Catecholamine Metabolism in Hypertensive Rats

By William J. Louis, Kenneth R. Krauss, Irwin J. Kopin, and Albert Sjoerdsma

ABSTRACT

The possible role of catecholamines in two forms of experimental hypertension in rats was investigated further. Inbred, spontaneously hypertensive (SH) Wistar rats had unchanged endogenous levels of norepinephrine (NE) in the three tissues studied, and a significantly ($P < 0.05$) decreased rate of NE synthesis ($^{14}$C-tyrosine technique) in heart and brainstem but not in gut, in comparison to normotensive Wistar rats. Also, the levels of free fatty acids (FFA) and major urinary catecholamine metabolites in plasma revealed no evidence of increased catecholamine turnover or release. Sprague-Dawley rats rendered hypertensive by treatment with desoxycorticosterone acetate and 1% salt had decreased cardiac NE concentration and increased cardiac NE turnover ($^{3}$H-NE technique) and cardiomegaly, confirming the work of others. However, urinary normetanephrine and plasma FFA did not differ from those in normal rats. These and other results fail to support but do not completely exclude a primary role for catecholamines in either type of hypertension.

ADDITIONAL KEY WORDS

norepinephrine
dopamine
homovanillic acid
3-methoxy-4-hydroxyphenylglycol
blood pressure
normetanephrine
salt
free fatty acids
desoxycorticosterone acetate

There is still uncertainty as to the role of norepinephrine in the pathogenesis of both human (1, 2) and experimental hypertension. de Champlain and co-workers (3-5) have observed changes in tissue levels and turnover of NE in rats made hypertensive with desoxycorticosterone acetate (DOCA) and salt, suggesting to them involvement of NE in the etiology of the hypertension. In contrast, our studies (6-8) on the hearts of spontaneously hypertensive (SH) rats, a different form of experimental hypertension, failed to reveal a primary abnormality of NE metabolism. We report here the results of further studies on both types of hypertension in the rat.

Methods

Experiments were carried out on male SH rats and normotensive rats weighing 140 to 200 g and aged 11 to 14 weeks (8). Each hypertensive rat was carefully matched for age and weight with a normotensive control rat. Systolic blood pressure was measured weekly and on the day before each experiment in the unanesthetized state using a tail plethysmographic technique (9). The question of a suitable control for a genetic strain of rats is of fundamental importance (8). We use normotensive Wistar rats of the same strain, bred brother to sister and housed in the same animal house under the same conditions as the hypertensive rats. Both groups of rats are then treated identically, have similar growth rates, and accept skin grafts.

In studies on the synthesis of NE from radioactive tyrosine, rats were given 0.5 ml 0.9% NaCl solution containing 20 µC of L-tyrosine-$^{14}$C (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 and labeled tyrosine. The heart, brainstem, and proximal ileum were removed rapidly, cooled on dry ice, homogenized in cold 0.4% perchloric acid, centrifuged, and analyzed for $^{14}$Clabeled and endogenous NE.
The methods used for determining endogenous and radioactive NE and endogenous and radioactive tyrosine have been reported (7).

In studies on urinary metabolites, rats were kept in individual metabolic cages for 3 days; urine was collected during the third day (during which the animals drank 30 ml 5% sucrose in water) and stored at 4°C until analyzed (within 1 week of collection). Homovanillic acid and 3-methoxy-4-hydroxyphenylglycol (MHPG) sulphate were estimated as described previously (10). Normetanephrine was measured using a modification of the method described by Haggendal (11).

Recently fed rats were killed by decapitation, and blood was collected in heparin and promptly cooled to 4°C. The unesterified fatty acids were extracted from plasma according to the method of Dole (12) and assayed by a modification of the method of Lorch and Gey (13). Some of the above procedures were also carried out on male Sprague-Dawley rats made hypertensive with daily subcutaneous injections of 1 mg DOCA in sesame oil and 1% NaCl in tap water to drink. Control rats were injected daily with sesame oil and given tap water. In addition, some of these rats were given 0.5 ml 0.9% NaCl solution containing 20 μg of 7-3H-DL-norepinephrine (New England Nuclear Corporation, 7.2 mc/mmole) into a tail vein; animals were killed by stunning at 5 minutes and 24 hours after injection. Hearts were removed and analyzed for 3H-NE and total endogenous NE (7). Some of these studies were carried out after 8 days of DOCA-salt treatment and others after 28 days.

Results

The results of studies on the synthesis of NE from 14C-tyrosine in hypertensive and control Wistar rats are summarized in Tables 1 and 2. The levels of 14C-NE in heart and brainstem but not in gut were significantly lower in hypertensive rats (Table 1). This indicates an apparent decrease in NE synthesis rate in heart and brain of hypertensive rats. There were no differences between the groups of rats in 14C-tyrosine in plasma and in the tissues, and plasma tyrosine levels did not change significantly during the experiment. Thus differences in 14C-NE were not attributable to differences in the distribution and uptake of 14C-tyrosine. Although these differences in apparent synthesis rate were statistically significant, they were not large. It is important to note, however, that with this technique there was no evidence of an increased synthesis rate of NE in any of the tissues studied. There were no differences in endogenous levels of NE in heart, brain, and gut of these animals (Table 2).

As shown in Table 3, the excretion of normetanephrine and MHPG was not significantly different in normotensive and hypertensive rats, nor was total catecholamine metabolite excretion as indicated by the sum of normetanephrine, MHPG and homovanillic acid (85.5 vs. 83.5 μg/24 hours). Normotensive rats excreted 3.6 times as much MHPG as homovanillic acid; in hypertensive rats, the ratio of these metabolites was about 2.3. As shown in Table 4, there were no significant differences in the plasma levels of free fatty acids between the two types of Wistar rats.

The effects of DOCA-NaCl treatment of Sprague-Dawley rats for 8 days and 28 days are shown in Table 5. After 8 days of treatment, systolic blood pressure levels were significantly elevated (P < 0.01) above those of control animals, and at 28 days the treated rats were obviously hypertensive (mean, 166 ± 2 mm Hg). Also, cardiac weight was slightly (P 0.05-0.1) increased in 28-day DOCA-NaCl animals. Significant (P < 0.05)
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TABLE 2

Status of Wistar Rats and Levels of Endogenous Norepinephrine in Organs

<table>
<thead>
<tr>
<th>Body wt (g)</th>
<th>Systolic BP (mm Hg)</th>
<th>Heart wt (mg)</th>
<th>Endogenous NE (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive</td>
<td>194 ± 7.7</td>
<td>122 ± 0.9</td>
<td>629 ± 24</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>189 ± 6.9</td>
<td>166 ± 1.0</td>
<td>680 ± 28</td>
</tr>
</tbody>
</table>

Animals are those in Table 1; all values are means ± se.

TABLE 3

Urinary Excretion of Major Metabolites of Norepinephrine and Dopamine in Wistar Rats

<table>
<thead>
<tr>
<th>Animal (no.)</th>
<th>NMN (µg/24 hr)</th>
<th>MHPG (µg/24 hr)</th>
<th>HVA (µg/24 hr)</th>
<th>HVA/MHPG ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive</td>
<td>1.61 ± 0.2</td>
<td>65.6 ± 4.3</td>
<td>18.3 ± 0.7</td>
<td>0.27 ± 0.01*</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>1.45 ± 0.2</td>
<td>57.4 ± 5.4</td>
<td>24.6 ± 2.3</td>
<td>0.43 ± 0.02*</td>
</tr>
</tbody>
</table>

NE metabolites are normetanephrine (NMN) and 3-methoxy-4-hydroxyphenylglycol (MHPG) sulfate; homovanillic acid (HVA) is the major metabolite of dopamine. Results shown are means ± se; excretion rates are in µg/24 hours.

*P < 0.001.

TABLE 4

Plasma Free Fatty Acids (FFA) in Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>FFA (µEq/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar (no.)</td>
<td></td>
</tr>
<tr>
<td>Normotensive (16)</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>Hypertensive (15)</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>Sprague-Dawley (no.)</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td>Control (8)</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td>DOCA-NaCl for 28 days (14)</td>
<td>0.41 ± 0.03</td>
</tr>
</tbody>
</table>

Results are expressed as means ± se.

Alternations in cardiac NE content were seen only in rats treated for 4 weeks (Table 5).

TABLE 5

Effects of DOCA and NaCl in Sprague-Dawley Rats

<table>
<thead>
<tr>
<th>Treatment group (no.)</th>
<th>Body wt (g)</th>
<th>Heart wt (mg)</th>
<th>Systolic BP (mm Hg)</th>
<th>3H-NE nC/heart</th>
<th>Urinary excretion (µg/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (10)</td>
<td>154 ± 9</td>
<td>510 ± 22</td>
<td>120 ± 2</td>
<td>790 ± 35</td>
<td>270 ± 24*</td>
</tr>
<tr>
<td>DOCA-NaCl (10)</td>
<td>140 ± 8</td>
<td>559 ± 34</td>
<td>138 ± 2</td>
<td>724 ± 51</td>
<td>245 ± 31*</td>
</tr>
<tr>
<td>28 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (10)</td>
<td>227 ± 11</td>
<td>590 ± 30</td>
<td>122 ± 1</td>
<td>869 ± 48</td>
<td>220 ± 18*</td>
</tr>
<tr>
<td>DOCA-NaCl (10)</td>
<td>181 ± 9</td>
<td>694 ± 44</td>
<td>166 ± 2</td>
<td>613 ± 42</td>
<td>203 ± 25*</td>
</tr>
</tbody>
</table>

Controls animals received daily injections of oil and tap water to drink; DOCA-NaCl animals were given 1 mg DOCA in oil subcutaneously daily and 1% NaCl to drink, for the time indicated. Values are means ± se.

*Radioactive NE in heart 5 minutes after 20 µc 3H-NE intravenously; 124 hours after.
about 30% in 28-day hypertensive rats. There were no significant ($P > 0.3$) differences in urinary normetanephrine and MHPG excretion (Table 5) and in plasma FFA levels (Table 4) between hypertensive and normotensive Sprague-Dawley rats, though the values were higher than in Wistar rats. This may be related to the daily injections received by Sprague-Dawley rats or to strain differences (8).

Discussion

The studies of NE metabolism in inbred hypertensive rats reported here confirm and extend our previous reports (7, 8) that there is no evidence of an increased turnover and release of NE in this form of hypertension. Kopin et al. (14) have demonstrated that newly synthesized NE is preferentially released by sympathetic nerve stimulation; thus measurement of levels of $^{14}$C-NE after injections or infusions of $^{14}$C-tyrosine may be the best means of studying this “active” pool of NE. The studies described here using the tyrosine technique indicate that there is a small but significant decrease in the apparent synthesis rate of NE in heart and brain of hypertensive Wistar rats but not in gut. In none of the tissues studied was there any evidence of an increased rate of NE synthesis. These results are supported by the finding of normal levels of free fatty acids in the plasma of these rats and of normetanephrine and MHPG in the urine.

Another method used to assess NE turnover rate was to measure excretion rates of homovanillic acid and MHPG in the urine. In the rat these two metabolites account for over 70% of the urinary metabolites of dopamine and NE, respectively, and their excretion may be used as an index of the total synthesis rate of catecholamines (10). Also, in this connection it has been found that the uptake of dopamine into nerve vesicles (with consequent $\beta$-hydroxylation) may be a rate-limiting step in the synthesis of NE and that changes in the homovanillic acid-MHPG ratio are a measure of the rate of conversion of dopamine to NE (10). In the hypertensive Wistar rats, there was no apparent change in total urinary catecholamine excretion, but there was an increase in the ratio of homovanillic acid to MHPG. This suggests that there may be a decrease in the conversion of dopamine to NE in these animals.

It can be argued that the normal physiologic response to elevated blood pressure should involve a diminution in sympathetic activity mediated either locally at nerve endings or via baroreceptors. The presence of normal or only slightly reduced sympathetic activity in hypertensive Wistar rats is suggested by the present findings; the sympathetics may thus still play a contributory role due to a resetting of baroreceptors. Moreover, our results do not completely exclude a primary role for NE; it is still possible that an increased turnover of NE at arteriolar nerve endings is masked in measurements of total tissue and body turnover. It is also possible that a change in NE metabolism initiated the hypertensive process earlier in the course of the disease but has now disappeared in the face of other adjustments. DeQuattro et al. (15) have proposed such a model in neurogenic hypertension following baroreceptor denervation.

Studies of $^3$H-NE turnover in DOCA-salt hypertensive rats present a somewhat different picture from that in genetic hypertension. Our results confirm in part the observations of de Champlain et al. (3-5) of normal cardiac uptake and diminished 24-hour cardiac levels of $^3$H-NE in sustained DOCA-salt hypertension. Our finding in such rats of “normal” levels of normetanephrine and MHPG in urine and free fatty acids in plasma, however, makes it difficult to accept their view that the more rapid disappearance of $^3$H-NE from heart reflects an increased rate of release of NE from cardiac nerve endings. Also, we cannot confirm the finding that these changes precede the onset of hypertension. In our studies, rats were not subjected to prior nephrectomy, and after treatment with DOCA and NaCl for 8 days, blood pressure was significantly elevated in the absence of
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statistically significant changes in the levels of NE or \(^{3}H\)-NE in heart. Even de Champlain et al. have had inconsistent results in this type of study. In one large series of animals (Table 4 in reference 16), statistically significant differences were seen only after treatment for 4 weeks and with blood pressure levels over 180 mm Hg. This is similar to findings shown here in Table 5. The rapid development of cardiac and vascular changes following injections of DOCA (17-19) makes it difficult to discuss a prehypertensive phase in this form of hypertension.

The purported degree of increased NE turnover and release in DOCA-salt hypertension seems inconsistent with the finding of normal NE synthesis rates (20). An interesting feature of this form of hypertension is the defect in granular storage of NE (4). This results in NE leaking from the vesicles into the soluble fraction of nerve endings, where it is rapidly destroyed by monoamine oxidase (MAO), with a resultant increase in deaminated catechols (4). This defect in itself may explain diminished tissue levels of NE (7, 8), and the increased turnover of NE without elevation of urinary normetanephrine. It seems to us that it is not the level of MAO (21) which matters but the availability of intragranular NE to extragranular MAO. In DOCA-salt hypertension, normal levels of MAO should be adequate to deaminate large quantities of NE without any release of intact NE, a situation similar to that following treatment with reserpine. An argument offered against this hypothesis and perhaps the key to the whole problem is the finding of grossly elevated levels of \(^{3}H\)-normetanephrine in the kidney of DOCA hypertensive rats 30 minutes after an intravenous injection of \(^{3}H\)-NE (0.9 \(\mu\)g NE) (4). de Champlain et al. state that these increased levels of \(^{3}H\)-normetanephrine in the kidneys indicate a greater release of physiologically active NE from nerve endings with preferential metabolism by catechol-O-methyltransferase (COMT). We disagree with this interpretation. The kidneys in such hypertensive rats are grossly hypertrophied (4) and probably receive a greater portion of the cardiac output and therefore of the injected \(^{3}H\)-NE; not only are there large increases in \(^{3}H\)-normetanephrine but in all other metabolites as well, the total \(^{3}H\)-catechol metabolite levels being three times greater per kidney in hypertensive rats (4). It is difficult to relate these large changes in \(^{3}H\)-normetanephrine to increased intraneuronal turnover of NE because at this time levels of \(^{3}H\)-normetanephrine in heart (4) and heart perfusate (3) are either normal or low. Furthermore, at the same time, tissue levels of \(^{3}H\)-NE are normal (4) in heart and kidney and not low as would be expected if these metabolites arose secondarily to a large release of \(^{3}H\)-NE from nerve endings. Finally, if we look at the levels of endogenous normetanephrine and MHPG in the urine, these are no different from control rats (Table 5).

A discrepancy between the excretion of endogenous normetanephrine and the levels of \(^{3}H\)-normetanephrine found in the urine after intravenous injection of \(^{3}H\)-NE may exist not only in DOCA-salt hypertension but under selected conditions also in human hypertension (22, 23). The difficulty with studying the fate of \(^{3}H\)-NE in this fashion is that its tissue distribution will be proportional to blood flow (24) so that a more rapid disappearance of \(^{3}H\)-NE from plasma may be due in large part to clearance and destruction in kidney and liver as well as uptake in organs such as heart (24). Results obtained with this procedure may thus be very sensitive to alterations in blood flow, renal clearance and rate of enzymatic degradation. Before it can be argued that changes in tritiated metabolites represent changes in release of \(^{3}H\)-NE from nerve endings, all these other factors must be shown to be constant. It also seems important to know what percent of the uptake and metabolism of the \(^{3}H\)-NE has taken place at nerve endings in arteriolar resistance vessels. Without this information, it seems unlikely that studies with \(^{3}H\)-NE will answer the fundamental question of what is going on at the arteriolar neuroreceptor complex in hypertension.
References


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