Further Studies on the Development of Two-Kidney, Two Clip Goldblatt Hypertension in 6-Hydroxydopamine-Treated Rats

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SUMMARY The development of two-kidney, two clip Goldblatt hypertension (2K2C) in the rat seems to be unaffected by chemical sympathectomy with 6-hydroxydopamine (6-HODA) and adrenal demedullation. Since this treatment only partially depletes vascular norepinephrine (NE) content, we evaluated the degree of sympathectomy achieved with the 6-HODA treatment and the structural vascular changes in treated and untreated animals. Litters of male Wistar rats were divided in two groups: 6-HODA-treated (6-HODA) animals (group 1) were injected with 6-HODA since the day of birth until the end of the experiment; control group (CG) animals (group 2) received the vehicle solution. When rats reached about 250 g, a silver clip (0.25 mm width) was placed on both renal arteries in half of them in each group; a sham operation was performed on the rest of the animals. Adrenal demedullation and denervation was performed in all 6-HODA animals. Blood pressure was followed weekly by the tail cuff method for 7 weeks. At the 8th week, Silastic cannulas were placed in the carotid artery and jugular vein in all the animals. Pressor responses to tyramine (0.05 and 0.1 mg), angiotensin (10, 40 and 160 ng), and norepinephrine (NE) (25, 100 and 400 ng) and the hypotensive effect of prazosin (1 mg/kg) were determined in the conscious rats. The pressor effect of carotid occlusion was registered 24 hours later. Animals were sacrificed, and the heart and artery weight as well as the nucleic acids and alkali soluble proteins content in the vascular wall were determined. Hypertension developed in all the 2K2C rats and direct blood pressure was not significantly different in the 6-HODA and CG rats at the end of the experiment. Results showed that the pressor response to tyramine was abolished, while the pressor effect of NE was enhanced more than a 100% in the 6-HODA rats. Prazosin determined the same decrease in blood pressure in all the animals whether or not they were hypertensive. Denervation of both carotid arteries produced a similar effect in 6-HODA and CG rats. Structural vascular changes were observed in all hypertensive animals. These data seem to indicate that 6-HODA treatment does not completely abolish the activity of the sympathetic nervous system and discard the participation of an overactivity of this system in the development of 2K2C hypertension. On the other hand, hyperplasia of the vascular wall does not seem to be dependent on an intact sympathetic nervous activity.

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Key Words • sympathectomy • pressor responses • tyramine • prazosin • carotid occlusion • vascular changes • norepinephrine • angiotensin

A N A L Y S I S of the development of different types of experimental hypertension has produced some evidence suggesting an increased activity of the sympathetic nervous system involved in the onset and development of high blood pressure (BP). In spite of these investigations, it is not yet clear whether the sympathetic nervous system is essential for the development of experimental hypertensive in the rat. Central and peripheral noradrenergic pathways appear to participate in the onset of one-kidney, one clip renovascular hypertension. In a previous report, we observed that the increase in BP and its maintenance in two-kidney, two clip Goldblatt hypertension was not altered by neonatal sympathectomy with 6-hydroxydopamine and additional bilateral adrenomedullectomy plus adrenal denervation to prevent an enhancement of the peripheral catecholamine system. Since this treatment only partially depleted the norepinephrine (NE) content at the vascular level, the present study was planned to evaluate the efficacy of the 6-hydroxydopamine treatment by the degree of sympathectomy achieved. The effect of the
neurotransmitter not depleted by the neurotoxin was also evaluated. We have also investigated the role of sympathoadrenergic mechanisms in the development of structural vascular changes in this type of hypertension since neural stimuli have been postulated to trigger cell proliferation in the vascular wall. 12-15

To fulfill these purposes, we analyzed the pressor effect of tyramine, exogenous NE, and sympathetic nerve stimulation. Also, we have examined the hypertensive effect of an α-adrenergic blocking agent, prazosin, on treated and untreated animals. To evaluate structural vascular changes, we have measured the amount of intracellular proteins and nucleic acids in the vascular wall.

Material and Methods

Male rats of the Wistar albino strain were used. Half of the rats were injected subcutaneously with 100 µg 6-hydroxydopamine (6-HODA) per gram of body weight on the day of birth and on the following day; 10 µl of 0.5% ascorbic acid in saline was used as vehicle. Further doses of 250 µg/g in 50 µl vehicle were given on Days 8 and 15, of 100 µg/g on Days 21, 28 and 35; thereafter, weekly doses of 50 µg/g were injected until sacrifice. Control animals were injected with vehicle following the same schedule. The 6-HODA schedule was a modification of that described by Provoost et al. 16

Blood pressure was measured by the plethysmographic tail method in the conscious animals beginning at 8 weeks of age. At 2 to 4 weeks later, a silver clip 0.25 mm width was placed on both renal arteries in 12 animals of the control group (clip) and in nine animals of the treated group (clip 6-HODA), at the same time, a sham operation was performed in 11 control rats (sham) and in seven treated animals (sham 6-HODA). Adrenal glands were enucleated and denervated during the surgical procedures in all the 6-HODA-treated rats. Blood pressure was measured at weekly intervals during the following 7 weeks. During the 8th week, the animals were anesthetized with ether and indwelling permanent cannulas of Silastic tubing were placed in the carotid artery and jugular vein. On the following day, carotid BP was recorded in the conscious animals by means of a Statham transducer (P23 Dc) and a Grass polygraph. Pressor responses to intravenous tyramine (0.05 and 0.1 mg) angiotensin II (Alli)(10, 40, and 160 ng) and NE (25, 100, and 400 ng) were measured before and after recording the hypotensive effect of two doses of 0.5 mg/kg i.v. prazosin. 17 Twenty-four hours later, the pressor effect elicited by carotid occlusion was recorded in eight rats of the treated and untreated groups. Animals were killed by cervical dislocation on the following day. The heart, thoracic and abdominal aortas, renal arteries, and mesenteric artery with its finest branches were removed; loosened adventitia, blood, and adipose tissue were eliminated. Heart and arteries were immediately weighed. Deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and alkali-soluble proteins were determined in the vascular wall, as mentioned in a previous report. 18

Values reported are means ± SEM. Means were compared by Student's t test. A probability level of less than 0.05 was the criterion of significance.

Results

Effect of 6-HODA Treatment on the Development of High Blood Pressure

The BP before clipping the renal arteries was significantly lower in the treated animals (86.5 ± 1.9 vs
118.3 ± 2.2 mm Hg; p < 0.0005). After clipping, BP gradually increased during the 7 weeks in treated and untreated animals. Direct BP recorded on the 8th week showed an elevation of 46 mm for the treated rats over control animals and of 38 mm Hg for untreated rats over control animals (fig. 1). The increased BP was further confirmed by the heart and arteries wet weights, which were higher in clipped rats whether or not they had received the 6-HODA treatment (table 1).

Pressor Responses to Vasoactive Agents

Pressor response to tyramine was markedly decreased in the treated animals (−82% and −86% in sham and clip animals) and was absent after prazosin administration in both treated and untreated rats (fig. 2A). Sensitivity to NE was enhanced in 6-HODA-treated animals; prazosin administration reduced the NE pressor response in all animals, but did not abolish it (fig. 2B). Pressor response to AII was not modified.

**TABLE 1. Heart and Artery Weights: Concentration and Total Content of DNA, RNA and Proteins, and RNA/DNA and Proteins/DNA Relationships in the Artery Wall**

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 10)</th>
<th>Clip (n = 11)</th>
<th>Sham 6-HODA (n = 4)</th>
<th>Clip 6-HODA (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart/body wt (mg/g)</strong></td>
<td>2.86 ± 0.07</td>
<td>3.44 ± 0.08*</td>
<td>3.08 ± 0.04</td>
<td>3.57 ± 0.15*</td>
</tr>
<tr>
<td><strong>Arteries/body wt (mg/g)</strong></td>
<td>0.58 ± 0.03</td>
<td>0.78 ± 0.06*</td>
<td>0.63 ± 0.05</td>
<td>0.83 ± 0.05*</td>
</tr>
<tr>
<td><strong>DNA μg/100 mg tissue</strong></td>
<td>248.7 ± 11.6</td>
<td>260.3 ± 8.1</td>
<td>260.4 ± 9.8</td>
<td>247.1 ± 15.3</td>
</tr>
<tr>
<td><strong>DNA μg/organ</strong></td>
<td>631.3 ± 37.9</td>
<td>834.7 ± 78.5*</td>
<td>566.8 ± 56.9</td>
<td>697.5 ± 48.4*</td>
</tr>
<tr>
<td><strong>RNA μg/100 mg tissue</strong></td>
<td>178.3 ± 9.8</td>
<td>205.8 ± 7.7</td>
<td>197.4 ± 22.4</td>
<td>187.2 ± 7.4</td>
</tr>
<tr>
<td><strong>RNA μg/organ</strong></td>
<td>448.4 ± 20.9</td>
<td>663.8 ± 66.9*</td>
<td>417.7 ± 26.5</td>
<td>530.4 ± 34.9*</td>
</tr>
<tr>
<td><strong>Protein μg/100 mg tissue</strong></td>
<td>8521.9 ± 331.7</td>
<td>8883.4 ± 282.5</td>
<td>8125.0 ± 835.0</td>
<td>8595.2 ± 263.3</td>
</tr>
<tr>
<td><strong>Protein μg/organ</strong></td>
<td>21692.1 ± 1336.1</td>
<td>26300.5 ± 2583.2*</td>
<td>17569.5 ± 2162.7</td>
<td>24320.5 ± 1794.0*</td>
</tr>
<tr>
<td><strong>RNA/DNA</strong></td>
<td>0.72 ± 0.01</td>
<td>0.79 ± 0.10</td>
<td>0.77 ± 0.10</td>
<td>0.77 ± 0.04</td>
</tr>
<tr>
<td><strong>Proteins/DNA</strong></td>
<td>34.4 ± 2.6</td>
<td>36.6 ± 1.4</td>
<td>31.2 ± 2.5</td>
<td>35.3 ± 1.7</td>
</tr>
</tbody>
</table>

*Sham = sham-operated rats; Clip = two-kidney, two clip Goldblatt hypertensive rats; Sham 6-HODA = sympathectomized sham-operated rats; clip 6-HODA = sympathectomized two-kidney, two clip Goldblatt hypertensive rats; DNA = deoxyribonucleic acid; RNA = ribonucleic acid.

*p < 0.05.

**FIGURE 2.** Pressor effects of tyramine 0.05 and 0.1 mg and of norepinephrine 25, 100, and 400 ng before and after prazosin (1 mg/kg) i.v. in sham and hypertensive untreated and 6-HODA-treated rats.
by 6-HODA treatment or prazosin administration, suggesting that cardiovascular reactivity was not affected by either agent.

**Hypotensive Effect of Prazosin**

The fall in BP elicited by the α adrenergic blocking agent was the same in the four groups of animals. The BP decreased -31.4 ± 3.6 mm Hg in sham animals and -40.4 ± 4.8 mm Hg in clipped rats. On the other hand, prazosin diminished the level of BP -26.7 ± 4.9 mm Hg in 6-HODA sham rats and -35.6 ± 6.4 mm Hg in 6-HODA clip animals.

**Effect of Neurogenic Stimulation**

Neurogenic stimulation was accomplished by occlusion of the sole remaining carotid artery. A significant rise in BP was observed in treated (52.6 ± 10.2 mm Hg) and untreated animals (32.0 ± 6.3 mm Hg). The difference did not reach statistical significance.

**Nucleic Acids and Proteins**

Since the arteries were enlarged, DNA, RNA, and protein contents were expressed in two forms, as the concentration per 100 mg of wet tissue and, in an absolute manner, as the total amount present in the vascular sample (table 1). DNA, RNA, and protein concentrations were not significantly different among the four groups. Absolute amounts of DNA, RNA, and proteins were significantly increased in both hypertensive groups. The relationships between RNA/DNA and protein/DNA were similar in all experimental groups (table 1).

**Discussion**

To analyze the participation of a neurogenic component in the development of 2K2C Goldblatt hypertension, we have tried to eliminate the peripheral sympathetic nervous system by treating the neonatal animals with 6-HODA and maintaining this treatment until the end of the experiment, to avoid incomplete or transient denervation of the vascular bed. At the same time, we performed adrenal demedullation and denervation to abolish increased activity of the adrenal medulla, which would have stimulated an enhanced amount of circulating catecholamines and replenishment of sympathetic nerve endings at the blood vessel walls.

Present results have confirmed our previous report, showing similar increments in BP in treated and untreated animals. Similar enlargement of the heart and the arteries as well as the structural vascular changes were observed in both groups of clipped rats, supporting our observation of high BP in sympathectomized animals. Changes in nucleic acids and proteins indicated that vascular hyperplasia may develop in the absence of an intact sympathoadrenergic system. Recently, Overbeck reported the same degree of hypertrophy of the thoracic aorta in guanethidine-sympathectomized and sham-injected rats with coarctation hypertension. Similar results were found by Simon using the same drug in one-kidney, one clip Goldblatt hypertensive rats. The hypertension-related stimulus for vascular hypertrophy appears to overcome the removal of the positive trophic effects of sympathetic innervation.

Compensatory mechanisms may play an important role in the development of high BP in 6-HODA-treated rats. We have investigated the peripheral renin-angiotensin system in these animals and did not find any significant difference in plasma renin activity among treated and untreated hypertensive and normotensive rats (unpublished observations). Therefore, increased circulating All does not seem to be the mechanism that compensates for the diminished neurogenic tone. On the other hand, pressor response to exogenous All was not altered by sympathectomy, excluding also a generalized increased sensitivity of the vascular tissue to vasopressor agents as an underlying mechanism to maintain BP levels.

Provoost et al. reported an almost complete degree of sympathectomy using a similar schedule of 6-HODA injections. We were not able to confirm their findings, since in a previous report we observed only 50% depletion of NE in mesenteric arteries, confirming the results previously reported by Finch. Profound inhibition of pressor response to tyramine and marked supersensitivity to exogenous NE actually indicated that we have attained an adequate degree of sympathectomy after continuous treatment with the neurotoxin. By contrast, the hypotensive effect of prazosin was similar in all the animals, suggesting that blockade of receptors to endogenous NE at the cardiovascular nerve endings was able to eliminate the same amount of adrenergic drive in treated and untreated animals. The dose of prazosin seemed to have been adequate since it completely abolished the pressor response to tyramine and significantly diminished the effect of NE in untreated animals, according to its characteristic action on adrenergic receptors. Further confirmation of partial efficacy of 6-HODA to induce total sympathectomy was afforded by the BP response to carotid occlusion, which showed the same increase in treated and untreated animals.

The present functional studies suggest that 6-HODA was not able to completely abolish the sympathetic nerve activity. Lack of tyramine pressor effect and supersensitivity to exogenous NE indicated a certain degree of sympathectomy. However, the hypotensive effect of prazosin and the pressor response to carotid occlusion in treated animals support residual sympathetic activity. Nevertheless, it is hard to accept an increased neurogenic impulse as the main component acting on the development and maintenance of high blood pressure.

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Further studies on the development of two-kidney, two clip Goldblatt hypertension in 6-hydroxydopamine-treated rats.

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