>
<td>0.048 (±0.020)</td>
<td>0.081 (±0.028)</td>
<td>1521 (±307)</td>
</tr>
<tr>
<td>Stimulation of crural nerves plus Norepinephrine (i.p.) 10 mg./Kg.</td>
<td>5</td>
<td>0.016 (±0.0059)</td>
<td>0.007 (±0.0036)</td>
<td>0.023 (±0.005)</td>
<td>1128 (±135)</td>
</tr>
<tr>
<td>Stimulation of crural nerves plus Epinephrine (i.p.) 10 mg./Kg.</td>
<td>5</td>
<td>0.015 (±0.0061)</td>
<td>0.033 (±0.0062)</td>
<td>0.047 (±0.007)</td>
<td>1290 (±106)</td>
</tr>
</tbody>
</table>

* These figures differ significantly at the 1 per cent probability level (t-test, Fisher) from the corresponding mean figures of the control group. (For the per cent relation between norepinephrine and epinephrine see fig. 1).

injection of epinephrine and norepinephrine. Correlation of the latter values with those obtained in the non-flushed hearts (table 1) shows that the norepinephrine plus epinephrine of the coronary blood constitutes only 0.4 to 3 per cent of the norepinephrine plus epinephrine values, obtained from the non-flushed myocardium. The corresponding percentages for the chromogenic total catechol readings range from 0.6 to 2 per cent. Thus, the coronary blood catechols are not a significant source of error and can be safely disregarded.

Both norepinephrine and total catechols were present in smaller amounts in the striated muscles of the thigh than in the heart (table 5). Epinephrine constituted a larger average fraction. Neither rhythmic electric stimulation of the supplying somatic nerves nor injection of norepinephrine and epinephrine nor a combination of these injections with rhythmic stimulation produced a major change of the active catecholamines, even though there seemed to be a slight tendency of injected epinephrine to accumulate in the muscles. The amounts of inactive chromogenic catecholamines were significantly augmented after all injections, suggesting a rapid enzymatic inactivation of the injected catecholamines, however with preservation of their adsorbable chromogenic catechol nucleus.

DISCUSSION

Our results confirm other observations and our own in various animal species and
in human hearts: norepinephrine forms the bulk of the pharmacodynamically active catecholamines in the normal mammalian heart. Beside norepinephrine and epinephrine, the heart muscle contains considerable quantities of chromogenic catechols with negligible pharmacodynamic activity (e.g., hydroxytyramine). This is also manifested by the large discrepancy between our colorimetric and bioassay readings. Cardiac sympathetic stimulation liberates norepinephrine at the post-ganglionic nerve terminals and thus accelerates the heart rate and increases myocardial oxygen consumption.

In striking contrast to the skeletal muscle, the myocardium displays an amazing avidity to absorb and store in an active form large quantities of injected norepinephrine and, even more so, of epinephrine. This appeared at a maximum when both were combined. The simultaneous augmentation of myocardial norepinephrine which was observed after the injection of epinephrine, could possibly be attributed to agonal discharges of norepinephrine from the postganglionic sympathetic nerve endings into the myocardium. However, in view of the inhibitory action of epinephrine on the sympathetic ganglionic synapses, this explanation appears unlikely. An enzymatic demethylation of excess epinephrine within the heart muscle is another possibility. That the large accumulations of epinephrine in the myocardium did not prove fatal in all instances, may be ascribed to the fact that epinephrine toxicity on the heart has an upper limit beyond which no further augmentation of toxic action on the myocardial cell surfaces seems to be possible.

The relatively good agreement between the colorimetric and the bioassay values after injection of norepinephrine and epinephrine suggests that norepinephrine and epinephrine are not materially inactivated during the extraction for bioassay.

The failure of some of the most potent sympatholytic drugs (benzodioxane, Dibenamine, Regitine) to diminish the quantity and pharmacodynamic effectiveness of the extractable active cardiac catecholamines, both under standard conditions and during sympathetic nerve stimulation, parallels the comparative ineffectiveness of these drugs as adrenergic blocking agents on the heart. It also suggests that the catecholamines exert a competitive rather than a destructive or membrane permeability-altering action. The augmentation of cardiac norepinephrine by Dibenamine and Regitine may serve as an explanation of the cardiac acceleration which is often elicited by these drugs; intramyocardially liberated norepinephrine produces a positive chronotropic effect, in contrast to parenterally administered, circulating norepinephrine which sets overwhelming inhibitory vagal counter-reflexes into motion via the peripheral vascular pressoreceptors. The somewhat diminished discrepancy between colorimetric and bioassay readings after administration of the sympatholytic drugs suggests that they promote an activation or re-activation of some of the pre-formed inactive cardiac catechols.

The low norepinephrine and total catechol contents of the striated muscle, the failure of its active catecholamine concentration to rise after catecholamine injections or nerve stimulation and the apparent ability of the skeletal muscle to rapidly inactivate absorbed injected catecholamines, set the skeletal muscle distinctly apart from heart muscle as far as catecholamine action and metabolism are concerned. These differences are believed to account for the comparatively minimal dynamic response of the skeletal muscle to injected or secreted catecholamines, and also to account for the absence of catecholamine-induced degenerative changes in the skeletal musculature, as contrasted with those which are so frequently encountered in the catecholamine-rich myocardium.

A variety of pathophysiologic observations concerning the potentially hypoxiating activity of myocardial catecholamines can be interpreted on the basis of the above findings. We are specifically referring to (1) the acute, painful myocardial hypoxia under conditions associated with adreno-sympathogenic stimulation (angina pectoris); (2) the cardiotoxic accumulation of myocardial catecholamines in severe renal insufficiency which is due to abnormally high catechol levels in the blood; (3) the "high cardiac output" congestive failure of the abnormally catecholamine-rich
beri-beri heart\(^1,\,18\); (4) the therapeutically beneficial diminution of the myocardial catecholamines following sympathectomy\(^8,\,19\) and (5) the relative therapeutic inefficacy of sympatholytic medication in such cardiac disorders as the tachycardias and angina pectoris.\(^1,\,11,\,14\)

**SUMMARY**

Heart muscle of the dog (like that of other mammalian species) contains norepinephrine and epinephrine in the approximate proportion of 5:1 and relatively large quantities of pharmacodynamically inactive catechol compounds.

Stimulation of the cardiac sympathetic nerves augments myocardial norepinephrine without altering the epinephrine concentration.

Injected, circulating norepinephrine, and even more so, epinephrine, are eagerly absorbed by the heart muscle and stored in an active form in large quantities.

Sympatholytic drugs (benzodioxane, Dibenamine, Regitine) do not diminish the amounts and pharmacodynamic effectiveness of myocardial norepinephrine and epinephrine. Dibenamine and Regitine even cause an increase of myocardial norepinephrine.

Skeletal muscle differs strikingly from heart muscle: It contains less norepinephrine and total catechols. Its catecholamine concentration remains practically unchanged by somatic nerve stimulation, by injections of norepinephrine or epinephrine and by combinations of these. It seems to inactivate its excess catecholamine deposits rapidly.

The significance of some of the enumerated findings in understanding the role of adreno-sympathetic catecholamines in the origin of certain clinical cardiac disorders is briefly mentioned.

**REFERENCES**

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