Norepinephrine Turnover in the Cardiovascular Tissues and Brain Stem of the Rabbit during Development of One-Kidney and Two-Kidney Goldblatt Hypertension

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SUMMARY To assess the role of the sympathetic and central noradrenergic neurons in one- and two-kidney Goldblatt hypertension, we examined the concentration and turnover of norepinephrine (NE) in the aorta, mesenteric artery, left ventricle, hypothalamus, midbrain, and pons medulla of hypertensive and control rabbits. Animals were made hypertensive by constriction of the left renal artery after right nephrectomy (1KGH group) or with the right kidney left intact (2KGH group), or were sham-operated on the renal artery (1KGC and 2KGC groups). At 14 days after the constriction, the blood pressure was increased to 136 ± 3 mm Hg in the 1KGH vs 98 ± 3 mm Hg in the 1KGC (p < 0.001), and 136 ± 2 mm Hg in the 2KGH vs 94 ± 2 mm Hg in the 2KGC group (p < 0.001). Turnover time in the aorta, mesenteric artery, and left ventricle in the 1KGH group was decreased to 47%, 45%, and 65% of that in the 1KGC group, respectively. Results suggest that enhanced sympathetic neuron activity in the cardiovascular system, especially in the arteries, contributes to the development of one-kidney Goldblatt hypertension. Norepinephrine turnover in the cardiovascular tissues in the 2KGH group and in the brain stem in the 1KGH and 2KGH group was not different from that in the control group. (Hypertension 4: 272-278, 1982)

KEY WORDS • norepinephrine turnover • cardiovascular tissues • brain stem • one-kidney Goldblatt hypertension • two-kidney Goldblatt hypertension

TWO types of renovascular hypertension in the rabbit, one-kidney Goldblatt (1KG) and two-kidney Goldblatt (2KG), show several contrasting features in pathophysiology, e.g., in serum potassium level, plasma renin activity, hematocrit, and circulating blood volume, or in the incidence of cerebral hemorrhage. It has been postulated that underlying pathogenetic mechanisms are also different between these two models of hypertension.

Hyperactivity of the sympathetic neurons has been implicated as one of the causal factors in experimental as well as essential hypertension. In Goldblatt hypertension, a few studies have been performed on the state of the sympathetic neuron activity. In chronic 1KG hypertensive dogs, norepinephrine (NE) turnover was decreased in the kidney and normal in the heart, and in chronic 2KG hypertensive rats, was slightly increased in the heart. Recently, Reid et al. reported that plasma NE levels are high in acute 1KG hypertension in the rat but normal in 2KG hypertension, indicating variable contribution of the sympathetic neurons. No data on NE turnover are available for the acute stage of Goldblatt hypertension, however.

There is a body of evidence that central noradrenergic mechanisms are also involved in hypertension. Norepinephrine turnover in the central nervous system decreased in deoxycorticosterone (DOC)-sodium hypertension and increased in sinoaortic denervation hypertension, and the altered activity of the central noradrenergic neurons has been considered a primary abnormality initiating hypertension. Again, there is a paucity of information about Goldblatt hypertension.

In the present study, we examined NE turnover in the cardiovascular tissues and brain stem of the rabbit.
with acute 1 KG and 2 KG hypertension to evaluate the role of the sympathetic and central noradrenergic mechanism in these models of hypertension.

Methods

Male New Zealand white rabbits weighing 2.0 to 2.5 kg were fed on 100 g pellets for rabbits (CR 1, Japan Clea), which provided 14 mEq sodium and 21 mEq potassium per day, and water ad libitum. After a control period of 7 days, rabbits were divided into the following four groups: a group with 1 KG hypertension (1KG group, n = 18), a control group for 1 KG hypertension (1KGC group, n = 15), a group with 2 KG hypertension (2KGH group, n = 37), and a control group for 2 KG hypertension (2KGC group, n = 40). In the 1KGH group, hypertension was produced by constriction of the left renal artery with a silver clip of 1.2 mm internal diameter 4 weeks after right nephrectomy. In the 1KGC group, a sham operation was performed on the left renal artery after nephrectomy. In the 2KGH group, hypertension was produced by constriction of the left renal artery with a silver clip of 0.9 mm internal diameter. In the 2KGC group, a sham operation was performed on the left renal artery. The right kidney was left untouched in these two groups. Previous studies have shown that these different internal diameters of the clip are suitable for obtaining 1 KG and 2 KG hypertension in the rabbit.\textsuperscript{*}20 Systolic blood pressure was measured in the conscious animal by an indirect method in the central ear artery 18 dilated by an application of small amount of xylol to the tip of the ear.

Norepinephrine Turnover

Fourteen days after renal artery constriction, NE turnover was examined in the aorta, mesenteric artery, left ventricle, hypothalamus, midbrain, and pons medulla. The rate constant of NE turnover was determined from the rate of decline in tissue NE concentration after the blockade of synthesis with DL-α-methyl-p-tyrosine methyl ester hydrochloride (α-MT, Aldrich).\textsuperscript{16, 20} Alpha-MT was injected intraperitoneally at a dose of 240 mg/kg, and the second dose of 120 mg/kg was given 3 hours after the first injection.\textsuperscript{20}

Animals were killed by rapid excision of the heart under pentobarbital anesthesia (30 mg/kg) before or 6 hours after the first injection in the experiments for 1 KG hypertension, and before or 2, 4, or 6 hours after the first injection in the experiments for 2 KG hypertension. The tissues were dissected as previously reported.\textsuperscript{20} The third to fifth branches were employed in the superior mesenteric arterial tree. The interventricular septum was included in the left ventricle. The dissected pieces of tissues were weighed and homogenized in 0.4 N perchloric acid. Norepinephrine was purified by the method of Anton and Sayre\textsuperscript{21} and measured spectrofluorimetrically.\textsuperscript{22} The recovery of NE averaged 81.3%, and the values of NE concentration were corrected for recovery. The interassay coefficient of variation of the NE determination was 3.78%. Turnover rate of NE was calculated as a product of tissue NE concentration and rate constant of turnover.

Statistical evaluation was made by Student $t$ test and analysis of covariance.\textsuperscript{23}

Results

One-Kidney Goldblatt Hypertension

Figure 1 shows changes in body weight and blood pressure in the 1KGH and 1KGC groups after renal artery constriction. There was no difference in body weight between the 1KGH and 1KGC group. The left renal artery was constricted with a silver clip on Day 0 4 weeks after right nephrectomy. Each point represents the mean ± S.E.M.
weight between the two groups. Constriction of the renal artery caused a gradual increase in blood pressure in the hypertensive group, which became higher than that in the control group on Day 2. On Day 14, blood pressure was 136 ± 3 mm Hg in the 1KGH group and 98 ± 3 mm Hg in the 1KGC group (p < 0.001).

The NE concentration in the left ventricle was lower in the 1KGH group than in the 1KGC group (6.01 ± 0.27 μg) did not differ from that in the 1KGC group (6.05 ± 0.42 μg), and the reduced concentration of NE was due to the increased left ventricular mass in the 1KGH group (3.74 ± 0.09 g) as compared with the 1KGC group (2.92 ± 0.07 g, p < 0.01). There was no difference in NE concentration in the other tissues.

Figure 2 shows the decline in NE concentration in the tissues after the administration of α-MT in the 1KGH and 1KGC group. The rate constant and turnover rate of NE, which are summarized in Table 1, were calculated from the data before and 6 hours after administration of α-MT. The rate constant of NE turnover in the central nervous system did not differ among the groups. Because of the greater rate constant, turnover rate of NE in the cardiovascular tissues of the 1KGH group was greater than that of the 1KGC group, indicating increased amount of NE liberated per each gram of tissue.

Two-Kidney Goldblatt Hypertension

Figure 3 shows changes in body weight and blood pressure in the 2KGH and 2KGC group. There was no difference in body weight. Similar to the 1KGH group, blood pressure in the 2KGH group increased gradually after the renal artery constriction and became significantly higher than that in the 2KGC group on Day 2. On Day 14, blood pressure was 136 ± 2 mm Hg in the 2KGH group and 94 ± 2 mm Hg in the 2KGC group (p < 0.001).

Table 2 summarizes the NE concentration in various tissues. The left ventricle in the 2KGH group had a significantly lower concentration of NE than in the

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**Table 1. Concentration, Rate Constant of Turnover and Turnover Rate of Norepinephrine in Tissues of Rabbits with One-Kidney Goldblatt Hypertension (1KGH) and of Control Rabbits (1KGC)**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1KGH (μg/g)*</th>
<th>1KGC (μg/g)</th>
<th>Rate constant (hr⁻¹)†</th>
<th>Turnover rate (μg/g · hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>0.74 ± 0.07</td>
<td>0.74 ± 0.11</td>
<td>0.180 ± 0.027†</td>
<td>0.083 ± 0.037</td>
</tr>
<tr>
<td>Mesenteric artery</td>
<td>10.09 ± 0.60</td>
<td>9.66 ± 0.47</td>
<td>0.107 ± 0.014†</td>
<td>0.048 ± 0.012</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>1.99 ± 0.12†</td>
<td>2.58 ± 0.22</td>
<td>0.105 ± 0.018</td>
<td>0.068 ± 0.018</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>1.71 ± 0.09</td>
<td>1.85 ± 0.10</td>
<td>0.189 ± 0.020</td>
<td>0.197 ± 0.026</td>
</tr>
<tr>
<td>Midbrain</td>
<td>0.28 ± 0.01</td>
<td>0.28 ± 0.02</td>
<td>0.178 ± 0.013</td>
<td>0.172 ± 0.024</td>
</tr>
<tr>
<td>Pons medulla</td>
<td>0.41 ± 0.01</td>
<td>0.39 ± 0.03</td>
<td>0.209 ± 0.014</td>
<td>0.193 ± 0.024</td>
</tr>
</tbody>
</table>

*Each value is the mean ± SEM of nine for 1KGH and seven experiments for 1KGC.
†Each value is the mean ± SEM calculated from the data depicted in figure 2.
§p < 0.05 compared with 1KGC.
¶p < 0.001 compared with 1KGC.

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**Table 2. Concentration, Rate Constant of Turnover and Turnover Rate of Norepinephrine in Tissues of Rabbits with Two-Kidney Goldblatt Hypertension (2KGH) and of Control Rabbits (2KGC)**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>2KGH (μg/g)*</th>
<th>2KGC (μg/g)</th>
<th>Rate constant (hr⁻¹)†</th>
<th>Turnover rate (μg/g · hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>0.66 ± 0.05</td>
<td>0.80 ± 0.08</td>
<td>0.105 ± 0.019</td>
<td>0.101 ± 0.024</td>
</tr>
<tr>
<td>Mesenteric artery</td>
<td>7.65 ± 0.59</td>
<td>7.71 ± 0.40</td>
<td>0.060 ± 0.019</td>
<td>0.060 ± 0.015</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>1.97 ± 0.11†</td>
<td>2.50 ± 0.16</td>
<td>0.084 ± 0.017</td>
<td>0.080 ± 0.019</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>1.93 ± 0.08</td>
<td>1.84 ± 0.13</td>
<td>0.154 ± 0.017</td>
<td>0.159 ± 0.015</td>
</tr>
<tr>
<td>Midbrain</td>
<td>0.34 ± 0.01</td>
<td>0.31 ± 0.02</td>
<td>0.188 ± 0.013</td>
<td>0.177 ± 0.012</td>
</tr>
<tr>
<td>Pons medulla</td>
<td>0.48 ± 0.02</td>
<td>0.44 ± 0.02</td>
<td>0.197 ± 0.014</td>
<td>0.210 ± 0.014</td>
</tr>
</tbody>
</table>

*Each value is the mean ± SEM of 11 experiments both for 2KGH and for 2KGC.
†Each value is the mean ± SEM calculated from the data depicted in figure 4.
‡p < 0.025 compared with 2KGC.
FIGURE 2. Declines in tissue norepinephrine concentration after the blockade of synthesis with \( \alpha \)-methyl-\( p \)-tyrosine methyl ester in rabbits with one-kidney Goldblatt hypertension (\( \bullet \) — \( \bullet \)) and control rabbits (\( о – о \)). Each point represents the mean ± SEM based on seven to nine experiments.

2KGC group. However, there was no difference in total amount of NE in the left ventricle between the 2KGH group (5.62 ± 0.34 \( \mu \)g) and the 2KGC group (5.48 ± 0.31 \( \mu \)g), and again the lower concentration of NE was due to the significantly greater left ventricular mass in the 2KGH group (3.5 ± 0.10 g) than that in the 2KGC group (2.72 ± 0.05 g, \( p < 0.01 \)). In the other tissues there was no difference in NE concentration between the two groups.

In this series of experiments, the decline in NE concentration after the administration of \( \alpha \)-MT was analyzed more precisely (fig. 4). The rate constant of NE turnover and turnover rate, which are presented in table 2, were calculated from the data before and 2, 4,

FIGURE 3. Changes in body weight and blood pressure of rabbits with two-kidney Goldblatt hypertension (2KGH group, \( \bullet \) — \( \bullet \), \( n = 37 \)) and of control rabbits (2KGC group, \( о – о \), \( n = 40 \)). The left renal artery was constricted with a silver clip on Day 0 with the right kidney untouched. Each point represents the mean ± SEM. Differences in blood pressure between the 2KGH and 2KGC group \( p < 0.05 \) on Day 2, \( p < 0.001 \) thereafter.
and 6 hours after administration of α-MT. The rate constant was not different between the groups both in the cardiovascular tissues and in the brain stem. Because of the lower concentration of NE, turnover rate of NE in the left ventricle was slightly lower in the 2KGH than 2KGC group.

**Discussion**

The present results showed that NE turnover was increased in the aorta and mesenteric artery and tended to be higher than normal in the left ventricle of the rabbit with 1KG hypertension of 14-day duration. An alteration in rate constant of NE turnover or its reciprocal, turnover time, indicates an alteration in the activity of the sympathetic neurons innervating the examined tissues. The greater rate constants in 1KG hypertensive animals suggest that the activity of the sympathetic neurons is increased in the cardiovascular system. It has also been shown that plasma NE concentrations are elevated in 1KG hypertension of the rat 7 to 28 days after the renal artery constriction, indicating increased sympathetic tone. Therefore, increase in activity of the sympathetic neurons is not peculiar to the rabbit model.

The enhanced sympathetic drive in the cardiovascular system alone could produce hypertension. In addition, there is an increase in circulating blood volume in 1KG hypertension and the effect of enhanced neuronal activity in arteries on blood pressure may be exaggerated by the expansion of the intravascular volume. Moreover, the pressor response to NE is increased in this type of hypertension. All these findings taken together are favorable for the view that the sympathetic nervous system plays an important role in 1KG hypertension.

The mechanism by which NE turnover is increased in 1KG hypertension is not clear. It has been shown in DOC-sodium hypertension of the rat that sodium retention diminishes the capacity of NE storage granules in sympathetic neuron terminals and hence causes an increase in NE turnover. The same mechanism might as well work in 1KG hypertension, since there is a tendency to sodium retention in acute phase of 1KG hypertension. Another possible mechanism is a participation of the central nervous system. Nakamura et al. and van Ameringen et al. have demonstrated the reciprocal changes in NE turnover in the brain stem and the heart of DOC-sodium hypertensive rats, namely, decrease in NE turnover in the central nervous system and increase in the periphery. They have pointed out that the lowering in activity of the depressor area in the brain stem enhances the peripheral sympathetic neuron activity. In the present study, however, no change in NE turnover in the brain stem was observed.

In 2KG hypertension, NE turnover was normal in the cardiovascular tissues. This was contrary to our expectation that increased circulating angiotensin II would facilitate the secretion of NE via presynaptic receptor on sympathetic neurons, leading to an increase in NE turnover, since plasma renin activity is high in this model. In agreement with our results, plasma NE levels are normal in acute 2KG hypertension of the rat.
Since peripheral vascular resistance as well as cardiac output are essential hemodynamic factors controlling blood pressure, it seems important to examine NE turnover in blood vessels, especially in resistance vessels like the mesenteric artery when NE turnover is studied to reveal the contribution of the sympathetic nervous system to hypertension. In this respect, most of the previous studies on NE kinetics in experimental hypertension have employed heart or kidney. While possible changes in NE kinetics in blood vessels have been inferred from results for these organs. However, the rate of turnover of NE is not homogeneous throughout the cardiovascular system, and in addition, there is no definitive evidence that sympathetic neurons in different parts of the cardiovascular system are involved in the same way under various pathological conditions. In fact, increased discharge rates of the sympathetic neurons that innervate the mesenteric arteries have been demonstrated in spontaneously hypertensive rabbits, in which NE turnover was decreased in the heart.

The kinetics of NE in peripheral vessels has been dealt with only by DeQuattro and Alexander. They reported complex changes in NE synthesis in the mesenteric region including the mesenteric arteries and veins in sinoaortic denervation hypertension of the rabbit. We used the aorta, an elastic conduit artery, the mesenteric artery, a muscular resistant artery, and the left ventricle. In 1 KG hypertension the alteration in NE turnover was most prominent in the mesenteric artery and least in the left ventricle as judged from turnover time. This implies that the change in sympathetic tone is greater in peripheral arteries than in the heart, which in turn suggests that the sympathetic neurons contribute more in raising peripheral vascular resistance than in augmenting cardiac output. In relation to this point, it is of interest to note that in the rabbit sodium loading produced an increase in NE turnover, and sodium deprivation a decrease, in the aorta, mesenteric vein, and left ventricle, whereas they did not grossly affect both blood pressure and NE turnover in the mesenteric artery.

The NE concentration was lowered in the heart in both 1 KG and 2 KG hypertensive rabbits. Decreased concentration of NE in the heart has been repeatedly demonstrated in studies of experimental hypertension. Several authors have shown a decrease in the total content of NE of the heart. In the present study, however, there was no change in the total amount of NE, and lower concentration of NE could be ascribed to dilution by greater ventricular mass because of cardiac hypertrophy.

In the aorta and mesenteric artery, NE concentration did not change significantly. Several studies have shown decreased concentration of NE in the peripheral arteries of 1 KG hypertensive dogs, in the peripheral arteries and aorta of 2 KG hypertensive dogs, and in the distal mesenteric region of rabbits with sinoaortic denervation hypertension. Norepinephrine concentration in the carotid artery of rats with hypertension by suprarenal aortic constriction is normal. Thus, the NE concentration may be reduced or remain unchanged depending upon the development of vascular wall thickening and the state of the sympathetic tone that modulate synthesis and secretion of NE.

As to the central nervous system, neither concentration nor turnover was altered in both types of Goldblatt hypertension. In the experiments with 1 KG hypertension, NE concentration was not examined at 2 and 4 hours after α-MT, at which time it should be markedly decreased since NE turnover is rapid in the central nervous system. One may consider that this could have contributed to the failure to detect any change in central NE turnover. However, such a possibility is unlikely since our previous study showed that the administration schedule of α-MT employed in the study lowered NE concentration linearly up to 6 hours, and therefore the NE concentration at 6 hours may be regarded as a sufficient index of NE turnover.

In accord with our results is the finding that blood pressure in 1 KG hypertensive rats was not affected by the administration of L-dopa combined with a peripheral decarboxylase inhibitor, which reduced blood pressure in DOC-sodium hypertensive and spontaneously hypertensive rats. On the contrary, a destruction of the catecholaminergic neurons by intracerebroventricular injection of 6-hydroxydopamine prevented the development of 1 KG hypertension in the rat, and the associated rise in plasma NE level. It was also noted that in the very early stage of 1 KG hypertension in the rat, NE concentrations were decreased in some nuclei of the brain stem. Studies of NE turnover in small and functionally homogeneous areas in the brain stem would give conclusive information to answer these inconsistencies, since it is possible that subtle changes in NE turnover in more localized regions of the brain stem failed to be detected in this study.

From the present study it seems unlikely that the central noradrenergic neurons primarily participate in developing 2 KG hypertension of the rabbit.

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