Catecholamine Metabolism in Experimental Hypertension in the Rat

By Jacques de Champlain, M.D., Ph.D., Lawrence R. Krakoff, M.D., and Julius Axelrod, Ph.D.

ABSTRACT

The role of catecholamines was investigated in uninephrectomized Sprague-Dawley male rats made hypertensive by administration of desoxycorticosterone and a high salt diet. The accumulation of tritiated norepinephrine and the endogenous norepinephrine levels were measured in the various organs. The content of endogenous norepinephrine and the accumulation of $^3$H-norepinephrine 1 hour after its injection were reduced in the heart, spleen, intestine, skeletal muscle, and kidney of hypertensive rats as compared to controls. Simultaneously, an increase in total $^3$H-metabolites was also found in the hypertensive animals.

A highly significant inverse relation was established between the level of blood pressure and the accumulation of $^3$H-norepinephrine or endogenous norepinephrine levels in the heart.

The abnormalities observed in the disposition of exogenously administered and endogenous norepinephrine do not appear to be secondary to a change in the distribution of cardiac output to the various organs. Since the initial uptake was normal in the hypertensive rats, the reduced capacity to accumulate norepinephrine seems to be related to a defect in the storage of norepinephrine.

ADDITIONAL KEY WORDS

DOCA and salt hypertension
fractional blood flow
uptake and binding of $^3$H-norepinephrine
endogenous norepinephrine
isolated perfused heart
uninephrectomized rats

Many factors, including norepinephrine, have been implicated in the pathogenesis of human and experimental hypertension (1). Previous studies on the significance of the urinary excretion of catecholamines and their metabolites in hypertensive diseases have been negative or contradictory (2-13). However, such studies may be misleading since they reflect a summation of events occurring in the entire body. Therefore, a defect which can occur locally may be obscured by measuring total excretion of catecholamines and their metabolites.

The observation that the blood pressure of hypertensive humans and animals is often lowered by the administration of drugs that alter the physiological disposition of norepinephrine, indicates the importance of this neurotransmitter and of the sympathetic nervous system in the maintenance of high blood pressure. In addition, the increased vascular reactivity to infused norepinephrine observed in some forms of human (14) and experimental (15) hypertension suggests that in these conditions, there is either an impaired inactivation of the amine or an increased sensitivity of the effector cells.

A major mechanism for the inactivation of circulating norepinephrine involves the uptake of the catecholamine across the sympathetic neuronal membrane and its subsequent binding in intraneuronal storage granules (16). It has been postulated that drugs or conditions that prevent the uptake or binding of norepinephrine would allow an increased amount of free catecholamine to be in the vicinity of the receptor and thus would explain the observed supersensitivity (17). This process of
inactivation, which depends upon uptake and binding of norepinephrine by the nerve has not been directly approached in the study of hypertensive diseases. On the basis of indirect evidence, however, Gitlow and co-workers (18) have recently suggested an abnormality in the storage of norepinephrine in essential hypertension.

The capacity to inactivate norepinephrine can be estimated by measuring the uptake and binding of norepinephrine by various tissues after injection of the radioactive amine. This approach was used in rats made hypertensive by the combined administration of desoxycorticosterone and sodium chloride. The present study describes a reduced accumulation of tritiated norepinephrine as well as lower endogenous norepinephrine levels in the various tissues of hypertensive animals. A preliminary report of these findings has been published elsewhere (19).

Methods

Male Sprague-Dawley rats weighing 200 to 250 g underwent a right nephrectomy and were divided into four groups as follows: one group, which became hypertensive, received daily subcutaneous injections of 1 mg of desoxycorticosterone acetate (DOCA) in oil and was given 1% saline (NaCl) in tap water ad libitum. Two other groups received either 1 mg DOCA daily or 1% saline. A fourth group, the control animals, were injected subcutaneously with sesame oil and were given tap water to drink. The systolic blood pressure had been directly determined simultaneously by carotid artery cannulation and blood pressure was measured weekly in prewarmed animals by tail pulse transducer. The appearance of unchanged \(^{3}\text{H}\)-norepinephrine into the tail vein of unanesthetized rats; they were killed 1 hour later in most experiments. Heart, spleen, upper intestine, kidney, skeletal muscle, liver and salivary glands were quickly removed, chilled on crushed ice, and analyzed for radioactivity in the storage of norepinephrine in essential hypertension.

The fractional cardiac output to the various organs was measured by the distribution of \(^{40}\text{K}\) (26). One hour after the injection of \(^{3}\text{H}\)-norepinephrine and 15 seconds before killing, 0.1 mg of \(^{40}\text{K}\) (182 mcg, Oak-Ridge River Laboratory) was injected in the tail vein. The rats were killed by a blow on the head and the hearts were removed within 5 seconds. The radioactivity in the hearts and other organs was measured in a well scintillation counter for 1 min and the appropriate corrections for \(^{40}\text{K}\) decay were made.
Results

1. Changes in Blood Pressure, Body and Organ Weights. The average systolic blood pressure for each group of animals is shown in Figure 1. The blood pressure of the control group showed little change throughout the 6 weeks of treatment. In animals treated with DOCA and NaCl, there was a significant increase in blood pressure as early as 1 week after the beginning of treatment ($P < 0.5$); the blood pressure continued to rise progressively during the following weeks, to reach a mean level of 184 mm Hg at the end of 6 weeks. In rats receiving either DOCA alone or drinking 1% saline, a slight increase in blood pressure occurred during the course of treatment.

The four groups of rats had a similar growth rate for the first 3 or 4 weeks of treatment, but thereafter the body weight of the hypertensive rats increased more slowly than that of the other three groups (Table 1), and at the end of treatment was significantly lower than that of the other rats.

There was no difference in mortality rate (20%) between the control and DOCA salt-treated groups during the 6 weeks of treatment. Observations made on any animals that died before completion of the study were not included in average systolic blood pressure or body weight given in Figure 1 and Table 1.

The hearts of hypertensive animals showed a slight to moderate increase in weight (Table 2). The mean heart weight was 21% greater in hypertensive rats; however, if expressed as fraction of body weight, it was 37% greater. No sign of congestive heart failure was observed in the hypertensive animals, as evaluated by the absence of hepatic or pulmonary congestion or effusion. The remaining left kidney was significantly enlarged in the rats treated with DOCA alone and further enlarged in the rats receiving both DOCA and NaCl (Table 2). A renal enlargement after desoxycorticosterone treatment has also been observed by others (27, 28). No significant differences were found in the spleen or adrenal weights among the groups of animals.

2. Accumulation and Metabolism of $^1$H-norepinephrine and Endogenous Levels in the

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Changes in Body Weight of Rats Treated with DOCA, Saline, or Both</strong></td>
</tr>
<tr>
<td><strong>No. rats</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>DOCA + NaCl</td>
</tr>
<tr>
<td>NaCl</td>
</tr>
<tr>
<td>DOCA</td>
</tr>
</tbody>
</table>

Results are expressed as mean in grams ± SEM.

* $P < .001$ vs. control.

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### Table 2

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. rats</th>
<th>Heart</th>
<th>Left kidney</th>
<th>Spleen</th>
<th>Adrenal glands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18</td>
<td>1079 ± 23</td>
<td>1471 ± 30</td>
<td>1009 ± 41</td>
<td>79 ± 4.0</td>
</tr>
<tr>
<td>DOCA + NaCl</td>
<td>18</td>
<td>1308 ± 38*</td>
<td>2039 ± 40†</td>
<td>911 ± 49</td>
<td>77 ± 4.0</td>
</tr>
<tr>
<td>DOCA</td>
<td>10</td>
<td>1087 ± 36</td>
<td>1884 ± 116*</td>
<td>959 ± 59</td>
<td>79 ± 4.5</td>
</tr>
<tr>
<td>NaCl</td>
<td>11</td>
<td>1106 ± 65</td>
<td>1564 ± 48</td>
<td>938 ± 88</td>
<td>86 ± 4.0</td>
</tr>
</tbody>
</table>

Results are expressed as mean in milligrams ± SEM.

*P < .01; †P < .001 vs. control.

### Figure 2

The accumulation and metabolism of H-norepinephrine in the hearts of rats receiving DOCA, saline, or both. H-norepinephrine and its metabolites were measured 1 hour after administration of the tritiated amine. From 10 to 18 rats were used in each group, and the results are expressed as the mean counts per minute ± SEM (vertical bars). ** = P < .01; *** = P < .001.

### Figure 3

Correlation between systolic blood pressure and the accumulation of H-norepinephrine in the heart of control and hypertensive rats. The systolic blood pressure for each value is the mean of two separate measurements done 3 and 1 days before the killing. The accumulation of H-norepinephrine is reported in counts per minute per heart.

The concentration and content of endogenous norepinephrine was also reduced in the hearts of the hypertensive rats (Fig. 4). The groups of rats receiving either NaCl or DOCA alone had endogenous norepinephrine levels which did not differ significantly from the control animals. A highly significant inverse relation was also observed between the level of blood pressure and the endogenous norepinephrine levels in the hearts from control and hypertensive rats (Fig. 5). Although inverse correlations were also found between heart weight and the accumulation of tritiated norepinephrine or endogenous norepinephrine levels, the degrees of correlation were less marked (r = -0.55 for H-norepinephrine; r = -0.34 for endogenous norepinephrine) than those shown in Figures 3 and 4.
1.2

Endogenous norepinephrine levels in the hearts of rats treated with DOCA, saline, or both. Groups containing 10 to 18 animals were used and the results are expressed as the mean in microgram ± SEM (vertical bars).

5 with respect to blood pressure (r = −.76 and r = −.66 respectively).

3. Accumulation and Metabolism of \(^{3}\)H-norepinephrine and Endogenous Levels in Various Organs. The concentration of \(^{3}\)H-norepinephrine and its metabolites and of endogenous norepinephrine in the various organs are summarized in Table 3. In the hypertensive rats, there was a significant reduction in the concentration of \(^{3}\)H-norepinephrine in the spleen, kidney, and intestine. There was no significant difference between the total content of \(^{3}\)H-norepinephrine in the kidney of hypertensive animals and that in control animals, indicating that the changes in concentration of the catecholamine in this organ were due to the difference in weight. With the exception of the salivary glands, in which there was a significant increase in the accumulation of \(^{3}\)H-norepinephrine in animals receiving DOCA alone, no significant changes could be demonstrated in the organs of rats receiving either DOCA or NaCl. The total tritiated metabolites were significantly increased in the intestine, and skeletal muscle of the hypertensive rats.

The endogenous norepinephrine levels in the various organs of hypertensive rats followed a pattern similar to that observed with the tritiated norepinephrine (Table 3). The concentration of endogenous norepinephrine was significantly reduced in the spleen, in the kidney, and in the intestine. A slight reduction was also observed in the skeletal muscle, but no difference could be found in the salivary glands. The total content of norepinephrine in the spleen and the kidney of the hypertensive rats was also significantly reduced, regardless of weight variations in the latter organ. The concentration of endogenous norepinephrine was increased in the intestine and salivary glands of rats treated with DOCA alone.

The close parallelism between the capacity to accumulate \(^{3}\)H-norepinephrine and the endogenous norepinephrine concentration in hypertensive rats is shown in Figure 6. Both tritiated and endogenous norepinephrine were reduced to about the same degree in the heart, intestine, skeletal muscle, spleen, and kidney of hypertensive rats, while in the salivary glands these values were both close to those of the controls.

4. Fractional Cardiac Output. Since it has been reported that the accumulation of \(^{3}\)H-norepinephrine in various organs may vary
TABLE 3

Concentration of $^3$H-Norepinephrine, $^3$H-Metabolites and Endogenous Norepinephrine in Various Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>DOCA + NaCl</th>
<th>DOCA</th>
<th>NaCl</th>
<th>DOCA + NaCl</th>
<th>DOCA</th>
<th>NaCl</th>
<th>DOCA + NaCl</th>
<th>DOCA</th>
<th>NaCl</th>
<th>DOCA + NaCl</th>
<th>DOCA</th>
<th>NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>15</td>
<td>17</td>
<td>8</td>
<td>10</td>
<td>15</td>
<td>17</td>
<td>8</td>
<td>10</td>
<td>15</td>
<td>8</td>
<td>10</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>$^3$H-norepinephrine ng/g</td>
<td>1.72 ± 0.18</td>
<td>1.37 ± 0.09*</td>
<td>1.90 ± 0.12</td>
<td>1.72 ± 0.20</td>
<td>1.88 ± 0.13</td>
<td>1.72 ± 0.13</td>
<td>0.91 ± 0.08</td>
<td>0.95 ± 0.03</td>
<td>1.1 ± 0.10</td>
<td>0.43 ± 0.06</td>
<td>0.38 ± 0.03</td>
<td>0.45 ± 0.06</td>
<td>1.68 ± 0.13</td>
</tr>
<tr>
<td>$^3$H-metabolites CPM × 10$^4$/g</td>
<td>5.6 ± 0.4</td>
<td>5.3 ± 0.5</td>
<td>6.8 ± 0.8</td>
<td>7.3 ± 1.2</td>
<td>10.1 ± 0.8</td>
<td>9.5 ± 1.3</td>
<td>1.0 ± 0.8</td>
<td>1.0 ± 0.8</td>
<td>11.4 ± 2.8</td>
<td>15.4 ± 1.0*</td>
<td>11.4 ± 2.8</td>
<td>9.5 ± 1.3</td>
<td>10.1 ± 0.8</td>
</tr>
<tr>
<td>Endogenous norepinephrine ng/g</td>
<td>790 ± 482</td>
<td>482 ± 46†</td>
<td>607 ± 105</td>
<td>691 ± 99</td>
<td>484 ± 35</td>
<td>301 ± 33†</td>
<td>164 ± 14</td>
<td>88 ± 8†</td>
<td>130 ± 15</td>
<td>170 ± 11</td>
<td>1,190 ± 78</td>
<td>1,250 ± 78</td>
<td>1,300 ± 120</td>
</tr>
</tbody>
</table>

*P < .05; †P < .01; ‡P < .001 vs. control.

with changes in the fractional distribution of cardiac output (29), such a study was made by measuring the disposition of $^4$K in the various tissues. No significant difference in the fractional distribution of $^4$K could be demonstrated in hypertensive and control animals in any of the organs studied (Fig. 7).

5. Initial Uptake of $^4$H-norepinephrine in Isolated Hearts and in Vivo. Hearts from control and hypertensive rats were perfused with $^3$H-norepinephrine by the Langendorff technique at a constant flow for a period of 10

levels (END. NE) in various tissues of hypertensive rats. The results are expressed as the percentage of the values found in the control (untreated) group. The accumulation of $^3$H-norepinephrine was measured 1 hour after an intravenous injection.

FIGURE 6

Relationship between the accumulation of $^3$H-norepinephrine (H$^3$NE) and endogenous norepinephrine

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min. Despite significant differences in the blood pressure and heart weight of both groups of rats, no reduction could be demonstrated in the initial uptake of \(^3\)H-norepinephrine in the hearts from hypertensive rats (Table 4). The amount of \(^3\)H-norepinephrine, the deaminated catechol and the O-methylated deaminated metabolites in the heart did not differ in the two groups, but the normetanephrine was slightly reduced in the hearts from hypertensive animals. There was no difference in the metabolites found in the perfusate outflow from the two groups of hearts (Table 4).

In order to study the early accumulation of \(^3\)H-norepinephrine in vivo, rats were killed 5 min after intravenous injection of 25 \(\mu\)g of \(^3\)H (S.A. 162 mc/g). The numbers in parenthesis indicate the number of animals studied in each group.

**TABLE 4**

<table>
<thead>
<tr>
<th>Uptake of (^3)H-Norepinephrine in Isolated Perfused Hearts of Control and Hypertensive Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Mean syst. B.P. (mm Hg)</td>
</tr>
<tr>
<td>Mean heart weight (mg)</td>
</tr>
<tr>
<td><strong>Total Uptake from the Perfusing Media</strong></td>
</tr>
<tr>
<td>Uptake for 10 min (ng (^3)H-NE)</td>
</tr>
<tr>
<td>Uptake as % of perfused amount</td>
</tr>
<tr>
<td><strong>(^3)H-Norepinephrine and Metabolites in Heart (% of Uptake)</strong></td>
</tr>
<tr>
<td>Unchanged (^3)H-norepinephrine</td>
</tr>
<tr>
<td>Normetanephrine</td>
</tr>
<tr>
<td>Deaminated catechol</td>
</tr>
<tr>
<td>O-methylated deaminated</td>
</tr>
<tr>
<td><strong>(^3)H-Metabolites in Perfusate (% of Uptake)</strong></td>
</tr>
<tr>
<td>Normetanephrine</td>
</tr>
<tr>
<td>Deaminated catechol</td>
</tr>
<tr>
<td>O-methylated deaminated</td>
</tr>
<tr>
<td>Total % in heart &amp; perfusate</td>
</tr>
</tbody>
</table>

\*\(P < .05; †P < .01; ‡P < .001\). Isolated hearts were perfused at 37°C with a medium containing 4.7 ng/ml of \(^3\)H-norepinephrine (S.A. 5 c/mmole) for 10 min at the rate of 3.7 ml/min. At the end of the perfusion the tissues and perfusates were analyzed for unchanged \(^3\)H-norepinephrine and metabolites. Deaminated catechol represents 3,4 dihydroxymandelic acid and the corresponding glycol. O-methylated deaminated metabolites represent 3 methoxy-4-hydroxymandelic acid and the corresponding glycol. The values are the mean ± SEM of 6 control and 6 rats treated with DOCA and NaCl.
findings demonstrate a decrease in the endogenous level of this amine in heart and several tissues. In addition, a highly significant inverse relation was observed between the accumulation of $^3$H-norepinephrine and endogenous norepinephrine and the level of blood pressure.

The decreased accumulation of $^3$H-norepinephrine may be the result of several factors. Changes in the tissue perfusion might have altered the delivery of $^3$H-norepinephrine to certain organs of the hypertensive rats (29). However, the fractional distribution of cardiac output as measured by the $^{42}$K technique showed no difference between the control and hypertensive rats. Furthermore, the initial accumulation of $^3$H-norepinephrine was similar in both groups of animals (Table 5).

It is possible that the reduced amount of tritiated norepinephrine in the various tissues of hypertensive animals might be associated with the development of vascular lesions (necrosis) which have been reported to appear between 2 and 3 weeks after chronic administration of DOCA and NaCl (27). Such lesions could decrease the vascular permeability, thus delaying the uptake of circulating norepinephrine into its storage sites. However, the lack of difference in early accumulation of $^3$H-norepinephrine in the isolated perfused hearts and 5 min after intravenous injection in hearts from hypertensive and control rats makes this possibility unlikely.

The reduced accumulation could be the consequence of an increase in heart size. There are recent reports (30, 31) that severe cardiomegaly induced by aortic constriction results in reduced accumulation of tritiated norepinephrine and lower endogenous norepinephrine levels in the heart. In mild or moderate cardiomegaly, however, of the same magnitude as that found in the present studies, the endogenous norepinephrine content remained unaffected (31). Furthermore, in the present studies, there was a reduced accumulation of $^3$H-norepinephrine and lower endogenous norepinephrine levels in several other tissues as well.

The changes in exogenous and endogenous levels of catecholamines in the hypertensive animals appear to be due to an impairment in either the transport, binding, release, or enzymatic degradation of norepinephrine. Normally, circulating norepinephrine crosses the membrane of the sympathetic nerve into the cytoplasm, where it is then taken up, stored, and bound within a dense core vesicle. In this bound form, norepinephrine remains physiologically inactive, and this appears to constitute the major mechanism of inactivation for circulating norepinephrine (16). Under normal conditions, norepinephrine is slowly released from the storage granules and is mostly deaminated and inactivated by monoamine oxidase present in the mitochondria of the sympathetic nerve. However, the norepinephrine that is released more rapidly by an increased sympathetic activity or by sympathomimetic amines leaves the nerve in a physiologically active form (32). It then diffuses away from the receptor and is inactivated by methylation via catechol-O-methyl transferase or by re-uptake and binding in the sympathetic neurone (33).

It appears unlikely that the decreased accumulation of $^3$H-norepinephrine in this form of hypertension resides in its inability to cross the neuronal membrane normally. The observation that both the initial uptake in the isolated perfused heart and the early accumulation of exogenous norepinephrine in hearts of hypertensive rats were the same as in control hearts would rule out a defect in the initial transport across the neuronal membrane.

Another explanation would be a decreased uptake of norepinephrine into the granules or

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**TABLE 5**

*Accumulation of $^3$H-Norepinephrine in Hearts 5 Minutes after Intravenous Injection*

<table>
<thead>
<tr>
<th>No. rats</th>
<th>Per gram</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive (DOCA + NaCl)</td>
<td>5</td>
<td>20.2 ± 2.2</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>22.2 ± 2.8</td>
</tr>
</tbody>
</table>

$^{25}$ mc $^3$H-norepinephrine (S.A. 5 c/mmole) was injected.

Results are expressed in ng $^3$H-norepinephrine ± SEM.
a decreased ability of these granules to retain the amine. The retention of norepinephrine in the granules is dependent on the binding capacity of the vesicle or on these factors that facilitate the release of the stored transmitter. Preliminary studies in which the disappearance rate and subcellular distribution of \(^3\)H-norepinephrine were compared suggest a decreased ability to retain norepinephrine in the storage granules of the hypertensive hearts (Krakoff, de Champlain and Axelrod, unpublished observations). Such a defect could explain the reduced accumulation of \(^3\)H-norepinephrine and the decrease in the endogenous content of the amine. Whether any alteration in the sympathetic tone or the synthesis rate of catecholamine is implicated in these changes remains open to investigation.

The concentration of norepinephrine in the granule is high as compared to the neural cytoplasm (34). This would indicate an active transport and retention of norepinephrine into these granules. Such a hypothesis is consistent with previous observations that the accumulation of norepinephrine into the granules requires ATP, is temperature dependent (35), and is stereospecific (36). It has been shown that in association with the production of hypertension by DOCA and high sodium intake, there is an increase in the sodium and potassium content of vascular tissues (37, 38). It is possible that the uptake and retention of norepinephrine in the intraneuronal granules depend on an optimal ionic environment. Disturbances in the electrolyte concentration might have an influence on the retention and release of the bound norepinephrine in the storage granules. Whatever factors are involved, the reduced ability to bind norepinephrine would make more of the neurotransmitter available to be released from the nerve to the adrenergic receptor, to be metabolized by the intraneural monoamine oxidase, or both.

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