Synthesis and Turnover of Norepinephrine in the Heart of the Spontaneously Hypertensive Rat

By William J. Louis, M.D., Sydney Spector, Ph.D., Ryo Tabei, M.D., and Albert Sjoerdsma, M.D., Ph.D.

ABSTRACT
The kinetics of norepinephrine (NE) metabolism in the heart was studied in genetically hypertensive and normotensive control Wistar rats. Concentrations of endogenous NE were similar in the two groups. However, rates of synthesis of NE were reduced in these hypertensive rats, whether calculated from the rate of decline (fractional turnover rate) of cardiac tritiated NE (\(^3\)H-NE) after intravenous injection, or estimated from levels of \(^{14}\)C-NE in the heart after injection of the precursor \(^{14}\)C-L-tyrosine. In experiments with \(^3\)H-NE the synthesis rate of NE was 30.2 ng/hour/g heart in control and 18.2 ng/hour/g heart in hypertensive rats. The levels of \(^{14}\)C-NE found in the heart of normotensive rats given \(^{14}\)C-tyrosine were up to 1.4 times those found in hypertensive rats. These findings indicate a reduced rate of release of NE in this form of hypertension and, rather than implicating NE as a primary factor, suggest a secondary, compensatory mechanism.

ADDITIONAL KEY WORDS catecholamines binding release vascular reactivity salt desoxycorticosterone acetate intraneuronal deamination

The role of catecholamines in the pathogenesis of hypertension, other than that due to pheochromocytoma, is still unclear. Recent observations have suggested that an increased rate of release of stores of endogenous norepinephrine (NE) might account for the hypertension which occurs in rats with unilateral nephrectomy given desoxycorticosterone and NaCl (DOCA/salt) (1, 2). The interpretation of changes in catecholamine metabolism in such animals is complicated, however, by the presence of cardiomegaly and variations in salt intake, which may in themselves affect NE metabolism (3-6).

In contrast with results reported in DOCA/salt hypertension in the rat, we have found evidence (7) of a reduced rate of release of NE in the hearts of a strain of genetically hypertensive rats (8), the so-called spontaneously hypertensive rat. These rats offer distinct advantages over other experimental preparations in that the hypertension can be studied before the occurrence of cardiovascular complications and in the absence of complicated manipulations such as clamping the renal artery, injecting steroids, and increasing dietary salt intake. We report here the results of further studies on the concentration, synthesis, and turnover rates of NE in the heart of the spontaneously hypertensive rat. The findings fail to implicate NE as a primary factor in this form of hypertension.

Methods
Experiments were carried out on male spontaneously hypertensive and normotensive inbred Wistar rats weighing 140 to 200 g and aged 11 to 14 weeks. At this age the rats have not yet developed cardiac hypertrophy or vascular complications (see below). Each hypertensive rat was carefully matched for age and weight with its normotensive control. Blood pressure
was measured weekly and on the day before each experiment in the unanesthetized state using a tail plethysmographic method (9).

In experiments with radioactive NE, rats were given 0.5 ml 0.9% NaCl solution containing 2.0 \( \mu \)c of 7-(\(^3\)H)-DL-norepinephrine (\(^3\)H-NE) [New England Nuclear Corporation, 7.2 c per mmole] into a tail vein and were killed by stunning at various times after injection. Hearts were rapidly removed, cooled on dry ice, weighed and homogenized in cold 0.4 N perchloric acid. After centrifugation, the clear supernatant fluid was analyzed for radioactive and total endogenous NE using the methods previously described by de Champlain et al. (1). Radioactive samples were counted in a scintillation mixture containing 60 g naphthalene, 100 ml methanol, 20 ml ethylene glycol, 8 g Omnifluor (New England Nuclear Corporation) and dioxane to 1,000 ml. This gave average counting efficiencies of 28%. Turnover rates were estimated from the rate of decline of radioactivity in the heart (10). The values for the tissue levels of NE and \(^3\)H-NE were logarithmically transformed for calculation of linearity of regression, standard error of the regression coefficient and significance of differences between regression coefficients (11).

In other experiments, rats were given 0.5 ml 0.9% NaCl solution containing 30 \( \mu \)c of L-tyrosine-14C (u.l., New England Nuclear Corporation, 370 mc/mmole) into a tail vein and were killed by decapitation at various times after injection. Blood samples were collected in heparin and centrifuged, and aliquots of plasma were analyzed for endogenous (12) and labeled tyrosine. Hearts were removed rapidly, cooled on dry ice, homogenized in cold 0.4 N perchloric acid and centrifuged, and endogenous and radioactive NE were determined essentially as above. The effluent from the alumina columns containing the \(^1\)C-tyrosine was acidified to pH 2 and passed over columns (0.6 X 8 cm) of Dowex 50 W-X8 in the sodium form. The columns were then washed with 10 ml of glass-distilled water and 25 ml 0.5 N HCl. Tyrosine was eluted with 25 ml 4 N HCl (13). After evaporation in nitrogen, aliquots were counted for radioactivity in the Omnifluor scintillation mixture and also assayed fluorometrically for endogenous levels of tyrosine. Efficiency of \(^1\)C counting averaged 80%.

Results

The course of hypertension in spontaneously hypertensive rats of the F\(_{13}\) generation up to the age of 40 weeks is illustrated in Figure 1. At all ages the systolic blood pressure of the hypertensive rats is significantly higher than that of normotensive controls. Male rats have slightly higher blood pressure than female rats of the same age; only male rats were used in the current studies. Until 10 to 15 weeks of age, spontaneously hypertensive rats show
### TABLE 1

Turnover of Norepinephrine in Hearts of Normotensive and Hypertensive Rats

<table>
<thead>
<tr>
<th></th>
<th>Normotensive</th>
<th>Hypertensive</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt. (g)</td>
<td>173.8 ± 1.4</td>
<td>173.5 ± 1.0</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Systolic B.P. (mm Hg)</td>
<td>119.5 ± 0.5</td>
<td>175.6 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart wt. (mg)</td>
<td>674.3 ± 10.8</td>
<td>665.8 ± 8.9</td>
<td>&gt;0.3</td>
</tr>
<tr>
<td>Endogenous NE (ng/g)</td>
<td>426 ± 15.0</td>
<td>439 ± 11.5</td>
<td>&gt;0.3</td>
</tr>
<tr>
<td>^3H-NE accumulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>48.5 ± 2.5</td>
<td>50.1 ± 3.0</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>2 hr</td>
<td>42.2 ± 1.7</td>
<td>44.5 ± 1.9</td>
<td>&gt;0.3</td>
</tr>
<tr>
<td>8 hr</td>
<td>28.1 ± 1.1</td>
<td>36.1 ± 1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24 hr</td>
<td>8.9 ± 0.9</td>
<td>17.5 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T½ (hr)</td>
<td>9.8</td>
<td>16.1</td>
<td></td>
</tr>
<tr>
<td>Rate constant (hr⁻¹)</td>
<td>0.071 ± 0.00058</td>
<td>0.043 ± 0.00087</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NE turnover time (hr)</td>
<td>14.1</td>
<td>23.2</td>
<td></td>
</tr>
<tr>
<td>Synthesis rate (ng/hr/g heart)</td>
<td>30.2</td>
<td>18.9</td>
<td></td>
</tr>
</tbody>
</table>

Twenty-four control and 24 hypertensive rats were given ^3H-NE and groups of 6 animals were killed at various times after injection. Results are expressed as means ± SEM. Half-lives and rate constants were derived (see text) from Figure 2. Synthesis rates were calculated from the rate constants and endogenous NE levels (10).

![Figure 2](image-url)

**Figure 2**

Disappearance rates of radioactive NE in the hearts of control and hypertensive rats after intravenous injection of ^3H-norepinephrine. Means ± SEM are shown; the actual data were converted to the logarithmic form and the curves depicted were derived by the method of least squares (11). Equations of the curves were:

- **Controls:** \( \text{dpm/g}_t = 50.3e^{-0.437t} \)
- **Hypertensives:** \( \text{dpm/g}_t = 48.9e^{-0.071t} \)

little difference in body weight from their control counterparts. After this age spontaneously hypertensive rats grow at a somewhat slower rate than controls. Progressive cardiomegaly, as measured by heart weight, occurs in spontaneously hypertensive rats after 16 ...
weeks of age but is not apparent in younger animals (14). Vascular and renal complications also occur but are uncommon in animals under 6 months of age (14).

The results of the experiments with $^3$H-NE are summarized in Table 1 and Figure 2. The 10-minute accumulation of $^3$H-NE, which is a measure of uptake, did not differ significantly in hypertensive and control rats ($P > 0.5$). It is apparent from Figure 2 that in both hypertensive and normotensive rats the disappearance of $^3$H-NE from the heart is exponential. In analyzing these data, advantage may be taken of the fact that under steady-state conditions rates of synthesis and removal of NE are equal. Accordingly, the rate of NE synthesis ($K$) can be expressed as $K = k(NE)_0$ where $k$, the rate constant of NE efflux, is the fraction of the total NE formed and lost per unit time (fractional turnover rate) and $1/k = (NE)_0/K$, the turnover time, and is the time required to synthesize an amount of NE equal to that stored in the tissue (10).

Visual inspection of the two curves (Fig. 2) indicates that the slopes are different and that the levels of $^3$H-NE at 8 hours and 24 hours are significantly higher ($P < .001$) in the spontaneously hypertensive rat. Applying curvilinear regression analysis it was calculated that

$$k_{\text{control}} = .071 \pm .00058;$$
$$k_{\text{hypertensive}} = .043 \pm .00087.$$  

These fractional turnover rates are substantially different ($P < .001$), as are the turnover times (14.1 and 23.2 hours) and the rates of synthesis of NE (Table 1). The synthesis of NE in the normotensive rat was 1.6 times that in the spontaneously hypertensive rat, being 30.2 ng/hour/g heart in controls and 18.9 ng/hour/g heart in hypertensive rats. These differences occurred in animals in which there were no significant differences in body weight, heart weight, or endogenous levels of NE (Table 1).

The rate of synthesis of NE was also determined using the precursor amino acid, $^{14}$C-tyrosine. Again there were no significant differences in body weight, heart weight, or endogenous levels of NE (Table 2), even though systolic blood pressure was substantially higher ($P < .001$) in the hypertensive rats. The levels of $^{14}$C-NE in the heart following injection of $^{14}$C-tyrosine were significantly lower in the hypertensive rats. This apparent decrease in NE synthesis rate was demonstrable at all times studied (Table 2) and was maximal at 10 minutes, when the level of $^{14}$C-NE in the heart following injection of $^{14}$C-tyrosine were significantly lower in the hypertensive rats. As shown in Figure 3, there were no differences in plasma $^{14}$C-tyrosine and $^{14}$C-tyrosine in the hearts of the two groups of rats and the plasma tyrosine concentration did not change significantly during the experiment. Thus, differences in $^{14}$C-NE levels in the heart were not at-

| TABLE 2 | Synthesis of Norepinephrine from $^{14}$C-Tyrosine in Hearts of Normotensive and Hypertensive Rats |
| Body wt. (g) | 169.7 ± 7.9 | 159.1 ± 8.1 | > 0.5 |
| Systolic B.P. (mm Hg) | 124.4 ± 0.8 | 169.3 ± 1.1 | < 0.001 |
| Heart wt. (mg) | 611.8 ± 18.6 | 582.6 ± 27.5 | > 0.3 |
| Endogenous NE (ng/g) | 396 ± 13 | 409 ± 22 | > 0.5 |
| $^{14}$C-NE accumulation (dpm/g heart) | 2047 ± 40 | 1483 ± 118 | < 0.001 |
| 10 minutes | 1482 ± 44 | 1192 ± 45 | < 0.001 |
| 60 minutes | 1404 ± 57 | 1168 ± 67 | = 0.02 |

Fifteen control and 15 hypertensive rats were given $^{14}$C-tyrosine and 5 animals were killed at various times after injection. Results are expressed as means ± SEM. dpm = disintegrations per minute.
Factors which complicate interpretation of our results include the concept that there may well be more than one dynamic pool of intraneuronal NE and that results obtained by different techniques may reflect changes in different pools (15). There is evidence that newly synthesized NE is turned over much more rapidly than stored NE and that data obtained using 14C-tyrosine may be a better index of the NE released from nerve endings onto receptors (16). The situation is complicated further by the fact that the 14C-tyrosine-method might give high or low estimates of apparent synthesis rate depending on the extent to which newly synthesized NE is "utilized" before measurements are made. There is evidence that monoamine oxidase activity is increased in both spontaneously hypertensive (17) and in DOCA/salt hypertensive rats (2). It might be argued that this increased monoamine oxidase activity could account for apparent differences in synthesis rate between the normotensive and spontaneously hypertensive rats using the tyrosine method, but it would not explain the diminished turnover rate of 3H-NE. In either case, the release of physiologically active NE from nerve endings onto cardiovascular receptors would be less in the hypertensive rat than in controls.

If the changes in turnover of NE in the hearts of spontaneously hypertensive rats reflect changes of NE turnover in other vascular areas, then they do not account for the hypertension, for release of NE in the hearts of these rats is diminished rather than increased. Nor do these changes in NE turnover explain the increased vascular reactivity to exogenous NE which occurs in hypertension (18), for there is no evidence of a diminished uptake of NE like that after administration of drugs such as cocaine and tricyclic antidepressants (19). It would seem that these changes in NE turnover are secondary to the hypertension and that they may represent a compensatory mechanism. Whether these changes are centrally or reflexly mediated or whether the unknown factor producing the hypertension leads to an increased
receptor sensitivity to NE, which in turn produces a feedback inhibition of NE synthesis, requires further study.

Since the findings in the spontaneously hypertensive rat are consistent with a diminished rate of release of physiologically active NE onto receptors, the question arises whether diminished NE release in the heart is also characteristic of other forms of experimental hypertension. In DOCA/salt hypertension, the fractional turnover of $^3$H-NE is not decreased but increased, and quite the opposite situation, i.e., an increased rate of release of physiologically active NE onto receptors, has been thought to exist and actually to account for the hypertension (1, 2). In unpublished observations we have confirmed some of the findings of de Champlain et al. (1, 2) in established DOCA/salt hypertension—in particular, the decreased endogenous levels of NE in heart, gut, and kidney and the diminished 24-hour tissue "accumulation" of $^3$H-NE. We have wondered, however, whether reported findings on the status of NE metabolism in DOCA/salt hypertension are really inconsistent with the presence of a decreased rate of release of "active" NE like that in the spontaneously hypertensive rat. The increased fractional turnover of cardiac NE in DOCA/salt hypertension is not associated with an increased total turnover (synthesis) rate (2) but is associated with a defect in granular retention of intraneuronal NE which makes the amine more accessible to deamination by monoamine oxidase (1, 2). This defect is similar to that produced by reserpine (19) and in itself seems sufficient to account for the increased fractional turnover of NE in these animals. It should be emphasized that it is not necessarily the level of monoamine oxidase (20) but the availability of the intraneuronal NE to monoamine oxidase which is important in this regard. The argument that, in addition to an increased metabolism of intraneuronal NE by monoamine oxidase, there also exists an increased rate of release of "active" NE is difficult to accept. In fact, the reported degree of increase in intraneuronal metabolism plus release seems inconsistent with the finding of a normal total NE turnover and synthesis rate (1, 2). An explanation for the difference in fractional turnover of $^3$H-NE in the two forms of experimental hypertension in the rat may be that there is in the spontaneously hypertensive animal, in addition to a diminished rate of synthesis and release of NE, an increased intraneuronal binding of NE in a form (e.g., granules) which is not accessible to monoamine oxidase.

Whatever the nature of changes in NE metabolism in DOCA/salt hypertension, it is apparent that in the spontaneously hypertensive rat the rate of release of NE in the heart is reduced. Whether similar changes in NE metabolism occur in the presence of accelerating or malignant hypertension with vascular damage remains to be determined. If the reduced rate of release of NE reported here represents a compensatory mechanism, then a breakdown in the mechanism would be expected to exacerbate the hypertension. Such a breakdown may be relevant to the problem of malignant hypertension.

Acknowledgments

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