Studies on the Metabolism of Catecholamines

By JULIUS AXELROD, PH.D., L. GORDON WHITBY, PH.D., GEORG HERTTING, M.D., AND IRWIN L. KOPIN, M.D.

CATECHOLAMINE HORMONES play an important role in the actions of the heart, yet the physiological disposition and metabolism of these compounds in this organ are poorly understood. Until recently there was relatively little information concerning the fate of these hormones after release from sympathetic nerve endings and the adrenal medulla or when given intravenously. Reports on the uptake of circulating epinephrine and norepinephrine by cardiac muscle have been contradictory. Raab and Gigele observed that heart muscle and other vascular tissues accumulated catecholamines selectively when large amounts of these compounds were administered. On the other hand, von Euler observed no significant elevation of catecholamine hormones in the heart after administration of large or small doses. For many years catecholamines were considered to be inactivated in the heart by monoamine oxidase. After the introduction of monoamine oxidase inhibitors, this enzyme was found to be relatively unimportant in the metabolism and inactivation of epinephrine and norepinephrine. With the recent isolation and characterization of the major metabolic products of epinephrine and norepinephrine as 3-methoxy-4-hydroxymandelic acid, metanephrine, normetanephrine, and 3-methoxy-4-hydroxyphenylglycol, it became apparent that O-methylation is an important pathway for the metabolism of catecholamine hormones. Studies with radioactive epinephrine and norepinephrine permit the following conclusion regarding the metabolism of these compounds in man. About 70 per cent of intravenously administered epinephrine is O-methylated to metanephrine. Part of the latter compound (about 25 per cent) is deaminated and oxidized to 3-methoxy-4-hydroxymandelic acid (20 per cent) or reduced to 3-methoxy-4-hydroxyphenylglycol (5 per cent). The remaining metanephrine is excreted unchanged or as sulfate and/or glucuronic acid conjugates. Approximately 20 per cent of the administered epinephrine is deaminated to 3, 4-dihydroxymandelic acid, most of which is then O-methylated to 3-methoxy-4-hydroxymandelic acid. A new metabolic product, 3, 4-dihydroxyphenylglycol, appears when catechol-O-methyl transferase is inhibited. Norepinephrine is metabolized in an analogous manner. Figure 1 shows the pathway for the metabolism of the catecholamines.

An enzyme, catechol-O-methyl transferase, that O-methylates catecholamines and other catechols has been described. S-adenosylmethionine serves as the methyl donor for this reaction. Catechol-O-methyl transferase was found in all tissues examined, including heart, muscle, and blood vessels. Unlike monoamine oxidase, the O-methylating enzyme when inhibited in vivo with pyrogallol blocks the metabolism of epinephrine and norepineph-
Table 1

Distribution of H³-catecholamines and Their O-Methylated Metabolites Two Minutes after the Intravenous Injection of H³-catecholamines

<table>
<thead>
<tr>
<th>Tissue</th>
<th>H³-epinephrine mg./Gm.</th>
<th>H³-norepinephrine mg./Gm.</th>
<th>H³-metanephrine mg./Gm.</th>
<th>H³-normetanephrine mg./Gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>40</td>
<td>44</td>
<td>35</td>
<td>5</td>
</tr>
<tr>
<td>Heart</td>
<td>150</td>
<td>383</td>
<td>156</td>
<td>44</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>4</td>
<td>32</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Spleen</td>
<td>190</td>
<td>148</td>
<td>229</td>
<td>37</td>
</tr>
<tr>
<td>Lung</td>
<td>14</td>
<td>122</td>
<td>126</td>
<td>124</td>
</tr>
<tr>
<td>Liver</td>
<td>39</td>
<td>432</td>
<td>48</td>
<td>65</td>
</tr>
<tr>
<td>Kidney</td>
<td>49</td>
<td>306</td>
<td>83</td>
<td>39</td>
</tr>
<tr>
<td>Small intestine</td>
<td>20</td>
<td>60</td>
<td>37</td>
<td>11</td>
</tr>
<tr>
<td>Brain</td>
<td>1.1</td>
<td>3.3</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>192</td>
<td>89</td>
<td>150</td>
<td>16</td>
</tr>
</tbody>
</table>

Cats, anesthetized with pentobarbital, were given 75 μg. (free base) H³-epinephrine/Kg. or 25 μg. (free base) H³-norepinephrine/Kg. I.V. within one minute. Two minutes after the injection the animals were killed and the tissues assayed for H³-catecholamines and their O-methylated metabolites.

Physiological Disposition of H³-catecholamines in Heart and Other Tissues

The availability of tritium-labeled epinephrine and norepinephrine of high specific activity made possible a study of the distribution and metabolism of the circulating amines under physiological conditions. H³-catecholamines and their metabolic products were separated by techniques involving column chromatography and solvent extraction, and the amount of radioactivity in the isolated products was determined by liquid scintillation spectrometry.

Cats were anesthetized with pentobarbital and the trachea was cannulated. H³-epinephrine (70 μg/Kg.) and H³-norepinephrine (25 μg/Kg.) were administered by a rapid intravenous injection to simulate a sudden discharge from the adrenal medulla and nerve endings. The animals were killed two minutes after the end of the injection, and tissues were removed immediately and assayed for unchanged H³-epinephrine and H³-norepinephrine and their major metabolic products, metanephrine and normetanephrine (table 1).

Both H³-catecholamines were found to be unevenly distributed in the various tissues. Within two minutes, heart, spleen, lungs, and adrenal glands selectively take up large amounts of circulating H³-epinephrine and H³-norepinephrine. Although the cats received three times as much H³-epinephrine as H³-norepinephrine, the concentration of both catecholamines in the heart and most tissues was the same. Within two minutes after the administration of the H³-catecholamines, the O-methylated metabolites metanephrine and normetanephrine were present in all tissues examined. Particularly noteworthy is the large amount of metanephrine found in the heart. The tissue concentration of metanephrine was higher than its parent compound, while the reverse was true for normetanephrine. The rapid appearance of large amounts of the O-methylated metabolites in the heart and other organs during the period after the physiological effects of the catecholamines had subsided indicates that catechol-O-methyl transferase is mainly responsible for the inactivation of these hormones. The large amounts of circulating catecholamines taken up by the heart would suggest that this organ selectively takes up these hormones as they are liberated from the adrenal medulla and the sympathetic nervous system.

Two hours after the administration of the catecholamine hormones, large amounts of these compounds were still present in heart, spleen, and adrenal gland. Larger amounts of norepinephrine were present in most tissues after two hours than after two minutes.

The large amounts of catecholamines retained in the heart and other tissues suggest that the hormones are bound to some constituent and protected from enzymatic attack. Since the bound catecholamines are physiologically inactive, binding as well as enzymatic O-methylation could be considered an important mechanism for the inactivation of the catecholamine hormones. Much larger amounts of norepinephrine than of epinephrine appear to be bound.
Effect of Enzyme Inhibitors on Metabolism and Disposition of Catecholamines

The role of monoamine oxidase and catechol-O-methyl transferase in the metabolism and inactivation of the catecholamine hormones was studied by the use of inhibitors for these enzymes. Mice were given tritium-labeled epinephrine and norepinephrine after the administration of iproniazid, a monoamine oxidase inhibitor, or pyrogallol, a catechol-O-methyl transferase inhibitor. Ten minutes later the mice were killed and assayed for the H\textsuperscript{3}-catecholamine remaining in the whole animal. In the untreated mice about 35 per cent of the H\textsuperscript{3}-epinephrine and 50 per cent of the H\textsuperscript{3}-norepinephrine were found in the whole animal. Treatment with the monoamine oxidase inhibitor did not affect the rates of metabolism of H\textsuperscript{3}-epinephrine or H\textsuperscript{3}-norepinephrine. However, treatment with a catechol-O-methyl transferase inhibitor dramatically slowed the metabolism of both catecholamines.

Pyrogallol and iproniazid markedly affected the excretion of the catecholamines and their metabolic products. Pretreatment with pyrogallol increased the excretion of catechols (unchanged catecholamines, 3, 4-dihydroxymandelic acid, and 3, 4-dihydroxyphenylglycol) and reduced the excretion of the O-methylated products (metanephrine, normetanephrine, 3-methoxy-4-hydroxymandelic acid, and 3-methoxy-4-hydroxyphenylglycol). Iproniazid pre-

Table 2

<table>
<thead>
<tr>
<th>Effect of Enzyme Inhibitors on the Metabolism of H\textsuperscript{3}-Norepinephrine in Cardiac and Skeletal Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{mg}./\text{Gm.})</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Heart</td>
</tr>
<tr>
<td>Skeletal muscle</td>
</tr>
</tbody>
</table>

Thirteen cats were given \(\mu\text{g.}\)/Kg. H\textsuperscript{3}-norepinephrine I.V., killed one hour later and tissues were examined for H\textsuperscript{3}-normetanephrine. Three cats were given 15 mg./Kg. JB 516 I.V. (a monoamide oxidase inhibitor) three, two, and one days before norepinephrine; and three cats received 50 mg./Kg. pyrogallol I.V. (a catechol-O-methyl transferase inhibitor) 3 minutes before the catecholamine injection and a second dose 30 minutes later.

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Effect of Drugs on the Uptake of $H^3$-norepinephrine by Heart and Skeletal Muscle

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Heart $H^3$-norepinephrine mg./gm.</th>
<th>Skeletal muscle $H^3$-catecholamines mg./gm.</th>
<th>Plasma $H^3$-norepinephrine mg./gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>165</td>
<td>3.6</td>
<td>49</td>
</tr>
<tr>
<td>Reserpine</td>
<td>5</td>
<td>3.3</td>
<td>109</td>
</tr>
<tr>
<td>Chlorpromazino</td>
<td>35</td>
<td>4.0</td>
<td>104</td>
</tr>
<tr>
<td>Imipramine</td>
<td>14</td>
<td>2.8</td>
<td>88</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>12</td>
<td>3.8</td>
<td>100</td>
</tr>
<tr>
<td>Cocaine</td>
<td>29</td>
<td>3.0</td>
<td>90</td>
</tr>
<tr>
<td>Phenoxybenzamine</td>
<td>74</td>
<td>4.5</td>
<td>134</td>
</tr>
</tbody>
</table>

All cats received 25 $\mu$g./Kg. $H^3$-norepinephrine I.V. and were killed one hour later. Blood samples were taken two minutes after the injection of the catecholamines. Details of drug treatment are described in the text.

One can conclude from these observations that catechol-O-methyl transferase is the principle enzyme for the metabolism of circulating catecholamines and that monoamine oxidase is mainly involved in the deamination of the O-methylated amines. Since pyrogallol also prolongs the responses of sympathetic nerve stimulation, while iproniazid does not, it is highly likely that O-methyl transferase is also concerned with the inactivation of locally released catecholamine hormones.

Effect of Drugs on the Cardiac Uptake of $H^3$-catecholamines

Many drugs affect the action of the heart but little is known about their actions at a biochemical level. Since some of these drugs act by affecting catecholamines, we examined the concentration of administered $H^3$-norepinephrine in heart, skeletal muscle, and plasma after pretreatment with reserpine, chlorpromazino, imipramine, amphetamine, cocaine, and phenoxybenzamine (Dibenzyline) (table 3). Drugs were given before the intravenous administration of 25 $\mu$g./Kg. $H^3$-norepinephrine to cats as follows: reserpine 3 mg./Kg. I.P., 24 and 1 hour; chlorpromazine 20 mg./Kg. I.P., 24 and 1 hour; 5 mg./Kg. I.V., 20 minutes; amphetamine 10 mg./Kg. I.V., 10 minutes; cocaine 5 mg./Kg. I.V., 10 minutes; phenoxybenzamine 20 mg./Kg. I.V., 90 minutes. Each drug was given to three cats; seven untreated cats served as controls. Blood samples were taken two minutes after the administration of the $H^3$-catecholamines and the animals killed one hour after. All drugs examined elevated plasma levels of $H^3$-norepinephrine and markedly reduced the concentration of the $H^3$-catecholamine in the heart but had little or no effect on the skeletal muscle.

The increased plasma levels and reduced heart concentration of $H^3$-norepinephrine suggest that these drugs interfere with binding mechanisms. Since these drugs have different actions on the heart, they might influence binding by releasing catecholamines or preventing uptake or both. Although acting by different mechanisms, these drugs would produce the same end result, elevated plasma levels and reduced concentrations of the hormone in cardiac muscle.

We have also found that reserpine, chlorpromazine, imipramine, and sympathomimetic amines markedly speed the disappearance of $H^3$-catecholamines from the whole animal. These drugs presumably prevent the binding of catecholamines, thus exposing the hormones...
to enzymatic attack and more rapid metabolic degradation.

Summary

Administered H\textsubscript{3}-epinephrine and H\textsubscript{3}-norepinephrine are rapidly and selectively taken up by heart muscle and retained for many hours. The uptake and retention of circulating H\textsubscript{3}-norepinephrine by the heart is greater than that of H\textsubscript{3}-epinephrine. Two minutes after the administration of H\textsubscript{3}-catecholamines large amounts of metanephrine and normetanephrine are found in the heart indicating rapid O-methylation of these hormones during the period of their inactivation. H\textsubscript{3}-epinephrine is more readily O-methylated than H\textsubscript{3}-norepinephrine.

Enzyme inhibition studies indicate that catechol-O-methyl transferase is the enzyme mainly concerned with the metabolism of catecholamines in the heart.

Reserpine, chlorpromazine, imipramine, amphetamine, cocaine, and phenoxybenzamine markedly inhibit the uptake of circulating H\textsubscript{3}-norepinephrine by the heart.

References

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