Turnover and Synthesis of Norepinephrine in Experimental Hypertension in Rats

By Jacques de Champlain, M.D., Ph.D., Robert A. Mueller, M.D., Ph.D., and Julius Axelrod, Ph.D.

ABSTRACT
The turnover of norepinephrine and the enzymes responsible for the synthesis of catecholamines were studied in various organs of rats made hypertensive by DOCA and NaCl. After inhibition of tyrosine hydroxylase by \(\alpha\)-methyl-\(p\)-tyrosine, an increase in the rate of disappearance of endogenous norepinephrine in the heart, intestine, and spleen of hypertensive rats indicated an increased norepinephrine turnover rate in these organs. Similarly, the rate of decline of \(\textsuperscript{3}\text{H}\)-norepinephrine endogenously formed from \(\textsuperscript{3}\text{H}\)-dopamine seemed to be increased in the same organs from hypertensive animals. In contrast, the salivary glands showed no change in turnover of norepinephrine.

The conversion of tyrosine to catecholamines was normal in the hearts of the hypertensive rats but it was increased in the adrenal glands. The \(\beta\)-hydroxylation of dopamine to norepinephrine was normal or slightly increased in the heart, spleen, intestine, and salivary glands of hypertensive animals. The phenylethanolamine N-methyl transferase activity was normal in the adrenal glands. It thus seems that in hypertension produced by DOCA and NaCl the turnover of norepinephrine is increased in various organs without any detectable change in the synthesis rate, with the exception of the adrenal gland, in which it was increased. This might explain the reduction of the endogenous norepinephrine levels observed in many tissues of animals made hypertensive by DOCA and sodium.

ADDITIONAL KEY WORDS
DOCA and sodium
\(\textsuperscript{3}\text{H}\)-dopamine \(\textsuperscript{14}\text{C}\)-tyrosine
phenyl-ethanolamine-N-methyl-transferase
tyrosine hydroxylase

A decreased norepinephrine storage by the sympathetic nerves has previously been observed in various organs of rats made hypertensive by desoxycorticosterone acetate (DOCA) and sodium (NaCl) (1-3). It was subsequently found that the state of sodium balance is closely associated with the norepinephrine storage capacity in the sympathetic neurones (4). High NaCl intake was associated with a decreased storage capacity and an increase in blood pressure, while a low intake or depletion decreased blood pressure and increased the norepinephrine storage capacity of the sympathetic nerves. In hypertensive animals, an increased turnover of norepinephrine in the heart was suggested by a study of the rate of decline of the specific activity of \(\textsuperscript{3}\text{H}\)-norepinephrine (2). To get additional information on the turnover and synthesis of norepinephrine in various organs of the rats made hypertensive with DOCA and NaCl, experiments using inhibitors of norepinephrine synthesis and precursors of norepinephrine were made. The present investigation indicates that the turnover of norepinephrine is increased but that its synthesis is apparently normal in various organs of the rats made hypertensive with DOCA and NaCl.
except for the adrenal gland, in which the synthesis of catecholamines increased.

Methods

Production of Hypertension.—Male Sprague-Dawley rats weighing 70 to 90 g, after a right nephrectomy, were made hypertensive by weekly subcutaneous injection of a suspension of desoxycorticosterone pivalate (Ciba) and 1% NaCl (in tap water) to drink ad libitum for 4 to 6 weeks. Both the control and hypertensive animals were given a regular laboratory diet (Purina chow) (4).

The systolic blood pressure was measured by a pulse transducer applied to the tail of the unanesthetized animal, as previously described (2). Inhibition of Norepinephrine Synthesis.—The rate-limiting step in norepinephrine synthesis (tyrosine hydroxylase) (5) was inhibited by administration of the ethyl ester of α-methyl-p-tyrosine (Sigma Chemical) diluted in physiologic saline at a dose of 250 mg/kg ip every 3 hours. The animals were killed by a blow on the head 0, 3, and 6 hours after the beginning of treatment, and the endogenous norepinephrine levels were measured in the heart, spleen, intestine, and salivary glands. The endogenous norepinephrine levels were determined by previously described methods (6, 7).

Conversion of [3H]-Dopamine to [3H]-Norepinephrine.—Rats were injected in the tail vein with 20 μc of [3H]-dopamine (3, 4 dihydroxyphenylethyl-2-[3H]-amine HBr, 1.6 c/mmole, New England Nuclear) and were killed at various time intervals (5, 15, 30, and 120 minutes later. Heart, spleen, intestine, and salivary glands were homogenized in 10 volumes of perchloric acid 0.4N. The homogenate was centrifuged and the supernatant was adjusted to pH 8.4 with NaOH in the presence of ascorbic acid and EDTA. The supernatant was poured on a column containing 800 mg of alumina (purified Woelm neutral). The column was washed with 20 ml of distilled water and 10 ml of 0.2N sodium acetate pH 8.4. The catechols were eluted from the column with 10 ml of HCl 0.2N. The Dopamine was separated from norepinephrine by a method similar to that described by Musacchio et al. (8). The alumina eluate was titrated to pH 2.0 and chromatographed on a column (0.6 x 4.0 cm) containing Dowex 50 WX 4, Na+ form. The column was washed with 10 ml of 0.1N phosphate buffer, pH 6.5, and 20 ml of distilled water. [3H]-norepinephrine was eluted with 11 ml of 1N HCl and [3H]-dopamine was eluted with 15 ml of 2N HCl. With this procedure, the contamination of [3H]-dopamine in the [3H]-norepinephrine fraction, and vice versa, was about 10%. Appropriate corrections were made in expressing the amount of [3H]-norepinephrine formed. Synthesis of 14C-Catecholamine from 14C-Tyrosine.—The rate of conversion of 14C-tyrosine to catecholamines was studied in heart and adrenal glands by measuring the amount of 14C-catecholamine formed either 1 hour after a single-pulse intravenous injection of 15 μc of 14C-tyrosine or at the end of a 1-hour continuous infusion of 14C-tyrosine at the rate of 0.33 μc/min (total 20 μc). Before injection or infusion, the 14C-tyrosine (New England Nuclear 0.37 c/mmole) was purified by passage on an alumina column. At the end of the experiment, the organs were homogenized in 0.4N perchloric acid and the extract was passed on an alumina column. The catecholamines formed were eluted from the column with 10 ml of HCl 0.2N. The results are expressed in terms of total catecholamines formed per hour. Endogenous tyrosine levels were measured by the method described by Wong et al. (9).

Phenylethanolamine-N-Methyl Transferase (PNMT).—Adrenal PNMT was measured by a method previously described (10).

Results

Inhibition of Norepinephrine Synthesis.—The endogenous norepinephrine levels were measured in the heart, spleen, intestine, and salivary glands of control and hypertensive rats, 3 and 6 hours after inhibition of the tyrosine hydroxylase by α-methyl-p-tyrosine (250 mg/kg). As previously reported (2), the initial norepinephrine levels were lower in the heart, spleen, and gut but slightly higher in the salivary glands of the hypertensive animals (Table 1). The rate of decline of norepinephrine levels was significantly faster in the heart (P < 0.05) of the hypertensive animals (Fig. 1). The decline of norepinephrine was slightly faster in the spleen and intestine but was unchanged in the salivary glands of the hypertensive rats.

Conversion of [3H]-Dopamine to [3H]-Norepinephrine.—To obtain information on the in vivo β-hydroxylation and on the turnover of the endogenously formed [3H]-norepinephrine, animals were injected with [3H]-dopamine and were killed at various time intervals (5, 15, 30, and 120 minutes). In the hearts of control animals, a rapid formation of [3H]-norepinephrine reached a maximum level at 30 minutes and then did not significantly change for the following 1.5 hours (Fig. 2). This pattern is similar to that previously reported by Björling.
TABLE 1

Endogenous Norepinephrine Levels in Tissues from Hypertensive Rats

<table>
<thead>
<tr>
<th></th>
<th>Control (20)</th>
<th>Hypertensive (13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>111 ± 2.4</td>
<td>200 ± 19.0*</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>248 ± 7.1</td>
<td>224 ± 9.9</td>
</tr>
<tr>
<td>Heart weight (mg)</td>
<td>802 ± 19</td>
<td>906 ± 49*</td>
</tr>
<tr>
<td>Endogenous norepinephrine (µg/g wet tissue)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>1.030 ± 0.06</td>
<td>0.640 ± 0.06*</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.566 ± 0.03</td>
<td>0.457 ± 0.06</td>
</tr>
<tr>
<td>Intestine</td>
<td>0.865 ± 0.06</td>
<td>0.337 ± 0.05*</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>1.31 ± 0.07</td>
<td>1.77 ± 0.09*</td>
</tr>
</tbody>
</table>

Data obtained from the group of rats used for the initial value in the study made on the inhibition of tyrosine hydroxylase by α-methyl-p-tyrosine (Fig. 1). Number in parentheses is the number of animals. The results are given as the mean ± SEM.

* P < 0.01.

Decline in endogenous norepinephrine, expressed as percentage of initial value (see Table 1), following inhibition of tyrosine hydroxylase. The animals were injected intraperitoneally with α-methyl-p-tyrosine (350 mg/kg every 3 hours) and were killed 0, 3, or 6 hours after the first injection. Each point is the mean of 6 to 13 individual values ± SEM. The curves were derived from the weighted least squares approximation of the log of the data point (3). The rats killed 3 or 6 hours after administration of α-methyl-p-tyrosine had comparable body weights and systolic blood pressure to those of the initial group of rats shown in Table 1.

The formation of 3H-norepinephrine at various times following administration of 20 µC of 3H-dopamine i.v. Each point is the mean of 5 to 6 individual values ± SEM. Because of the cardiac hypertrophy found in hypertensive rats (1.005 ± 0.05 g vs. 0.830 ± 0.035 g for hearts of control animals) the 3H-norepinephrine levels were expressed per total heart. Values for other organs are per gram of tissue. The mean blood pressure of the hypertensive rats was 186 ± 7.0 mm Hg before the start of the experiment.
Catecholamine Synthesis from $^{14}$C-Tyrosine in Hypertension Induced by DOCA and NaCl

<table>
<thead>
<tr>
<th>Groups</th>
<th>Organ weight (mg)</th>
<th>Endogenous NE (mcg/organ)</th>
<th>Tyrosine (mcg/organ)</th>
<th>$^{14}$C-catecholamines (counts/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>After injection</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>879 ± 24</td>
<td>0.844 ± 0.05</td>
<td>26.2 ± 1.0</td>
<td>110 ± 15</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>1143 ± 58*</td>
<td>0.555 ± 0.035*</td>
<td>33.7 ± 1.4*</td>
<td>133 ± 12</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>90 ± 12</td>
<td>3.12 ± 0.34</td>
<td>1.4 ± 0.2</td>
<td>517 ± 25</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>114 ± 8</td>
<td>2.94 ± 0.03</td>
<td>1.7 ± 0.1</td>
<td>1050 ± 200*</td>
</tr>
</tbody>
</table>

The tissues were examined for $^{14}$C-catecholamines either 1 hour after injection of 15 mcg $^{14}$C-tyrosine iv or after a 1-hour infusion of 20 mcg $^{14}$C-tyrosine. Groups of 6 or 7 animals were used with either technique; results are expressed as the mean ± SEM. There was no significant difference in the specific activity of tissue tyrosine either after injection or infusion. The blood pressure of hypertensive animals was 215 ± 7.5 mm Hg and that of the control animals was 118 ± 1.5 mm Hg.

* $P < 0.01$.
† The results are expressed per pair of adrenal glands; the epinephrine content was 20.3 ± 1.9 mcg in the glands of the hypertensive rats and 15.9 ± 0.8 mcg in those of the control rats, but these values are not significantly different.

$P < 0.05$.

Discussion

The results reported in this paper demonstrate that the turnover of norepinephrine is increased in the heart, spleen, and gut of animals made hypertensive with DOCA and NaCl. These conclusions are supported by the faster disappearance of endogenous norepinephrine after inhibition of tyrosine hydroxylase, the rate-limiting step in norepinephrine synthesis, and by the increased decline of the $^{3}$H-norepinephrine formed from the precursor dopamine in tissues from hypertensive rats. These observations corroborate previous observations showing an increased rate of decline of the specific activity of $^{3}$H-norepinephrine in the hearts of hypertensive animals (3).

Such an increase in the turnover of norepinephrine could be the consequence of several mechanisms involved in the synthesis, storage, and metabolism of that amine. Norepinephrine synthesis involves hydroxylation of tyrosine to dopa, decarboxylation to dopamine, and β-hydroxylation to norepinephrine (12). Our results indicate that neither the hydroxylation of tyrosine nor the β-hydroxylation of dopamine was significantly affected in the hearts from hypertensive animals. It is unlikely that a change in the decarboxylation of dopa would affect the turnover of norepinephrine synthesis since that...
enzyme does not appear to be rate limiting (13). In the adrenal glands of hypertensive rats, however, the rate of conversion of tyrosine into catecholamine increased, an indication of greater norepinephrine synthesis rate in this tissue.

Since the norepinephrine levels were normal in the adrenal glands of the hypertensive animals, the increased synthesis rate would suggest an increased discharge of catecholamines into the blood stream. The increased norepinephrine turnover in the heart, spleen, and intestine could be due to an increased metabolism by monoamine oxidase or by catechol-O-methyl transferase. Catechol-O-methyl transferase has been shown to be unchanged in the hearts of rats made hypertensive by DOCA and NaCl (14), but there was an increase in monoamine oxidase in the hearts, but not in the other tissues, of hypertensive animals. It was also shown that monoamine oxidase was not related to hypertension or to heart content of endogenous norepinephrine but was related to heart mass (14). Tissues such as gut, kidney, and spleen from hypertensive animals showed a decrease in their capacity to retain $^{3}$H-norepinephrine as well as a lowering of their endogenous norepinephrine levels (2) without any elevation of the concentration of monoamine oxidase (14). In addition, the increased turnover in hearts of hypertensive rats could be rapidly reversed by treatment with a ganglion blocker without any changes in the concentration of monoamine oxidase (14).

Norepinephrine is stored in dense core vesicle localized at the sympathetic terminals. This permits the storage of the amine and prevents its destruction by intraneural monoamine oxidase (15). If the increased turnover observed in hypertension caused by DOCA and NaCl is a consequence of a reduced capacity to store and retain norepinephrine, such an abnormality could possibly explain the elevation of blood pressure in these animals. The abnormalities in ionic concentration in vascular tissues that have been observed with hypertension induced by DOCA and NaCl (16-19) might prevent the proper retention of norepinephrine by the storage granules, or ionic disturbance in central or peripheral nervous tissue might increase the sympathetic activity. Either mechanism would result in a greater output of norepinephrine from the storage vesicles, part of it being metabolized by intraneural monoamine oxidase and part of it leaving the nerve terminal to react with the receptors, thus leading to an elevation of blood pressure. Several observations support this hypothesis. It has been shown that the norepinephrine storage capacity is reduced in several organs of the hypertensive animals (2), mainly because of an increased turnover in the norepinephrine storage particles (3). An increase in the O-methylated and deaminated metabolites as well as in norepinephrine in the kidney (3) and urine (20) of hypertensive rats suggests an increased output of active norepinephrine from the nerve endings. The electrolytes, as well as the sympathetic tone, seem to play an important role in the pathogenesis of this type of hypertension, since it was shown that treating these animals with a sodium-deficient diet or with a ganglionic blocking agent resulted in a decrease in blood pressure and restoration of a normal norepinephrine storage capacity (4). An unchanged rate of synthesis of norepinephrine in the hearts of hypertensive animals does not rule out a faster and more efficient use of this neurotransmitter. The organs from hypertensive rats would be protected against a rapid depletion of the endogenous store because of a suggested slightly more efficient mechanism of uptake or reuptake in hearts of hypertensive rats in vivo and in vitro (2). In addition, the increased synthesis of norepinephrine by the adrenal glands might serve to increase the supply of endogenous catecholamines to these organs.

There have also been reports of changes in the turnover of norepinephrine in other forms of experimental hypertension. In the neurogenic type of hypertension (21), in the peri-nephritic type of hypertension (22), and in hypertension associated with kidney infarction (23), an increased turnover of norepinephrine was also observed in the hearts of the hyperten-
sive animals. More recently, in hypertension caused by unilateral renal artery stenosis associated with contralateral nephrectomy, a similar increased norepinephrine turnover in the heart was also observed (J. de Champlain, R. A. Mueller, and J. Axelrod, unpublished observations). In contrast, the turnover of norepinephrine was decreased in the hearts of spontaneously hypertensive animals (24, 25), but in this form of hypertension, it would seem that the changes in norepinephrine synthesis are secondary rather than primary to the hypertension. Moreover, this form of hypertension differs from the others, since spontaneously hypertensive rats do not show cardiac hypertrophy (25) and since the development and evolution of their hypertension is rather independent of sodium intake (26).

Whether these models are relevant to human hypertension is difficult to decide. There is, however, no doubt that the study of a multiplicity of models can only give more insight into the interrelationship of blood pressure regulation and sympathetic nervous activity.

References
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