Relationship between Sodium Intake and Norepinephrine Storage during the Development of Experimental Hypertension

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ABSTRACT

It has been reported that storage of norepinephrine by the sympathetic nervous system was decreased in rats made hypertensive by the administration of desoxycorticosterone trimethylacetate (DOCA) and sodium chloride. The present investigation indicated that the storage of norepinephrine was impaired at an early stage of treatment with DOCA and NaCl (1 week), and preceded the appearance of hypertension. The role of sodium and sympathetic activity, the two major factors suspected of contributing to the development of the abnormality in storage of norepinephrine, was studied in normotensive and hypertensive rats. The withdrawal of sodium from the diet of hypertensive rats for 2 weeks lowered the blood pressure to normotensive levels and simultaneously restored to normal the storage and binding capacity as well as the endogenous norepinephrine content of the sympathetic storage granules in the heart. Sodium restriction or depletion in normotensive rats caused a slight decrease in blood pressure and the retention of norepinephrine in the storage granules was increased. These findings suggested that the capacity of the sympathetic granules to bind and store norepinephrine was influenced by the state of sodium balance and showed that the capacity of storage could be correlated with the level of blood pressure. The finding that treatment with a long-acting ganglionic blocker could restore the blood pressure and the norepinephrine storage capacity in hypertensive animals to normal suggested a neurogenic component in the development of this form of hypertension.

ADDITIONAL KEY WORDS  

- H-norepinephrine storage granule
- sodium restriction and depletion
- DOCAsalt hypertension
- reversal of experimental hypertension
- subcellular distribution
- endogenous norepinephrine
- rat

A defect in the storage of norepinephrine by sympathetic nerves has been found in rats made hypertensive by the administration of desoxycorticosterone trimethylacetate (DOCA) and sodium (NaCl) (1-3). The present investigation was initiated to obtain further information concerning the sequence of events occurring during the development of this form of hypertension and the role of sodium intake in the production of this abnormality in norepinephrine storage. The production of hypertension in animals treated with DOCA and NaCl is associated with sodium retention. Furthermore, the sodium and potassium contents of vascular tissues are increased in this form of hypertension as well as in other forms of hypertension in humans and experimental animals (4-8). It is therefore possible that a disturbance in the intraneural ionic balance is responsible for the decreased capacity of the adrenergic nerve to store norepinephrine.
rine during this condition (2). To examine this possibility, we studied the effect of depletion of sodium on blood pressure and storage of norepinephrine in normotensive and hypertensive rats. We have demonstrated that dietary restriction of sodium, which reverses DOCA and sodium hypertension, also restored the norepinephrine storage capacity to normal in the hearts of these animals. We also observed that treating the hypertensive rats with a ganglionic blocking agent could restore the blood pressure and norepinephrine storage capacity to normal, thus indicating the presence of a neurogenic component in the development and maintenance of DOCA and NaCl hypertension.

Methods

Production of Experimental Hypertension.—Male Sprague-Dawley rats weighing 70 to 90 g were prepared by removing the right kidney. Hypertension was induced by weekly subcutaneous injections of 0.4 ml of a suspension containing 25 mg DOCA, 10.5 mg methylcellulose, 3 mg carboxymethylcellulose, 1 mg polysorbate 80, and 8 mg NaCl per ml. The rats were given a regular laboratory diet (Purina Chow) and 1% NaCl solution (in tap water) ad libitum. Although the body weight of the hypertensive rats was slightly lower than that of the control rats at the end of 4 to 6 weeks of treatment, none showed signs of cardiac failure (ascites, liver enlargement, or pulmonary effusions) at the time they were killed. The hearts of the hypertensive rats were 15 to 25% larger than normal.

Production of Sodium Restriction and Depletion.—Rats weighing 150 to 200 g were given a synthetic sodium-deficient test diet, composed of sucrose (72%), casein (18.0%), butter fat (5%), sodium-free salt mixture (5%) supplemented with vitamins (Nutritional Biochemical Co.), and distilled water for 2 weeks ad libitum. At the start of the sodium restriction, control animals received the same synthetic diet to which 22 g of NaCl per kg was added.

Measurement of Systolic Blood Pressure.—The systolic blood pressure was measured by means of a pulse transducer applied to the tail of the anesthetized rats (E and M Instruments, Houston, Texas) as previously described (2).

Measurement of Uptake and Storage of \(^{14} \)H-Norepinephrine and Endogenous Norepinephrine.

The rats were injected via the tail veins with 15 to 25 \( \mu \)g of \(^{14} \)H-norepinephrine (New England Nuclear, Boston, Mass., 7.3 c/mmole) and then killed 5 minutes, 1 hour, 4 hours, or 24 hours later by a blow on the head. Their hearts were rapidly removed, blotted free of blood, and homogenized with a glass pestle in 10 ml of ice-cold perchloric acid. The homogenates were centrifuged at 12,000 \( \times g \) for 1 hour. The precipitate was removed, blotted free of blood, and homogenized in 10 volumes of chilled 0.25M sucrose for 1 hour. The homogenate (2.5 ml) was acidified with 0.1 volume of 4N perchloric acid. Each subcellular fraction was then transferred to cellulose nitrate tubes and was centrifuged rapidly at 100,000 \( \times g \) for 1 hour. This centrifugation yielded a pellet containing the catecholamine storage granules and other microsomal elements. The supernatant containing the microsomal and soluble fractions was then transferred to cellulose nitrate tubes and was centrifuged at 12,000 \( \times g \) for 1 hour. The supernatant was then dialyzed against cold water, the norepinephrine was eluted with 6 ml of 0.2N acetic acid, and the remainder of the homogenate (7.5 ml) was used for subcellular studies by a modification of the method of Iversen et al. (3, 12). The homogenates were centrifuged at 12,000 \( \times g \) in a refrigerated Sorval RC-2 centrifuge for 10 minutes to remove the nuclei, unbroken cells, mitochondria, and debris. The supernatant containing the microsomal and soluble fractions was then transferred to a glass filter containing 400 mg of alumina, EDTA, and sodium metabisulfite, then poured on a glass column containing 400 mg of alumina. After being washed with water, the norepinephrine was eluted with 6 ml of 0.2N acetic acid. The tubes were wiped dry and the pellets were resuspended and homogenized in 5 ml of 0.4N perchloric acid. Each subcellular fraction of the homogenates was analyzed for \(^{14} \)H-norepinephrine and endogenous norepinephrine by previously described methods (9-11).

Results
to that of control, untreated rats, and the blood pressure and heart weight of the treated animals were slightly but not significantly increased (Table 1). The reduction in endogenous norepinephrine concentration and content of the heart was negligible in the treated group but the retention of tritiated norepinephrine 24 hours after the injection was significantly reduced (26% per heart and 33.5% per gram of heart). Rats maintained on DOCA and NaCl usually show a significant rise in systolic blood pressure on or about the tenth day of treatment.

**THE EFFECT OF SODIUM INTAKE ON CARDIAC STORAGE OF NOREPINEPHRINE AND BLOOD PRESSURE**

Relationship of Sodium Intake, Blood Pressure and Norepinephrine Retention.—Groups of rats were subjected to various sodium regimens, and the endogenous norepinephrine levels of the hearts of these animals as well as the capacity of the hearts to retain exogenous norepinephrine were studied. Two groups of rats were fed a normal diet for 4 weeks and then were subjected to sodium depletion or restriction for 2 weeks before they were killed. Two other groups were fed a normal diet and either were given 1% saline to drink or were treated with DOCA for 6 weeks, and one group of rats was made hypertensive by treatment with DOCA and 1% saline for 6 weeks. At the end of the treatment, a striking inverse relationship could be observed between the mean systolic blood pressure of these various groups of rats and the capacity of the heart to retain exogenous *H*-norepinephrine (Fig. 1, Table 2), and its endogenous norepinephrine content (Fig. 2). Both the endogenous and tritiated norepinephrine contents were also inversely proportional to the degree of sodium intake or retention. The retention of *H*-norepinephrine and endogenous norepinephrine levels were highest during sodium depletion (induced by the administration of a natriuretic agent at the beginning of the sodium restriction) while the blood pressure was also the lowest. Rats subjected to sodium depletion alone also showed a greater retention of

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**TABLE I**

<table>
<thead>
<tr>
<th>Group</th>
<th>Sodium Regimen</th>
<th>Systolic Blood Pressure (mm Hg)</th>
<th>Heart Weight (g)</th>
<th>M.E. Endogenous Norepinephrine (μg/g Heart)</th>
<th>M.E. Tritiated Norepinephrine (μg/g Heart)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal Diet</td>
<td>110 ± 5</td>
<td>2.5 ± 0.5</td>
<td>0.080 ± 0.010</td>
<td>0.060 ± 0.005</td>
</tr>
<tr>
<td>DOCA 1% NaCl</td>
<td>1% Saline</td>
<td>110 ± 5</td>
<td>2.5 ± 0.5</td>
<td>0.080 ± 0.010</td>
<td>0.060 ± 0.005</td>
</tr>
</tbody>
</table>

The treated group was given the substaneous injection of 1 μg of DOCA and a solution of 15 μCi of *H*-norepinephrine and were killed 24 hours later. The results represent the mean ± S.E. of 8 animals.

*P < 0.001, P < 0.005.*
Effect of Sodium Intake on Blood Pressure and Cardiac Storage of Endogenous and Trinitated Norepinephrine

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Body wt (g)</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Heart wt (g)</th>
<th>3H-Norepinephrine (counts/min * 10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>327 ± 6.0</td>
<td>113 ± 2.2</td>
<td>1070 ± 23</td>
<td>102.6 ± 8.5</td>
</tr>
<tr>
<td>Na depletion</td>
<td>7</td>
<td>304 ± 9.5</td>
<td>102 ± 2.1</td>
<td>1154 ± 43</td>
<td>133.4 ± 5.7</td>
</tr>
<tr>
<td>Na restriction</td>
<td>7</td>
<td>328 ± 6.2</td>
<td>106 ± 2.7</td>
<td>1177 ± 50</td>
<td>110.8 ± 3.9</td>
</tr>
<tr>
<td>DOC 1.0%</td>
<td>11</td>
<td>347 ± 11.0</td>
<td>117 ± 5.5</td>
<td>1100 ± 65</td>
<td>87.1 ± 9.6</td>
</tr>
<tr>
<td>NaCl 1.0%</td>
<td>10</td>
<td>328 ± 21.0</td>
<td>133 ± 5.5</td>
<td>1087 ± 56</td>
<td>85.5 ± 5.9</td>
</tr>
<tr>
<td>DOC + NaCl</td>
<td>10</td>
<td>285 ± 10.8</td>
<td>184 ± 4.8</td>
<td>1308 ± 38</td>
<td>60.4 ± 2.5</td>
</tr>
</tbody>
</table>

Numbers represent the mean values ± 1 sem. The rats were injected intravenously with 25 μc of 3H-norepinephrine and were killed 1 hour later.

*P < 0.01; **P < 0.001.
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**Figure 2**

Relationship between systolic blood pressure and endogenous norepinephrine (NE) content in the hearts of the same groups of rats illustrated in Figure 1.

**Table 3**

Effect of Sodium Restriction and Depletion on Uptake and Storage of Norepinephrine (NE)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sodium restriction</th>
<th>Sodium depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 minute</td>
<td>24 hours</td>
<td>1 minute</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>257 ± 5.5</td>
<td>264 ± 4.4</td>
<td>223 ± 0.2*</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>122 ± 2.8</td>
<td>123 ± 1.1</td>
<td>112 ± 5.8</td>
</tr>
<tr>
<td>Tritiated NE μg/μg heart</td>
<td>369.5 ± 54.0</td>
<td>54.1 ± 6.4</td>
<td>364.5 ± 27.7</td>
</tr>
<tr>
<td>Total heart (μg)</td>
<td>312.7 ± 39.5</td>
<td>43.4 ± 5.0</td>
<td>305.0 ± 27.7</td>
</tr>
<tr>
<td>Endogenous NE μg/μg heart</td>
<td>1.30 ± 0.08</td>
<td>1.37 ± 0.07</td>
<td>1.57 ± 0.08*</td>
</tr>
<tr>
<td>Total heart (μg)</td>
<td>1.10 ± 0.07</td>
<td>1.05 ± 0.04</td>
<td>1.14 ± 0.04</td>
</tr>
<tr>
<td>Specific activity (μmol/μg)</td>
<td>25.4 ± 22.1</td>
<td>45.4 ± 4.4</td>
<td>301.9 ± 23.2</td>
</tr>
<tr>
<td>μS/μs G</td>
<td>1.72 ± 0.15</td>
<td>1.27 ± 0.09</td>
<td>1.10 ± 0.17*</td>
</tr>
</tbody>
</table>

Male Sprague-Dawley rats were subjected to sodium restriction or depletion for 2 weeks. To study the uptake and storage of norepinephrine in these animals, 15 μg of [3H]-norepinephrine was injected iv and one group was killed 5 minutes later, while another group was killed 24 hours later. The results represent the mean ± SEM of 6 animals in each individual group.

*P < .05; **P < .01.
*The hearts were analyzed for subcellular distribution and μS/μs G represents the ratio of the specific activity of the supernatant to the specific activity of the granules (100,000 × g/pellet).
Effect of sodium intake and DOCA administration on systolic blood pressure. The numbers and letters adjacent to each curve identify groups referred to in Figures 3 and Table 4. Group 1 represents the control group which received a regular laboratory diet for 4 weeks. Group 2 was given a regular diet for the first 2 weeks and was treated with DOCA and 1% saline for the subsequent 2 weeks. Group 3 was treated with DOCA and 1% saline for 3 weeks after 1 week of regular diet. Group 4 was treated with DOCA and 1% saline for 4 weeks. Group 5 received a normal diet for the first 2 weeks at a sodium free diet with distilled water for the last 2 weeks. Group A refers to rats treated with DOCA and 1% saline in the first 2 weeks, after which this treatment was stopped and the animals were given a normal laboratory diet for the remaining 2 weeks. Group B was also treated with DOCA and 1% saline for the first 2 weeks but was given a sodium-deficient diet and distilled water for the following 2 weeks after DOCA administration was stopped.

Each point represents the mean of 6 to 14 individual values. At the end of the period of 4 weeks of treatment, each blood pressure with the exception of group B and group 5 was significantly different from the control value.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Effect of Sodium Restriction on Blood Pressure and Norepinephrine Retention in the Heart of Normotensive and Hypertensive Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group no. and regimen</td>
<td>No.</td>
</tr>
<tr>
<td>1—Control</td>
<td>14</td>
</tr>
<tr>
<td>5—Low Na diet</td>
<td>6</td>
</tr>
<tr>
<td>2—DOCA + NaCl (2 weeks)</td>
<td>8</td>
</tr>
<tr>
<td>3—DOCA + NaCl (3 weeks)</td>
<td>6</td>
</tr>
<tr>
<td>4—DOCA + NaCl (4 weeks)</td>
<td>12</td>
</tr>
<tr>
<td>A—DOCA + NaCl Normal lab. diet</td>
<td>8</td>
</tr>
<tr>
<td>B—DOCA + NaCl Low Na diet</td>
<td>7</td>
</tr>
</tbody>
</table>

The numbers preceding the groups refer to numbers in Figures 3 and 4. Animals were injected intravenously with 25 μg of 3H-norepinephrine and were killed 4 hours later. Values are means ± sem.

*P < 0.001; †P < 0.01; *P < 0.05.
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norepinephrine, 4 hours after iv injection, was also inversely related to the mean systolic blood pressure in the hearts of these rats under various treatments (Fig. 4). Rats given DOCA and saline during the 2 to 3 weeks preceding the experiment (Groups 2 and 3) showed cardiac 3H-norepinephrine levels intermediate between that of the normotensive (Group 1) and that of the hypertensive rats given the same treatment for 4 weeks (Group 4). The hearts from normotensive rats subjected to a sodium depletion had a greater capacity of retention (Group 5). The rats in which DOCA and saline treatment was replaced with a normal laboratory diet (Group A) had a reduced capacity to retain norepinephrine as well as an elevated blood pressure. In the group of hypertensive rats treated with a low-sodium diet (Group B), the capacity of retention was restored to near normal levels (Fig. 4).

Subcellular Distribution of Norepinephrine during Sodium Restriction in Normotensive and Hypertensive Rats.—The norepinephrine storage by the sympathetic granules was examined in the hearts of normotensive and hypertensive rats administered a sodium-restricted diet. The preparation of Groups 1, 4, and 5 and Group B was the same as that just reported and showed similar variations of blood pressure (Fig. 5). The rats in Group C were treated like those in Group B except that they received DOCA, 10 mg/week, throughout the entire experiment. In the latter group, the maintenance of DOCA treatment during the period of sodium depletion slightly delayed but did not prevent the drop in blood pressure, which reached the same level as that in the animals in Group B after 2 weeks of sodium restriction (Fig. 5). At the end of the treat-

![Figure 4](http://circres.ahajournals.org/)

**FIGURE 4**

Relationship between the retention of tritiated norepinephrine (NE) in the whole heart 4 hours after iv injection and the mean systolic blood pressure in groups of rats submitted to various sodium intake and treatment. The number and letters correspond to the same groups and curves illustrated in Figure 3. The results are the means of 6 to 14 animals and are expressed as the mean percentage of control values.

![Figure 5](http://circres.ahajournals.org/)

**FIGURE 5**

Effect of sodium intake and DOCA administration on systolic blood pressure. The letters and numbers adjacent to each curve identify the groups of rats referred to in Figures 6 and 7 and Table 5. Each group contains six to eight rats. Groups 1, 4, 5, and B were treated in a similar way to those illustrated in Figure 3. Group C was treated with DOCA for 4 weeks in association with 1% saline in the first 2 weeks and with a sodium-deficient diet and distilled water during the last 2 weeks.
The animals were injected with $^3$H-norepinephrine and were killed 24 hours later. The hearts were homogenized in isotonic sucrose, and tritiated and endogenous norepinephrine were analyzed in the various subcellular fractions.

As previously found, the retention of tritiated norepinephrine and the endogenous norepinephrine content of the heart of the hypertensive animals (Group 4) were markedly reduced (Fig. 6). However, the retention of $^3$H-norepinephrine and endogenous content of the amine in the hearts of the sodium-depleted animals (Group 5) and in the hearts of the hypertensive rats treated with a low-sodium diet, independent of DOCA administration (Groups B and C), were slightly higher or equal to the control levels.

The tritiated norepinephrine and the endogenous norepinephrine found in the microsomal fraction (which contains the norepinephrine storage granules) of these hearts showed a pattern similar to that in the whole heart (Fig. 7 and Table 5). An estimate of the subcellular distribution of the neurotransmitter was made by measuring the ratio of the specific activity of the supernatant to the specific activity of the granular fraction. This ratio was lower in the hearts of sodium-depleted rats (Group 5), indicating a greater capacity for the storage of norepinephrine; the ratio in hearts from the hypertensive rats (Group 4) was slightly higher (Fig. 7). In the hearts of hypertensive rats treated with a low-sodium diet (Groups B and C) this ratio was intermediate between that of the hypertensive animal (Group 4) and that of the controls (Group 1).

The greater retention of norepinephrine in the granular fraction during sodium depletion could also be observed in another study illustrated in Table 3. Five minutes or 24 hours after the injection of tritiated norepinephrine, the ratio of the specific activity of the supernatant to the specific activity of the granular fraction was significantly lower in rats subjected to sodium restriction or depletion.

**EFFECTS OF GANGLIONIC BLOCKADE ON BLOOD PRESSURE AND STORAGE OF NOREPINEPHRINE**

To establish whether a neurogenic component was involved in the development of DOCA and NaCl hypertension, the effect of a long-lasting ganglionic blocking agent was studied in normotensive and hypertensive rats.
### TABLE 5
Effect of Sodium Depletion on Blood Pressure and Subcellular Distribution of Norepinephrine

<table>
<thead>
<tr>
<th>Group no and regimen</th>
<th>Body wt (g)</th>
<th>Systolic Blood pressure (mm Hg)</th>
<th>Heart wt (g)</th>
<th>Tritiated norepinephrine (ng)</th>
<th>Endogenous norepinephrine (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Supranatant</td>
<td>Granule</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>207 ± 4.9</td>
<td>119 ± 7.8</td>
<td>107 ± 8.4</td>
<td>90.5 ± 3.5</td>
</tr>
<tr>
<td>Low Na diet</td>
<td>8</td>
<td>180 ± 4.1</td>
<td>110 ± 3.3</td>
<td>106 ± 15.3</td>
<td>64.5 ± 6.4</td>
</tr>
<tr>
<td>DOCA + NaCl (4 weeks)</td>
<td>7</td>
<td>192 ± 4.1</td>
<td>179 ± 6.0</td>
<td>179 ± 6.0</td>
<td>50.5 ± 6.0</td>
</tr>
<tr>
<td>DOCA + NaCl</td>
<td>6</td>
<td>188 ± 4.3</td>
<td>129 ± 6.4</td>
<td>140 ± 10.4</td>
<td>24.5 ± 5.1</td>
</tr>
<tr>
<td>Low Na diet + DOCA</td>
<td>6</td>
<td>192 ± 7.1</td>
<td>179 ± 6.0</td>
<td>90 ± 3.6</td>
<td>35.0 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>188 ± 4.3</td>
<td>129 ± 6.4</td>
<td>140 ± 10.4</td>
<td>24.5 ± 5.1</td>
</tr>
</tbody>
</table>

The number preceding the group corresponds to numbers in Figures 5, 6, and 7. Animals were injected with 20 µCi 3H-norepinephrine iv and were killed 24 hours later. The values given are means ± 1 SEM.

### TABLE 6
Effect of Canglionic Blockade on Blood Pressure and Norepinephrine Storage

<table>
<thead>
<tr>
<th>Group</th>
<th>Untreated</th>
<th>Hypertensive</th>
<th>Chlorisondamino</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body wt (g)</td>
<td>Systolic Blood pressure (mm Hg)</td>
<td>Heart wt (g)</td>
<td>Tritiated norepinephrine (ng)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total heart</td>
<td>Supranatant</td>
<td>Granule</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>247 ± 7.5</td>
<td>124 ± 2.6</td>
<td>827 ± 22</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>7</td>
<td>206 ± 11</td>
<td>120 ± 9.0</td>
<td>116 ± 32</td>
</tr>
<tr>
<td>Chlorisondamino</td>
<td>8</td>
<td>247 ± 7</td>
<td>88 ± 5.5</td>
<td>751 ± 23</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>8</td>
<td>205 ± 11</td>
<td>120 ± 5.8</td>
<td>1078 ± 49</td>
</tr>
</tbody>
</table>

Values given are means ± SEM.

*P < 0.05; tP < 0.001; tP < 0.0001.
Endogenous and tritiated norepinephrine content in the subcellular fraction, containing the norepinephrine storage granules, of the hearts from the same groups illustrated in Figure 5. The last group of data at the right end of the figure represents the ratio of the specific activity of the supernatant (100,000 x g supernatant) over the specific activity of the granular fraction (100,000 x g pellet). (See Methods.) The numbers and letters at the bottom of the figure identify the groups as illustrated in Figures 5 and 6. Each group contains 6 to 8 rats and the results are expressed as the mean ± SEM. Twenty-five microcuries of tritiated norepinephrine were injected iv 24 hours before killing the animals.

Discussion
The present study suggests that the capacity of the heart to store norepinephrine is reduced prior to the appearance of hypertension in rats treated with DOCA and sodium. This indicates that the storage abnormality appears early in the sequence of events leading to or associated with the development of this form of hypertension. At this early stage, the endogenous norepinephrine content of the heart is still normal but it is reduced in rats treated for a longer period (1-3), probably as a consequence of the reduced ability of the nerve to store the amine.

It also appears that sodium intake or the state of sodium balance can influence the capacity of the nerve granules to bind and store norepinephrine. At an early stage of...
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CONTROL CHLORISONDAMINE (10 mg/kg/8 hours)

FIGURE 8

The effect of chlorisondamine on the norepinephrine storage and on the blood pressure of normotensive and hypertensive rats. Twenty-five microcuries of tritiated norepinephrine were injected first, then the treatment with chlorisondamine (10 mg/kg every 8 hours) was started 15 minutes after the injection of norepinephrine. The animals were sacrificed 24 hours later. Each group contains 7 to 8 rats and the results are expressed as the mean ± SEM.

DOCA and sodium hypertension, the blood pressure as well as the storage capacity of the nerve granules can be restored completely to normal by feeding the animals a sodium-free diet for 2 weeks. The maintenance of DOCA administration during sodium depletion does not prevent either the reversal of hypertension or the restoration of the storage capacity of the granules in hypertensive rats. In addition, normotensive rats undergoing sodium restriction or depletion have a tendency towards a greater storage capacity for norepinephrine in the heart, and their blood pressure is slightly lowered. The direct effect of the natriuretic agent used during sodium depletion can probably be discounted since the last administration of the drug was made 11 days before the rats were killed. The decreased ratio of the soluble to the granule-bound norepinephrine also indicates a greater avidity of the nerve granules for the amine during sodium restriction or depletion. These observations are the converse of those made in the hypertensive animals, in which a reduced retention of norepinephrine in the storage granules was found.

The uptake, binding, and storage of norepinephrine by the nerve ending constitute a major means of inactivation of this amine (13, 14). A change in the efficiency of the nerve in performing these functions will bring about variations in the amount of physiologically active norepinephrine available to react with the receptors or pass into the circulation or both. The present study suggests that the state of sodium balance may be important in modulating the storage capacity of the nerve and in regulating the availability of physiologically active norepinephrine. In the hypertensive animals, the reduced retention and storage of norepinephrine, the greater proportion of soluble norepinephrine (presumably unbound), and the increased amount of O-methylated metabolites in the kidney (1-3) are consistent with the concept of an increased amount of norepinephrine released from the nerve. During sodium depletion, the increased capacity of storage and the lesser proportion of soluble norepinephrine lead to the opposite conclusion. The striking inverse relationship observed between the blood pressure and the norepinephrine storage capacity in the heart suggests a role for the sympathetic nervous system in the regulation of blood pressure under these conditions. It is therefore possible that the hypotensive effect of sodium restriction or treatment by natriuretic agents is mediated through an increased inactivation of norepinephrine by a more efficient binding and storage in the nerve.

The vascular reactivity to catecholamines has been found to increase in DOCA and sodium hypertension (15-17) as well as in...
DE CHAMPLAIN, KRAKOFF, AXELROD

other forms of hypertension (15-20), and to
decrease during sodium deprivation (21, 22)
and after treatment by natriuretic agents
(20, 23, 24). It is not known to what extent
the subcellular distribution of norepinephrine
may be related to the state of vascular re-
activity, but in these conditions it seems that
the change in responsiveness could be as-
associated with changes in subcellular distri-
bution of the catecholamine.

The mechanisms regulating the binding,
storage, and release of norepinephrine by the
nerve are not fully understood. However,
since active transport and ionic shift are in-
volved in the numerous functions of the
nerve, it may be that the intraneural ionic
balance is critical for proper functioning of
the storage and release of the nerve trans-
mitter. There is indirect evidence that the
ionic balance may be perturbed in the con-
ditions we studied. The sodium and potas-
sium contents of vascular tissues have been
found to be increased during the develop-
ment of DOCA and sodium hypertension
(4-7) and sodium content has been found to
be decreased during sodium depletion
(25). It is possible that, in these conditions,
the variations in norepinephrine storage and
in the levels of blood pressure are initiated
through discrete intraneural ionic distur-

Similar ionic disturbances have been ob-
served in tissues of animals with other forms
of experimental hypertension (4-6, 8) and
in human hypertension (26). It is possible that, in these conditions,
the variations in norepinephrine storage and
in the levels of blood pressure are initiated
through discrete intraneural ionic distur-

in other forms of hypertension as it is in
DOCA and saline hypertension.

The reversal of hypertension and of the
defective norepinephrine storage capacity by
ganglionic blockade suggests a central neuro-
genetic component in the development of this
form of hypertension. It is not known, how-
ever, to what extent the state of sodium bal-
ance can influence the activity of the sympa-
thetic nervous system.

Our experiments demonstrate that the ca-
pacity of the sympathetic granules to bind
and store norepinephrine can be influenced
by the state of sodium balance and that nor-
epinephrine could play an important role in
the pathogenesis of DOCA and sodium hyper-
tension. The degree of the sympathetic ac-
tivity appears to be of great significance in
the development of the norepinephrine stor-
age abnormality but more studies are re-
quired to understand the relationship that
exists between sodium balance and the activity of the sympathetic nervous sys-
tem.

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References

1. DE CHAMPLAIN, J., KRAKOFF, L. R., AND
AXELROD, J.: Reduction in the accumula-
tion of 3H-norepinephrine in experimental hyperten-
2. DE CHAMPLAIN, J., KRAKOFF, L. R., AND
AXELROD, J.: Catecholamine metabolism in ex-
perimental hypertension in the rat. Circulation
3. KRAKOFF, L. R., DE CHAMPLAIN, J., AND
AXELROD, J.: Abnormal storage of norepinephrine
in experimental hypertension in the rat. Circulation
4. TOBIAN, L.: Interrelationship of electrolytes, jux-
taglomerular cells and hypertension. Physiol.
5. COB, J. F., AND FREMENT, A.: Le sodium dans les
6. TAKAMATSU, H.: Aortic wall electrolyte and the
adrenal cortex in experimental hypertensive rats.
7. COB, J. F., AND SCHMIDT, H.: Natrium und Kaliumgehalt von Plasmas und Geweben beim

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Relationship between Sodium Intake and Norepinephrine Storage during the Development of Experimental Hypertension

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