Catecholamine Synthesis in Rabbits with Neurogenic Hypertension

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ABSTRACT
Norepinephrine synthesis was determined in the heart and adrenals of rabbits made hypertensive by complete denervation of the carotid sinuses and aortic arch. There was a 24% reduction of norepinephrine concentration in the left ventricle 3 weeks after denervation, despite evidence of enhanced synthesis as determined by three methods: (1) an 18% increase in apparent synthesis rates (from 0.76 to 0.90 μmoles/g/hour) calculated from norepinephrine turnover rates after infusion of DL-norepinephrine-3H; (2) a 124% increase in synthesis rates (from 2.1 to 4.7 μmoles/g/hour) estimated from the incorporation of label from infused tyrosine-3H; (3) a 50% increase (from 4.0 to 6.0 μmoles/g/hour) in the activity of the regulatory enzyme tyrosine hydroxylase. Additionally, in the denervated rabbits there was a 43% reduction in the amount of epinephrine in the adrenal gland 2 days after denervation and a return to normal values at 3 weeks. Adrenal tyrosine hydroxylase, phenylethanolamine-N-methyl transferase, and the incorporation of label from tyrosine-3H into epinephrine were increased at 3 weeks. A smaller number of hypertensive rabbits studied at 2 days showed a reduction in left ventricular norepinephrine concentration. Catecholamine reductions, due only in part to dilutional hypertrophy, may be related to increased utilization and may serve to enhance synthesis of the neurotransmitter and maintain the increase in blood pressure.

ADDITIONAL KEY WORDS biosynthesis baroreceptor denervation catecholamines carotid sinus phenylethanolamine-N-methyl transferase tyrosine hydroxylase hypertension

Biochemical evidence of altered sympathetic nerve function has been reported in essential hypertension (1, 2). Defective neurotransmitter storage has also been demonstrated in a salt-loaded, DOCA-treated, uninephrectomized animal model with experimental arterial hypertension (3). However, the pathophysiologic conditions induced in that model are not present in essential hypertension (4). This report describes decreased storage and increased synthesis of norepinephrine and epinephrine, which is direct evidence of sympathoadrenal hyperactivity, in the rabbit made hypertensive by this denervation.

Methods

Preparation of the Rabbits
We cut the afferent fibers arising from the baro-
and chemoreceptors in the carotid sinus and carotid body (carotid sinus nerves) and from the baroreceptors in the aortic arch and subclavian artery (aortic nerves) (6). New Zealand female rabbits weighing 2 to 3 kg were secured on a board with the head extended, and 2% procaine was injected subcutaneously along the midline from clavicle to jaw. After a midline incision and the separation of the sternohyoid muscles over the trachea, the common carotid artery on one side was retracted to expose the sympathetic, aortic, and vagus nerves. The denervation procedure was carried out with the aid of a dissecting microscope (7x). The aortic nerve was resected from its junction with the superior laryngeal to a level just above the clavicle. (The aortic nerve is completely separate from the sympathetic and vagus nerves in rabbits.) Next, the carotid bifurcation was exposed and about 1 cm of both the internal and external carotid arteries was dissected out and cleared from surrounding tissue. Two ligatures were placed around all tissue between the internal and external carotid arteries, including the carotid sinus nerve. One ligature was tied as close as possible to the bifurcation; the other was tied close to the origin of the carotid sinus nerve, and the tissue between the two ligatures was dissected away. The severed nerve ends, carotid sinus wall and bifurcation area were painted with 5% phenol. The procedure was repeated on the other side of the neck. The skin incision was closed with silk thread and the animal was given 600,000 units of penicillin im. The nerves were located and exposed in sham-operated controls, but they were not handled or dissected. Sham-operated animals remained supine for the same length of time as the denervated animals.

Arterial Pressures and Pulse Rates

A central ear artery was cannulated with no. 50 polyethylene tubing after local nerve block with 2% procaine. The animal was placed in a small cage within a sound-attenuated chamber and the cannula was connected to a Sanborn Model 267B gauge by a tube filled with saline plus heparin. The ear pressures were recorded while the animal sat quietly in a normal position. Systolic and diastolic pressures were recorded continuously on a Sanborn Model 7700 recorder (paper speed 0.5 mm/sec) for 20 minutes. Electrically integrated mean pressure and pulse rates were obtained for 10-second periods at increased paper speed (5 mm/sec). Pressures and pulse rates of the control animals were stable but those of the denervated animals were labile; therefore, measurements were taken from all records at 5-minute intervals, and the mean values are reported. Arterial pressures and pulse rates were recorded on each of the 2 days before operation in 57 animals. Postoperative recordings were made on days 1 and 2 in 8 denervated and 7 control rabbits and on days 19 to 21 in 23 denervated and 19 control rabbits. They were decapitated 2 days and 3 weeks postoperatively.

Catecholamine Assays

The organs were removed rapidly, blotted dry, and stored on dry ice until the time of assays. After thawing, the heart was separated into combined atria, right ventricle, and left ventricle. The left ventricles and the adrenals were weighed and homogenized separately in glass in 0.25M sucrose. Aliquots of the homogenates were rehomogenized in 5% trichloracetic acid prior to fluorimetric assay (7). These measurements were made in all of the animals that had physiologic measurements except for 6 denervated and 5 control animals 2 days postoperatively. Catecholamine studies were performed 2 days postoperatively in an additional 6 denervated and 6 control animals in which no physiologic data were obtained. In some instances, assays were made of the excised atra and other sympathetically innervated organs.

Norepinephrine Turnover Studies

Three weeks after operation, DL-norepinephrine-\(^3\text{H}\) (14 c/mm\(^2\), \(^1\) 100 \text{mcg/kg}, was injected into 19 denervated and 15 control animals. The animals were killed in batches of 6 to 12 at intervals from 5 minutes to 24 hours later. The specific activity of the recovered left ventricular norepinephrine-\(^3\text{H}\) was determined from aliquots of alumina eluates. The mean norepinephrine-\(^3\text{H}\) specific activities were plotted semilogarithmically and the curves of decline of specific activity were fitted to the values according to the method of least squares. Significance was determined by analysis of variance. The fractional decay (percent/hour) (k) of the decline of specific activity of norepinephrine was calculated from the half-life (0.693/k). Synthesis rates were calculated for both control and denervated animals as [NE]k, where [NE] is the left ventricular norepinephrine concentration for each animal.

Norepinephrine Synthesis

In-Vivo Tyrosine-\(^3\text{H}\) Incorporation Studies.—L-tyrosine-\(^3\text{H}\) (3, 5T) (25 c/mm\(^2\)), 350 \text{mcg/kg}, was injected either two days or three weeks postoperatively. The animals were killed 30 minutes after injection, and tyrosine and norepinephrine specific activities were determined from alumina

\(^1\)This and the other radioisotopes were obtained commercially from the New England Nuclear Corp., Boston.
effluents and eluates of protein-free filtrates of left ventricle and whole adrenal glands. Tyrosine-$^{3}$H in the alumina effluent was separated from phenolic acids by adsorption and elution from Dowex H$^{+}$. Norepinephrine-$^{3}$H and epinephrine-$^{3}$H were separated from dopa and dopamine by adsorption and differential elution from Dowex Na$^{+}$. Details of these methods and of that for measuring nonradioactive tyrosine are described elsewhere (8). Norepinephrine synthesis rates were estimated from the specific activities of tyrosine and norepinephrine at 30 minutes. The following formula was used. The norepinephrine synthesis rate ($\text{nmmoles/g/hour}$) =

\[
\frac{\text{cpm labeled norepinephrine}}{\text{mean tyrosine specific activity}} \times 4,
\]

correction factor to convert the 30-minute value to synthesis rate per hour and also to adjust for the loss of 50% of the label with the hydroxylation of tyrosine, 3, 5T. The mean specific activity of left ventricle tyrosine was calculated from the 30-minute tyrosine specific activity and the slope of decay of labeled tyrosine ($-1.56$) in rat heart after intravenous administration (9).

**In-Vitro Tyrosine Hydroxylase Assay.**—The activity of the enzyme tyrosine hydroxylase, which is rate-limiting in norepinephrine biosynthesis, was assayed in sucrose homogenates of ventricle and adrenal by measuring the conversion of L-tyrosine-$^{14}$C to L-dopa-$^{14}$C (10). The incubation mixture contained 200 $\mu$moles of phosphate buffer, pH 6.4, 100 $\mu$moles of L-tyrosine-$^{14}$C ($300 \text{ mc/mM} ; 6 \times 10^{5}$ cpm), 1 $\mu$ mole of 2-amino-4-hydroxy-tetrahydropteridine, 100 $\mu$ moles of mercaptoethanol and 100 $\mu$ moles of brocresine and 0.4 ml of the homogenate (a 1:4 dilution for heart and 1:16 dilution for adrenal) in a final volume of 1.0 ml.

**In-Vitro Phenylethanolamine-N-Methyl Transferase Activity.**—Phenylethanolamine-N-methyl transferase (PNMT) is responsible for the last step in the biosynthetic sequence to epinephrine. PNMT was assayed in supernatant fluid of adrenal glands homogenized at 50,000 $\times$ g using S-adenosyl-L-methionine (S-methyl-$^{14}$C) (30-50 mc/mM) and normetanephrine as substrates (11). The

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**FIGURE 1**

*Increased arterial pressures and pulse rates after sinoaortic denervation (SAD).* Values are means ± SEM of pressures at 1 to 2 postoperative days for 7 sham-operated and 8 denervated rabbits and at 19 to 21 days for 19 sham-operated and 23 denervated rabbits. Postoperative systolic (top value in each column), mean (middle value), and diastolic (lowest value) pressures were greater in denervated than in control rabbits ($P < .02, .01, .001; P < .001$ for all) at both time periods. Postoperative pulse rates were greater in denervated than in control rabbits ($P < .001$) at both time periods.

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TABLE 1  
Effect of Sinoaortic Denervation on Left Ventricle Weight and Norepinephrine Content

<table>
<thead>
<tr>
<th>Time after operation</th>
<th>Weight g</th>
<th>Body wt* (g/kg)</th>
<th>Norepinephrine μg/g</th>
<th>total μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operation (8)</td>
<td>3.35 ± .13</td>
<td>1.44 ± .06</td>
<td>1.64 ± .16</td>
<td>5.53 ± .61</td>
</tr>
<tr>
<td>Denervation (8)</td>
<td>4.19 ± .25</td>
<td>1.84 ± .08</td>
<td>1.08 ± .25</td>
<td>4.26 ± .71</td>
</tr>
<tr>
<td>% Difference</td>
<td>+25</td>
<td>+28</td>
<td>-34</td>
<td>-23</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Three weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operation (19)</td>
<td>4.11 ± .11</td>
<td>1.42 ± .04</td>
<td>1.72 ± .09</td>
<td>6.97 ± .31</td>
</tr>
<tr>
<td>Denervation (23)</td>
<td>4.52 ± .16</td>
<td>1.58 ± .06</td>
<td>1.31 ± .08</td>
<td>5.76 ± .33</td>
</tr>
<tr>
<td>% Difference</td>
<td>+10</td>
<td>+11</td>
<td>-24</td>
<td>-17</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;.05</td>
<td>&lt;.05</td>
<td>&lt;.01</td>
<td>=.01</td>
</tr>
</tbody>
</table>

*At each time period, the mean body weights of sham-operated and denervated animals were identical; values were 2.3 and 2.9 kg at 2 days and 3 weeks, respectively. Values are means ± SEM for numbers of animals given in parentheses.

PNMT incubation mixture contained 12.5 μmole of phosphate buffer, pH 8.0, 110 μmole of nor-metanephrine, 2 μmole of S-adenosyl-L-methionine (S-methyl-14C) and 10 μl of the supernatant fluid from a 1:16 sucrose homogenate of the whole adrenal gland in a final volume of 50 μl. The incubation was continued for 60 minutes at 37°C. Metanephrine-14C formed was isolated by extraction and counted.

Results

Blood Pressure and Pulse Rate Increases after Sino-Aortic Denervation

The operation resulted in sustained increases in systolic, diastolic, and mean arterial pressures and pulse rates of 22%, 30%, 24%, and 26%, respectively, at 2 days, and 26%, 40%, 29%, and 17%, respectively at 3 weeks. Pre- and postoperative pressures and heart rates and the statistical significance of their differences are indicated for both the denervated and control groups in Figure 1.

Catecholamine Concentration and Left Ventricle Weights and Adrenal Catecholamines after Denervation

Mean concentration of norepinephrine in left ventricles was 34% and 24% less at 2 days and 3 weeks, respectively, after denervation than in the control rabbits (Table 1). The norepinephrine content was only 23% and 17% less. Thus part of the reduction in norepinephrine concentration was dilutional, consistent with the increase in mean left ventricle weights of 28% and 11% at 2 days and 3 weeks, respectively. The combined weight of the atria at 2 days and 3 weeks was 24% and 12% greater than that of the controls (P < .02). Right ventricle weights did not differ in the two groups. Left ventricle hypertrophy, with increased thickness of 2 to 3 mm, was apparent in some denervated rabbits. However, ventricle weights of the two groups overlapped considerably, and some denervated animals showed no evidence of hypertrophy. An inverse relationship between norepinephrine content and left ventricle weights in the denervated rabbits is shown in Figure 2 (r = -0.60; P < .001). However, several denervated animals had low norepinephrine content in the absence of hypertrophy. No significant correlation was found in denervated animals between left ventricle weights (γ = -0.41) nor norepinephrine concentration (γ = -0.27) and cardiac work index (MAP × PR^-1/2). (MAP is mean arterial pressure and PR is pulse rate.)

Total adrenal epinephrine and norepinephrine were 34% less than controls 2 days after denervation (60 ± 4 μg compared to 106 ± 10 μg; P < .001). The 3-week values were not significantly different (116 ± 8 μg for controls and 121 ± 9 μg for denervated animals). In each instance epinephrine constituted more than 90% of the total adrenal catecholamines. Adrenal weights were unaffected by denervation at either time period.

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549

2.0

SAD, r = -0.60

Sham SAD

2 day

3 week

0.5 1.5

Left Ventricle Wt (g / kg Body Wt)

FIGURE 2

Relationship of norepinephrine concentration in left ventricle to left ventricular weight per kg body weight. The correlation was significant (P < .001) for 31 rabbits with sinoaortic denervation (SAD) (8 at 2 days and 23 at 3 weeks). Values for sham-operated controls were entered for comparison.

Norepinephrine Turnover

Cardiac norepinephrine specific activity of denervated animals had a half-life of 6 hours compared to 9.5 hours in controls (Fig. 3). The slopes of decline, 0.0495 and 0.0305, respectively, were not significantly different. The mean 24-hour values for specific activity in denervated animals significantly decreased (P < .02). The calculated mean turnover rates for denervated animals were 0.90 μmoles/g/hour and for controls 0.76 μmoles/g/hour.

Tyrosine Hydroxylase Studies

In Vivo.—Synthesis was also evaluated in vivo after intravenous injection of tyrosine-3H. There was increased formation of labeled catecholamines in both the left ventricles and the adrenals (Table 2) after denervation, although tissue tyrosine specific activities did not differ from controls. The increased incorporation of norepinephrine-3H in ventricle (cpm/g) 2 days after denervation differed less from controls than it did at 3 weeks. The increase at both time intervals was magnified when expressed as total content because the ventricles of denervated rabbits were heavier than those of controls at both times. However, more importantly, there was a twofold increase in norepinephrine specific activity (cpm/μg) at both time intervals, reflecting the increased proportion of newly synthesized norepinephrine (Table 3). The increase at 3 weeks was significant (P < .05). The cardiac norepinephrine synthesis rate at 3 weeks, as estimated from counts per minute of norepinephrine and tyrosine specific activity, was 4.7 μmoles/g/hour after denervation compared to 2.1 for controls. The rate of formation of labeled epinephrine and norepinephrine was greater in adrenals after sinoaortic denervation at both time periods (Table 2). The adrenal values were significantly higher than control values (P < .05) only in the rabbits denervated for 2 days because of the variability of the values after denervation and the small number of animals. The total catecholamines in the adrenals 2 days after denervation were 106 μg compared with 60 μg in the control adrenals (P < .001), resulting in a larger increase in the catecholamine specific activity of the denervated animals (Table 3).

The formation of labeled catecholamine and the specific activities of the ventricles and adrenals at 2 days in sham-operated animals were greater than values at 3 weeks. The batch of tyrosine-3H used for the animals at 3 weeks was different; it was first evaporated under N2 and then redissolved and injected, whereas the batch for the animals studied at 2 days was administered without prior evaporation. Less than 0.16% of the impurity (l-dopa-3H) was present in these batches (both had same lot number) after paper chromatography in butanol, acetic acid, and water (4:1:1). Perhaps this small amount of l-dopa-3H or some other impurity was removed.
TABLE 2
Effect of Sinoaortic Denervation on Formation of Labeled Catecholamines* in Ventricle and Adrenals after Administration of Tyrosine-3H

<table>
<thead>
<tr>
<th>Time after operation</th>
<th>Left ventricle</th>
<th>Both adrenals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm/g</td>
<td>total cpm</td>
</tr>
<tr>
<td>Two days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operation</td>
<td>324 ± 40</td>
<td>1,078 ± 152</td>
</tr>
<tr>
<td>Denervation</td>
<td>438 ± 67</td>
<td>1,683 ± 320</td>
</tr>
<tr>
<td>Three weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operation</td>
<td>175 ± 33</td>
<td>724 ± 107‡</td>
</tr>
<tr>
<td>Denervation</td>
<td>312 ± 53</td>
<td>1,420 ± 124‡</td>
</tr>
</tbody>
</table>

*Values are means ± SEM for three animals. Norepinephrine-3H in ventricle and norepinephrine-3H and epinephrine-3H in adrenals. †P < .05. ‡P < .01. Higher values 2 days after sham operation than at 3 weeks may be due to differences in batches of L-tyrosine-3H (see text).

FIGURE 3
Effect of sinoaortic denervation on norepinephrine turnover in left ventricle; 19 denervated and 15 sham-operated animals were studied at 5 minutes, 4, 14, and 24 hours; numbers of animals were 4 and 3, 3 and 3, 6 and 4, and 6 and 5 for the respective time periods. The mean norepinephrine specific activities are plotted semilogarithmically; only the 24-hour values are significantly different (P < .02). Their slopes of decline, fitted to the individual values by the method of least squares, are not significantly different (P = 1.35).

In Vitro.—The tyrosine hydroxylase activity of the ventricles and adrenals of the denervated animals was greater than that in the sham-operated animals at both time periods. At 2 days, the activity was 83% greater in

during the N2 evaporation of the 3 week batch. The magnitude of the increased norepinephrine-3H formation after sinoaortic denervation was similar at each time period, although the absolute values were different.
TABLE 3
Effect of Sinoaortic Denervation on Catecholamine Specific Activity in Rabbit Ventricle and Adrenals after Administration of Tyrosine-3H

<table>
<thead>
<tr>
<th>Time after operation</th>
<th>Left ventricle NE-3H (cpm/µg)</th>
<th>Both adrenals NE-3H + E-3H (cpm/µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operation</td>
<td>256 ± 43</td>
<td>8.3 ± 0.9</td>
</tr>
<tr>
<td>Denervation</td>
<td>509 ± 126</td>
<td>55 ± 22</td>
</tr>
<tr>
<td>Three weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operation</td>
<td>99 ± 21</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Denervation</td>
<td>242 ± 38*</td>
<td>4.1 ± 1.1</td>
</tr>
</tbody>
</table>

All values are means ± SEM for three animals. Higher values 2 days after sham operation than at 3 weeks may be due to differences in batches of l-tyrosine-3H (see text).

*Difference from control is significant, P < .05.

ventricle and 100% greater in adrenals; at 3 weeks the tyrosine hydroxylase activity of both organs was 50% more than that of the controls (Table 4). Only the differences in mean total tyrosine hydroxylase content of left ventricle are significant (P < .05). However, when the means of the combined 2-day and 3-week values of the denervated animals were compared with control values, there was a 60% increase in tyrosine hydroxylase activity of both organs (P < .02 for ventricle and < .01 for adrenals). This increase in tyrosine hydroxylase activity reflected change in amounts rather than activity per se, since the conditions of assay were optimal for both sham-operated and denervated animals. In the denervated heart at 3 weeks, this maximal norepinephrine synthesis increased from 4.0 to 6.0 µmole/g/hour.

PNMT activity did not differ from controls at 2 days but at 3 weeks was 24% greater (in 9 controls, 150 ± 12, in 10 denervated rabbits, 186 ± 10 µmole/g/hour; P < .05). As shown in Figure 4, there was good correlation (r = 0.82) between the concentrations of the two enzymes in the adrenals for both sham-operated and denervated groups. Interestingly, the activities of the two enzymes were of similar magnitude and they were increased proportionally after denervation of the sinoaortic regions.

Discussion

Complete sinoaortic denervation in the rabbit induced elevations of systolic and diastolic blood pressure which were near maximal at 24 hours and then persisted throughout the 3-week study period. This hypertension is less than that of experimental renal hypertension and is associated with brief episodes of hypotension often precipitated by body movements (6). Unpublished observations in our laboratory and reports of others indicate that sinoaortic denervation is followed by immediate elevation of arterial pressures and heart rates (12) which persist.

TABLE 4
Effect of Sinoaortic Denervation on Tyrosine Hydroxylase Activity in Ventricles and Adrenals

<table>
<thead>
<tr>
<th>Time after operation</th>
<th>µmole/g/hr Left ventricle</th>
<th>µmole/g/hr Both adrenals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operation (3, 6)</td>
<td>3.5 ± 1.1</td>
<td>11 ± 4.2*</td>
</tr>
<tr>
<td>Denervation (3, 6)</td>
<td>6.4 ± 0.5</td>
<td>32 ± 4.3*</td>
</tr>
<tr>
<td>Three weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operation (9, 9)</td>
<td>4.0 ± 0.5</td>
<td>17 ± 2.4</td>
</tr>
<tr>
<td>Denervation (9, 9)</td>
<td>6.0 ± 1.0</td>
<td>30 ± 5.4</td>
</tr>
</tbody>
</table>

Values are means ± SEM, numbers in parentheses represent experiments in ventricles and in adrenals.

*P < .05. If values for all sham-operated animals are compared with those for all denervated animals, the increases were significant at P < .02 and < .01 for ventricles and adrenals, respectively.
Co-regulatory enzymes in adrenal epinephrine biosynthesis. Values for adrenal medulla phenylethanolamine-N-methyl transferase (PNMT) and tyrosine hydroxylase are given for 13 denervated and 12 sham-operated rabbits.

for long periods (4). The tachycardia may result from a reduction in vagal tone as well as enhanced sympathetic nerve activity, and it declined somewhat over the 3-week period. Minimal changes in pressure and pulse rate in some rabbits may have been a result of incomplete surgical ablation or anatomic variations of the buffer nerves. Large elevations in pressure and pulse rate in several animals were often associated with marked enlargement of the left ventricle and decrease in cardiac norepinephrine content. However, there was no significant overall correlation of the physiologic variables with hypertrophy or norepinephrine concentration. There was no gross pathophysiologic evidence of heart failure in denervated animals at either time period.

Left ventricular norepinephrine concentration was decreased at both 2 days and 3 weeks in denervated animals, and dilutional hypertrophy contributed to the reduction. In general, hypertrophy was associated with decreased norepinephrine content. However, the latter was also present in left ventricles of normal size. Cardiac norepinephrine depletion occurs in congestive heart failure and after sympathetic denervation, with an associated (perhaps causative) marked reduction of tyrosine hydroxylase activity (13). If sympathetic denervation had occurred unintentional-

TABLE 5
Effect of Sinoaortic Denervation on Comparative Rates of Cardiac Norepinephrine Synthesis

<table>
<thead>
<tr>
<th>Method</th>
<th>Sham operation (mumoles/g/hr)</th>
<th>Denervation (mumoles/g/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine turnover (34)</td>
<td>0.76 ± .05</td>
<td>0.90 ± .08</td>
</tr>
<tr>
<td>Tyrosine hydroxylase activity (18)</td>
<td>4.0 ± 0.5</td>
<td>6.0 ± 1.0</td>
</tr>
<tr>
<td>In-vivo tyrosine-3H (6)</td>
<td>2.1 ± 0.4</td>
<td>4.7 ± 0.9*</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM for numbers of animals given in parentheses.

*This is the only increase which is significant (P < .05).
ly during buffer denervation (unlikely because of the location of sympathetic nerves relative to the resected nerves), it might account for the reduction in cardiac norepinephrine but not that of adrenal medulla epinephrine. The restoration at 3 weeks of the 2-day adrenal epinephrine deficit in denervated rabbits and the intact cardiac uptake of exogenous DL-norepinephrine-\(^3\)H, suggest that storage mechanisms were intact but that utilization surpassed synthesis for a time following sinoaortic denervation.

There has been controversy regarding the accuracy of norepinephrine turnover rates after DL-norepinephrine-\(^3\)H in quantifying norepinephrine synthesis. Studies with perfused rat spleen (14) indicate that newly synthesized norepinephrine does not mix evenly with storage norepinephrine but is preferentially released as neurotransmitter. The present findings tend to be in agreement with this concept, since the increase in synthesis rates after DL-norepinephrine was not as striking as in the in-vivo precursor method (Table 5). For calculating the latter, it was assumed that none of the formed amines were released during the 30-minute period. Further, and critical to the validity of the comparative increase, equal distribution of the precursor was required. The identical tyrosine specific activities of the two groups were evidence for this. This method afforded an estimation of synthesis rates only, since the determination of absolute values requires knowledge of the mean specific activity of tyrosine at the intraneuronal locus of norepinephrine synthesis. While mean specific activity of tissue tyrosine following the injection of the isotope can be determined mathematically from its rate of tissue decay, this is more accurately determined following a constant infusion. Further, as discussed by Sedvall and associates, (9), the specific activity of tyrosine in the tissue at the intraneuronal locus of norepinephrine synthesis is probably not greater than the average tyrosine specific activity which is determined after homogenization. The latter is assumed to be a minimal tyrosine specific activity and thus yields a maximal synthesis rate.

This is the first report of increased tyrosine hydroxylase activity in intact animals. Its assay at optimal conditions with saturating amounts of substrate indicates the capacity for maximal norepinephrine synthesis. Recently, increased activity of tyrosine hydroxylase was reported after stimulation of the isolated vas deferens (15), contrary to previous findings of unchanged tyrosine hydroxylase activity in stimulated isolated organs (16, 9) and animals (17). The increased activity of tyrosine hydroxylase in the tissues of denervated rabbits may be accounted for by the long duration of the stimulus and the physiologic intactness of these rabbits. The enzyme increase may be a result of inducer substances formed after increased sympathetic activity or to derepression following reduction of norepinephrine at a critical cell locus. It is presumed from the magnitude of increased in vivo incorporation of labeled tyrosine into norepinephrine that norepinephrine reduction also occurred at an allosteric site, resulting in diminished feedback inhibition. Similar mechanisms and perhaps the additional implication of increased steroid formation in the adrenal cortex may explain the changes in PNMT activity. The PNMT increase and the restoration of adrenal epinephrine to normal suggest that this enzyme is co-regulatory with tyrosine hydroxylase for epinephrine synthesis.

Autonomic dysfunction, as reflected by abnormal sympathetic neurotransmitter metabolism, has been described infrequently in human essential hypertension (1, 2). Numerous studies (e.g., 2, 18) have failed to demonstrate altered catecholamine metabolism in this disorder. The findings, relating a sympathoadrenal role to human hypertension, both positive and negative, are difficult to interpret since the catecholamine studies were performed indirectly on plasma and urine. Direct tissue investigations of catecholamine metabolism have been correlated with the hypertension of three animal models: (1) the salt-loaded, DOCA-treated uninephrectomized rat, having defective norepinephrine
storage (19); (2) the rat with renal artery stenosis having increased norepinephrine turnover (20); and (3) the spontaneously hypertensive rat having increased norepinephrine binding (21). The findings in the respective models suggest that, in the first case, integrity of norepinephrine storage granules is influenced by the state of sodium balance; in the second, the sympathetic nervous system may serve as a secondary causative factor in renal hypertension, and studies of the inbred rat indicate that reduced cardiac catecholamine turnover may be secondary to the elevated blood pressure (22).

The present paper describes abnormalities of tissue catecholamine metabolism in another experimental model—rabbit with sinoaortic denervation—which has some physiologic characteristics of the early stages of uncomplicated essential hypertension (4, 6). Further, neither the human disorder nor this model (4) has the gross alteration in sodium metabolism or renal function present in the first two models (19, 20). The data from the present studies are consistent with the following postulated sequence of events: Increased sympathetic outflow results in hypertension and intraneuronal catecholamine reduction. This is rapidly followed by a compensatory enhancement of neurotransmitter synthesis via increased activity and concentration of the regulatory enzymes. These adjustments serve to maintain the increased blood pressure. Similar rapid increments in in-vivo cardiac norepinephrine synthesis have been described after stimulation of the cardiac nerves (16) and in stress (17). The relation of the biochemical changes to the nature and course of the hypertension after sinoaortic denervation is of additional importance. The cardiac and adrenal catecholamine concentrations and rates of biosynthesis may become normal while adjustments in the peripheral arterioles sustain the initial blood pressure elevation. In some humans with hypertension, a high cardiac output pattern at the onset reverts toward a normal output and higher total peripheral resistance (5). The clinical implication of the biochemical findings of enhanced norepinephrine synthesis in the hypertensive sinoaortic denervated rabbit is directed to the genesis of essential hypertension in man, whereby a neurogenic stimulus (23) may play a dominant but transient role.

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References


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