Effect of neonatal sympathectomy with 6-hydroxydopamine on reactivity of the renin–angiotensin system in spontaneously hypertensive rats

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Summary

1. Chemical sympathectomy was produced in spontaneously hypertensive rats by intra-peritoneal injection of 6-hydroxydopamine hydrobromide (6-OHD) (100 µg/g body weight) on days 1, 4 and 7 of neonatal life and weekly thereafter until 6 weeks of age. The dose was then reduced to 50 µg/g body weight and injected every 2 weeks.

2. Studies were performed at 6 and 12 weeks of age in pentobarbital-anaesthetized rats. Plasma renin activity was measured before and 5 and 15 min after the administration of intravenous hydralazine (5 mg/kg). Tissue noradrenaline concentration was determined in brains, hearts and kidneys from rats killed immediately upon completion of blood sampling.

3. The blood pressure of 6-OHD-treated rats was significantly lower than in untreated rats at 6 weeks (117 ± 3 and 146 ± 2 mmHg respectively) and 12 weeks (151 ± 5 and 196 ± 4 mmHg respectively).

4. Kidneys and hearts from 6-OHD-treated rats demonstrated a highly significant reduction in noradrenaline concentration at both 6 and 12 weeks; brain noradrenaline in treated rats was normal at 6 weeks and reduced only to 80% of normal at 12 weeks.

5. Control (pre-hydralazine) plasma renin activity was significantly lower in 6-OHD-treated rats at 6 and 12 weeks. Nevertheless, renin release in response to intravenous hydralazine, expressed in terms of absolute values of plasma renin activity, was not significantly different in the treated and untreated rats. When the percentage increase in plasma renin activity from control to 15 min post-hydralazine samples was calculated, the response of the 6-OHD-treated rats was found to be significantly greater than the response in the untreated rats at each age.

6. These data show that treatment of neonatal rats with 6-OHD results in a significant reduction in basal activity of the renin–angiotensin system but does not interfere with the release of renin in response to hypotensive stress. In cases of severe compromise of adrenergic nervous system function, alternative mechanisms exist which allow the renin–angiotensin system to manifest a maximal response in order to maintain cardiovascular homeostasis.

Key words: hypertension, renin–angiotensin system, spontaneous hypertension, sympathectomy.

Abbreviations: ANG I, angiotensin I; 6-OHD, 6-hydroxydopamine hydrobromide.

Introduction

The activity of the renin–angiotensin system is regulated, in part, by the sympathetic nervous system. Adrenergic fibres entering the kidney via the renal nerve have been shown to innervate the juxtaglomerular apparatus in a manner similar to that of other adrenergic neuroeffector systems.
(Barajas, 1978). Electrical stimulation of the renal nerve (Vander, 1965) or administration of β-adrenergic receptor agonists into the artery of isolated perfused kidneys (Sinaiko & Mirkin, 1978) causes the secretion of renin, a response that can be blocked by the prior administration of β-adrenergic receptor antagonists (Tahe, McLain, McDonald & Schrier, 1976).

In addition to exerting a direct effect on activity of the renin–angiotensin system, the sympathetic nervous system also influences renin secretion mediated by non-neural mechanisms. Stimulation of renal nerves at frequencies which are usually sub-threshold for renin secretion enhances the output of renin induced by activation of the renal baroreceptor or macula densa (Thames & DiBona, 1979). Conversely, sectioning of the renal nerves inhibits the usual release of renin observed in response to constriction of the aorta proximal to the renal arteries when this stimulus is applied within hours of renal denervation (Stella, Calaresu & Zanchetti, 1976). The effect of elimination of renal adrenergic input on renin secretory mechanisms may be vastly different, however, if studies are conducted in chronically, rather than acutely, denervated animals. Investigations of end-organ response after denervation in other adrenergic neuroeffector systems have clearly established that receptor hypersensitivity does not appear until 7–14 days after interruption of adrenergic innervation and that the response elicited under these conditions is many-fold greater than that noted before or within 1 or 2 days after denervation (Trendelenburg & Weiner, 1962).

The present study investigated the effect of chronic denervation produced by chemical sympathectomy on activity of the renin–angiotensin system. Spontaneously hypertensive rats treated with 6-hydroxydopamine hydrobromide (6-OHD) beginning 1 day after birth were subjected to severe hypotension with hydralazine at 6 and 12 weeks of age to evaluate the capacity of the renin–angiotensin system to respond to stimuli under conditions of cardiovascular stress.

**Methods**

**Animals**

*Maintenance.* Pregnant spontaneously hypertensive rats were purchased at 16 days gestation from Taconic Farms Inc., Germantown, New York, U.S.A., and housed in light- and temperature-controlled conditions. The rats were fed tap water *ad libitum* and regular rat chow throughout the period of gestation and nursing. Offspring were similarly housed and fed after weaning at 3 weeks of age.

*Chemical sympathectomy.* This was initiated in newborn male and female rats at 1 day of age with intraperitoneal injections of 6-OHD (100 μg/g body weight) dissolved in 0.9% NaCl containing 1% ascorbic acid. This dose was repeated on postnatal days 4 and 7 and weekly thereafter until 6 weeks of age. The dose was then reduced to 50 μg/g body weight and administered every 2 weeks until 12 weeks of age. Untreated animals received 0.9% NaCl containing 1% ascorbic acid in a similar schedule.

*Studies.* Studies were performed with 6 or 12 week old male and female rats anaesthetized with pentobarbital (30 mg/kg). Equivalent numbers of each sex were included in the 6-OHD-treated groups and untreated groups at each age. Cannulae were placed in the left jugular vein for drug administration and in the right carotid artery for blood sampling and continuous monitoring of mean blood pressure with a Statham P23DB transducer and Beckman type RS Dynograph.

After steady-state conditions were achieved, a blood sample (0.3 ml) was obtained for measurement of control plasma renin activity and hydralazine (5 mg/kg) was injected intravenously. Blood samples were collected, at 5 and 15 min after injection of hydralazine, in tubes containing EDTA and immediately centrifuged at 4°C. The plasma samples were stored at −20°C for 1–2 weeks until assayed for plasma renin activity.

Upon completion of blood sampling the rats were killed and the heart, brain and kidneys removed and placed immediately on ice. The tissues were weighed and stored at −20°C until assayed for noradrenaline content.

*Analytical procedures*

*Plasma renin activity.* Rat plasma (0.05 ml) was incubated with 0-1 ml of maleate buffer (pH 6), 0-01 ml of 2,3-dimercapto-1-propanol (BAL) (1.7%) and 0-01 ml of 8-hydroxyquinoline (6-6%) at 37°C for 3 h. Plasma renin activity was determined by radioimmunoassay (Haber, Koerner, Page, Kliman & Purnode, 1969) and results were expressed as nanograms of angiotensin I h⁻¹ ml⁻¹ of plasma (ng of ANGI h⁻¹ ml⁻¹ of plasma).

*Tissue noradrenaline.* Noradrenaline was determined by using a modification of a previously described method incorporating high-pressure liquid chromatography with electrochemical detection (Keller, Oke, Mefford &
Renin activity in 6-hydroxydopamine-treated rats

Adams, 1976). Tissue samples were homogenized in perchloric acid (0.1 mol/l)/sodium bisulphite (0.4 mmol/l) and dihydroxybenzylamine was added to the solution as an internal standard. After centrifugation of the homogenate at 12,000 g for 2 min, an aliquot of supernatant was mixed with an equal volume of Tris buffer (0.5 mol/l, pH 9.6) and 10 mg of acid-washed alumina immediately added. This mixture was agitated for 10 min and the supernatant was aspirated. The alumina was then washed three times and the catecholamines were eluted from the alumina with perchloric acid (0.1 mol/l).

Concentrations of noradrenaline were expressed as ng/g wet weight of tissue. The detection threshold of this assay is 5 ng of noradrenaline. The intra-assay and interassay coefficients of variation are 3 and 5.4% respectively.

Statistical analysis

Analysis of data was performed with Student’s t-test (unpaired); a P value of less than 0.05 was considered to be significant.

Results

Effect of chemical sympathectomy on tissue noradrenaline concentration (Table 1)

After administration of 6-OHD, the noradrenaline concentration of kidneys and hearts in both 6 and 12 week old rats was less than 5% of that assayed in comparable tissues of untreated control rats. The only exception noted was in kidneys obtained from 6 week old rats in which the noradrenaline concentration was 14% of control values.

The effect of 6-OHD on depletion of brain noradrenaline was not as pronounced. No significant difference was observed between treated and untreated rats at 6 weeks of age; although a statistically significant reduction in brain noradrenaline was noted in the treated rats at 12 weeks of age, the values were reduced to only 80% of those found in the untreated rats.

Effect of chemical sympathectomy on weight (Table 2)

The weights of the female rats were consistently lower than those of the male rats in both the 6-OHD-treated and control groups at 6 and 12 weeks of age. In addition, evidence of growth retardation was present in the 6-OHD-treated rats. The weights of both the female and male rats which received 6-OHD were significantly less than the weights of the untreated female rats of the same age.

<table>
<thead>
<tr>
<th>Table 1. Tissue noradrenaline concentrations in 6-hydroxydopamine-treated and untreated spontaneously hypertensive rats</th>
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</thead>
<tbody>
<tr>
<td>Values are expressed as ng of noradrenaline/g wet weight of tissue ± SEM. * P &lt; 0.01, compared with untreated rats of same age; ** P &lt; 0.05, compared with untreated rats of same age.</td>
</tr>
<tr>
<td>Age (weeks)</td>
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<td>6 Heart</td>
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<td>6 Kidney</td>
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<td>12 Heart</td>
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<td>12 Kidney</td>
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<td>12 Brain</td>
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</tbody>
</table>

Effect of chemical sympathectomy on mean blood pressure (Table 2)

The blood pressures of the female and male rats did not differ significantly within any of the groups, with the exception of the 12 week old untreated rats. Although blood pressures of the male rats in this group were significantly greater than those of the female rats (205 ± 3 vs 192 ± 4 mmHg, P < 0.05), the pressures recorded for both sexes were in the severely hypertensive range. Therefore data of similarly treated male and female rats were combined for purposes of group comparisons at each age.

Chemical sympathectomy with 6-OHD significantly delayed but did not prevent the development of hypertension in the spontaneously hypertensive rats. The mean blood pressure of untreated rats reached 146 ± 2 mmHg by 6 weeks of age, whereas the mean blood pressure of 6-OHD-treated rats was 117 ± 3 mmHg at this age. The mean blood pressures of the untreated rats increased significantly between 6 and 12 weeks of age (196 ± 4 mmHg), whereas the mean blood pressure of the treated rats reached only 156 ± 5 mmHg (equivalent to that recorded in the 6 week old untreated rats).

After the injection of hydralazine, the blood pressure fell significantly in each group of rats. In the 6 week old rats this response, expressed either as the lowest blood pressure (mmHg) achieved or as percentage reduction in blood pressure from pre-hydrallazine values, was similar in both the 6-OHD-treated and untreated groups. However, in the 12 week old rats, the hypotensive response was significantly greater in the untreated rats when this was expressed in terms of either absolute blood pressures or percentages of control values.
TABLE 2. Effect of 6-hydroxydopamine on weight and mean blood pressure (mmHg) of spontaneously hypertensive rats at 6 and 12 weeks of age

Results are expressed as mean values (±SEM when n ≥ 3). P values were calculated by comparing data for males and females of 6-OHD-treated and untreated rats of the same age. N.S., Not significant.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Weight (g)</th>
<th>Blood pressure (mmHg)</th>
<th>Reduction in blood pressure (%)</th>
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<tr>
<td></td>
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<td>Before hydrallazine</td>
<td>15 min after hydrallazine</td>
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<tr>
<td>Untreated rats (6 weeks)</td>
<td>Male</td>
<td>3</td>
<td>115 ± 2</td>
<td>145 ± 3</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>2</td>
<td>110</td>
<td>143</td>
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<td></td>
<td>Male + female</td>
<td>5</td>
<td>114 ± 3</td>
<td>146 ± 2</td>
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<td>52 ± 4</td>
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<tr>
<td>6-OHD-treated rats (6 weeks)</td>
<td>Male</td>
<td>3</td>
<td>102 ± 1</td>
<td>118 ± 4</td>
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<tr>
<td></td>
<td>Female</td>
<td>2</td>
<td>97</td>
<td>115</td>
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<tr>
<td></td>
<td>Male + female</td>
<td>5</td>
<td>101 ± 2</td>
<td>117 ± 3</td>
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<td>50 ± 3</td>
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<tr>
<td>Untreated rats (12 weeks)</td>
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<td>3</td>
<td>274 ± 7</td>
<td>205 ± 3</td>
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<tr>
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<td>Female</td>
<td>3</td>
<td>202 ± 26</td>
<td>192 ± 4</td>
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<tr>
<td></td>
<td>Male + female</td>
<td>6</td>
<td>238 ± 20</td>
<td>196 ± 4</td>
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<td>62 ± 2</td>
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<tr>
<td>6-OHD-treated rats (12 weeks)</td>
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<td>2</td>
<td>187</td>
<td>155</td>
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<tr>
<td></td>
<td>Female</td>
<td>2</td>
<td>159</td>
<td>157</td>
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<tr>
<td></td>
<td>Male + female</td>
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<td>173 ± 14</td>
<td>156 ± 5</td>
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<td>46 ± 1</td>
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Effect of 6-hydroxydopamine (6-OHD) on plasma renin activity (Fig. 2)

Treatment with 6-OHD caused a significant reduction in control (pre-hydrallazine) plasma renin activity in the 6 week old rats (6-OHD-treated, 15 ± 2 ng of ANGI h⁻¹ ml⁻¹ of plasma; untreated, 50 ± 7 ng of ANGI h⁻¹ ml⁻¹ of plasma; P < 0.0005). Between 6 and 12 weeks of age, control plasma renin activity decreased significantly in the untreated rats (P < 0.0005). Nevertheless, plasma renin activity remained significantly lower in 6-OHD-treated rats at 12 weeks of age (6-OHD-treated, 14 ± 4 ng of ANGI h⁻¹ ml⁻¹ of plasma; untreated, 24 ± 2 ng of ANGI h⁻¹ ml⁻¹ of plasma; P < 0.0005).

Plasma renin activity increased significantly in all groups of rats after the administration of hydrallazine. The activities achieved 15 min after hydrallazine were greater at each age in the untreated than in 6-OHD-treated rats (6 weeks: 304 ± 68 vs 188 ± 16 ng of ANGI h⁻¹ ml⁻¹ of plasma respectively; 12 weeks: 143 ± 11 vs 130 ± 26 ng of ANGI h⁻¹ ml⁻¹ of plasma respectively); however, these differences were not statistically significant (P > 0.05).

Although the absolute values for plasma renin activity were greater in the groups of rats which did not receive 6-OHD, when the percentage increase in plasma renin activity from the control (pre-hydrallazine) to 15 min (post-hydrallazine) period was calculated, renin secretion was found to be significantly greater in the sympathectomized rats at each age (Fig. 2).

Discussion

Results obtained in this study and others (Jaim-Etcheverry & Zieher, 1971; Provoost & de Jong, 1976; Clark, Jones, Phelan & Devine, 1978) have established that ablation of the adrenergic nervous system by administration of 6-OHD during early neonatal life retards the development of systemic hypertension. It has been suggested from studies evaluating haemodynamic responsiveness of perfused hind-limb and tail vessels in New Zealand spontaneously hypertensive rats treated with 6-OHD that the development and maintenance of genetic hypertension may be dependent upon an intact peripheral vasomotor neuroeffector system (Clark et al., 1978). In the present study, the almost total absence of noradrenaline in cardiac...
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FIG. 1. Plasma renin activity of 6-OHD-treated (□) and untreated (○) spontaneously hypertensive rats at 6 and 12 weeks of age. After a control (C) blood sample was obtained for plasma renin activity, hydralazine (5 mg/kg) was injected intravenously and additional blood samples were obtained 5 and 15 min thereafter. * P < 0.05, compared with control rats of same age.

FIG. 2. Percentage increase in plasma renin activity after intravenous hydralazine in 6-OHD-treated (□) and untreated (○) spontaneously hypertensive rats. At each age where direct comparisons were made between treated and untreated rats a significantly greater degree of renin secretion could be demonstrated in the rats receiving 6-OHD.

Coincident with the reduction in systemic blood pressure, the administration of 6-OHD to neonatal rats also causes significant alterations in activity of the renin–angiotensin system. The direct anatomical (Barajas, 1978) and functional (Vander, 1965) relationship existing between the adrenergic neuron and renin-storage sites in the renal juxtaglomerular apparatus suggests that the plasma renin activity of peripheral blood might be expected to reflect changes in the adrenergic activity. Fluorescent histochemical and electron-microscope studies have shown that degeneration of renal adrenergic nerves occurs shortly after treatment with 6-OHD (Barajas & Wang, 1975), and this effect is confirmed in the present study by the virtual elimination of kidney noradrenaline in the sympathectomized rats. There is sufficient evidence therefore to support the concept that the reduction in resting plasma renin activity observed in 6-OHD-treated rats results from the attenuation in efferent renal adrenergic activity.

The maximal plasma renin activities achieved in the untreated rats after the administration of hydralazine were not significantly different from those of 6-OHD-treated rats at 6 and 12 weeks of age. Therefore, despite the presence of a peripheral sympathectomy and a reduction in resting plasma renin activity, the capacity of the renin–angiotensin system to respond to vasodilator therapy was not compromised. The induction of renin release by hydralazine has been attributed to reflex sympathetic activation, since it can be

and renal tissue of 6-OHD-treated rats, compared with only a modest reduction in whole-brain catecholamine concentration, also suggests that peripheral adrenergic mechanisms influence the development of genetic hypertension.
blocked by propranolol when hydralazine is given in a dose of 1 mg/kg (Pettinger, Campbell & Keeton, 1973). Although peripheral adrenergic neuronal activity was virtually eliminated in the 6-OHD-treated rats, it has recently been shown that the adrenal gland responds to chemical sympathectomy with 6-OHD by a compensatory secretion of noradrenaline (Micalizzi & Pals, 1979) to re-establish normal plasma concentrations. Because we have been unable to prevent increases in plasma renin activity in rats pretreated with propranolol when doses of hydralazine equivalent to those administered in the present study (5 mg/kg) were used (unpublished data), it would appear that the increase in renin activity noted after this dose of hydralazine resulted from the activation of mechanisms not related to the adrenergic nervous system.

Pentobarbital anaesthesia has been shown to increase plasma renin activity in rats (Pettinger, Tanaka, Keeton, Campbell & Brooks, 1975). However, in the present study, control plasma renin activities were significantly lower in the 6-OHD-treated rats at 6 and 12 weeks of age, despite the use of pentobarbital. Since it is clear that the potential response of the renin–angiotensin system of 6-OHD-treated rats is equivalent to that of untreated rats, as noted above, the significantly reduced response to pentobarbital noted in the sympathectomized rats suggests that renin release stimulated by this agent is mediated, at least in part, via the adrenergic nervous system.

The data from this study were further analysed by calculating percentage increases in plasma renin activity from control to 15 min post-hydralazine samples. With this method of analysis, the response in the 6-OHD-treated rats was found to be significantly greater than the response in the untreated rats at each age, suggesting a pattern of receptor hypersensitivity in the sympathectomized rats. Receptor hypersensitivity after either pre- or post-synaptic denervation is a well-known phenomenon in other autonomic neuroeffector systems (Trendelenburg & Weiner, 1962) or angiotensin to denervated hind-limb vessels in the rat (Clark & Phelan, 1975). The finding of a hypersensitivity-type pattern of renin release to non-adrenergic stimuli in the 6-OHD-treated rats despite the apparent absence of sympathetic input would be consistent with these previously reported results; however, whether a percentage increase in plasma renin activity or increase in absolute values of plasma renin activity is the more appropriate method of analysis to describe the response observed after the administration of hydralazine remains speculative. Nevertheless, the studies reported herein demonstrate that despite severe compromise in adrenergic nervous system function, alternative mechanisms exist which allow the renin–angiotensin system to manifest a maximal response in order to maintain cardiovascular homeostasis.

Acknowledgments

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References


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