Blood Pressure and Vascular Resistance in Genetically Hypertensive Rats Treated at Birth with 6-Hydroxydopamine

DAVID W.J. CLARK, DAVID R. JONES, E. LINTON PHELAN, AND CARRICK E. DEVINE

SUMMARY Genetically hypertensive (GH) rats of the New Zealand strain and normotensive (N) rats were sympathectomized from birth with 6-hydroxydopamine (100 mg/kg, s.c., on alternate days, seven treatments). In adult treated rats from each strain (GHTr and NTr), blood pressure was lower than normal. Functional tests and electron microscopy showed that denervation was virtually complete in mesenteric and hindlimb arteries; the innervation of the renal artery was little affected. Ganglionic blockade still caused a large fall in blood pressure in treated rats. Vascular resistance was higher in blood-perfused hindlimbs and tails of GH rats than in those of N rats; in contrast, resistance was similar in limbs and tails of GHTr and NTr rats and was greater than that found in untreated N rats. Saline-perfused limb vessels had neither neurogenic nor myogenic tone and resistance was higher in GH limbs (whether these were from treated rats or not) than in untreated N limbs. In saline-perfused NTr limbs, there was a paradoxical structural adaptation (probably luminal narrowing) of the hindlimb blood vessels and resistance was higher than in untreated N rats. The resistance of saline-perfused GH and GHTr limbs was similar. A high peripheral resistance appears to be the main mechanism sustaining genetic hypertension, and the integrity of the vasomotor sympathetic nerves is necessary for the development of this form of experimental hypertension.

A FEW DAYS after birth, the blood pressure of genetically hypertensive (GH) rats of the New Zealand strain is significantly higher than that of random-bred normotensive (N) rats. Blood pressure rises quickly in young rats, and pressures near those of the adult are present in 4-week-old N rats and 6-week-old GH rats. The discovery that injection of newborn rats with 6-hydroxydopamine hydrobromide (6-OHDA) permanently destroys or prevents the development of noradrenergic nerves has made possible a study of the etiological role of the sympathetic nervous system in genetic hypertension.

When mature rats are treated for the first time with 6-OHDA, destruction is confined to the terminal portions of the noradrenergic neuron and, as the perikaryon is unaffected, regeneration of the nerves occurs a few weeks after treatment is stopped. In newborn rats treated with high doses of 6-OHDA, the lesion is irreversible and affects the cell body and axons of many peripheral noradrenergic neurons, as well as some noradrenergic terminals in the brain. However, chronic sympathectomy...
from birth with 6-OHDA does not deprive all peripheral organs of their adrenergic innervation to the same extent; the adrenals appear to be unaffected and it is claimed that vasomotor nerves are particularly resistant to attack by 6-OHDA. Effector organs also vary in their ability to compensate for their loss of sympathetic nerves.

This paper describes the noradrenergic sympathectomy which persists into maturity when rats are treated at birth with 6-OHDA. Blood pressure, heart rate, vascular resistance in tissues perfused, either with blood or physiological saline, residual adrenergic nerve function, and the effects of adrenalectomy were compared in chronically sympathectomized (Tr) and untreated control rats of the GH and N strains. Electron microscopy of periarterial nerves and assays of endogenous noradrenaline content were performed in some tissues. Part of this work has appeared as a preliminary publication.

Methods

6-OHDA Treatment

Newborn male albino rats from the normotensive Otago stock colony and from the New Zealand strain with genetic hypertension were injected subcutaneously on alternate days from birth to the age of 13 days (seven treatments) with 6-OHDA, 100 mg/kg, freshly dissolved in a 0.9% sodium chloride solution containing ascorbic acid (0.5 mg/ml). These treated rats will be referred to as NTr or GHTr rats. Litter mates were injected with medium only (untreated rats). The rats were used for the various experiments when 13-26 weeks old (i.e., 11-24 weeks after the last treatment with 6-OHDA). Rats were fed a standard pellet diet and drank tap water.

Mean intra-arterial (femoral) blood pressures and pressor responses to tyramine (200 mg/kg, iv) and norepinephrine (0.5 µg/kg of base, iv) were measured under chloralose anesthesia (75 mg/kg, iv) in treated rats and their untreated littermates.

Heart rate was measured under light ether anesthesia by electronically counting the peaks of the QRS complexes of the ECG.

Adrenalectomy

Some treated and untreated rats were adrenalectomized and injected intraperitoneally with 0.1 mg of dexamethasone. All adrenalectomized rats drank sodium chloride solution. One week after adrenalectomy, intraarterial blood pressure was measured under light chloralose anesthesia (50 mg/kg, iv); the usual dose of chloralose markedly depressed respiration and blood pressure in adrenalectomized rats.

Vasomotor Nerve Function in Isolated Mesenteric Artery and Renal Artery Preparations

The superior mesenteric artery and the right renal artery of treated and untreated rats were isolated under ether anesthesia and perfused at a constant rate (2 ml/min, 37°C) with an oxygenated Locke's solution (composition, mmol/liter: NaCl, 154; KCl, 5.6; CaCl₂, 2.1; NaHCO₃, 2.0; glucose 5.5, gassed with 95% O₂, 5% CO₂). In both preparations, the periarterial nerves were stimulated (74 V, 1 msec, 16 Hz) for periods of 20 seconds. Responses were recorded as increases in perfusion pressure.

Vascular Resistance

Hindlimb Blood Vessels

A hindlimb was isolated from the main circulation in treated and untreated rats anesthetized with chloralose by dissecting and dividing the muscles just below the head of the femur, leaving only the femur, the femoral and sciatic nerves, and the femoral vein intact. The isolated innervated hindlimb was perfused through the cannulated femoral artery with blood taken from the opposite femoral artery. Control rats were both untreated littermates, which were heavier than treated rats, and younger untreated rats matched for weight with the 6-OHDA-treated animals.

Perfusion pressure was measured twice for periods of 3-6 minutes at each of six constant flow rates (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml/min). Flow rates were changed first in ascending order and then in descending order. The nerves to the hindlimb then were cut and, after 30 minutes, the series of flow rate changes repeated. In the perfused innervated hindlimb there was a gradual rise in vascular resistance with time; therefore, in another group of NTr and N rats, the nerves were not cut and the second series of flow rate changes were studied with the nerves intact.

At the end of some blood perfusion experiments, the rats were killed and blood was quickly washed from the pump with Locke's solution. The hindlimbs then were perfused with Locke's solution at 1.0 ml/min until the outflow from the cut femoral vein was free of blood and the perfusion pressure was stable for at least 10 minutes (about 15 minutes after the start of Locke perfusion). The limbs were perfused at seven flow rates (1, 2, 3, 4, 5, 6, and 8 ml/min), first in ascending and then in descending order. Results were rejected if there was a spontaneous rise in perfusion pressure or there was visible edema. Acetylcholine or sodium nitroprusside injected into the Locke-perfused limb caused no decrease in perfusion pressure.

The relative mean internal radius, assumed proportional to the fourth power of the flow conductance from Poiseuille's law, of a hypothetical single vessel with resistance equivalent to that of the maximally dilated hindlimb vessels, was calculated for each flow rate.

Tail Blood Vessels

Under chloralose anesthesia, the ventral artery was cannulated and the tail perfused with blood from a cannulated femoral artery at a series of six constant flow rates (0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 ml/min) as described above. The remainder of the blood supply to the tail (venous return and a collateral arterial supply) was untouched. The series of flow rate changes was repeated after ganglionic blockade with pentolinium bitartrate, 25 mg/kg, iv. In this series, the N and GH rats used as controls were matched for weight with, and thus were younger than, the NTr and GHTr rats.
The blood perfusion pump, similar in essential to what previously described, causes no detectable hemolysis, and its output is pressure-independent. Perfusion pressures were measured by Statham P23Gb transducers and displayed on a two-channel pen-recorder. The perfusion pressure due to cannula resistance was subtracted from observed perfusion pressures at each flow rate.

**Assay of Endogenous Noradrenaline**

Rats were killed under ether anesthesia by intravenous injections of air. Tissues (heart, vas deferens, spinal cord, brain stem, and cortex) for assay of endogenous norepinephrine were removed, frozen over dry ice, and stored at -15°C. Norepinephrine was measured fluorimetrically after homogenization of the tissue in acetic acid and separation of catecholamines on Dowex-50 columns.

**Electronmicroscopy of Blood Vessels**

The saphenous and muscular branches of the femoral artery supplying the gracilis anticus muscle, the small mesenteric arteries supplying the jejenum, and the right renal artery were removed from untreated and 6-OHDA-treated rats. The tissues were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), postfixed with osmium tetroxide, blocked with aqueous uranyl acetate, embedded in Epon 812, sectioned transversely, stained with lead citrate, and examined with a Hitachi HU11A electron microscope.

**Drugs**

Drugs used were angiotensin Val\(^3\)-amide (Ciba), \(\alpha\)-chloralose (BDH), dexamethasone sodium phosphate (Decadron, MSD), 6-hydroxydopamine hydrobromide (Roche Ltd and Regis Chemical Co.), (\(\alpha\))-norepinephrine bitartrate (Levophed, Winthrop), pentolinium bitartrate (May & Baker), and tyramine hydrochloride (Koch-Light). Doses are expressed as salt except for norepinephrine, which is expressed as base.

**Statistics**

Values are means with standard errors of the mean. Means were compared by Student's t-test or one-way analysis of variance.

Perfusion pressure (mean of the ascending and descending values) was plotted against flow rate for each of the four groups. Analysis of variance was used to compare perfusion pressures in pairs of groups.

### Summary of analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>No.</th>
<th>Degrees of freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>2</td>
<td>2-1</td>
</tr>
<tr>
<td>Flow rate (blood)</td>
<td>6</td>
<td>6-1</td>
</tr>
<tr>
<td>Interaction (group x flow)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Animals (the homogenous, (n_1 + n_2); within-group, animal variances were pooled)</td>
<td>Error</td>
<td>5((n_1 + n_2) - 10)</td>
</tr>
</tbody>
</table>

If the interaction term was not significant, the analysis allowed a direct test of the null hypothesis for the group effect, i.e., that the perfusion pressures in the two groups were identical at each flow rate; the level of rejection was set at 5%. A significant interaction term indicated that, for at least one identical stepwise increase in flow, the increase in perfusion pressure was statistically different in the two groups compared. The level of rejection of the null hypothesis was set at 1%. When interaction was significant, perfusion pressures at each flow rate were compared by one-way analysis of variance.

**Results**

Rates chronically sympathectomized with 6-OHDA appeared normal apart from marked ptosis; they were, however, considerably lighter than their untreated littermates (see Table 2). The food and water intake of treated rats was not abnormal.

**Intra-arterial Blood Pressure (Table 1)**

Blood pressure was significantly lower (\(P < 0.001\)) in GHTTr rats (BP range 120-155 mm Hg) than in untreated littermates (GH rats, BP range 160-193 mm Hg) and pressure also was lower (\(P < 0.001\)) in NTr rats (BP range 90-115 mm Hg) than in untreated littermates (BP range 113-140 mm Hg). Heart rate and heart weight were not significantly affected by treatment with 6-OHDA. In treated rats, pressor responses to norepinephrine were increased (\(P < 0.05\)) and responses to tyramine decreased (\(P < 0.01\)). Also consistent with widespread sympathectomy, the reduction, often to undetectable levels, of the norepinephrine content of the heart; in several other tissues not listed in Table 1 (kidney, vas deferens, spinal cord, and cerebral cortex), norepinephrine content was less the 25% of the levels in untreated controls.

Adrenalectomy did not lower blood pressure in N rats whether treated or not. In contrast, in adrenalectomized GH and GHTTr rats, blood pressure was about 15 mm Hg lower (\(P < 0.05\)) than in similar rats with intact adrenals. Thus, in GHTTr rats, the magnitude of the fall in pressure after adrenalectomy was not significantly enhanced by 6-OHDA treatment.

**Vasomotor Nerve Function**

Sympathetic vasomotor control was not completely abolished in rats treated with 6-OHDA at birth. There was a large fall in blood pressure when a ganglionic blocker (pentolinium bitartrate) was given to treated and untreated rats used for tail perfusion experiments (Table 2). Blood pressure after ganglionic blockade was about the same in N rats (93 ± 11 mm Hg), NTr rats (88 ± 8 mm Hg), GH rats (76 ± 5 mm Hg), and GHTTr rats (66 ± 6 mm Hg).

The effects of 6-OHDA on nerve function were assessed in three vascular regions. In Locke-perfused mesenteric arteries of treated rats, perfusion pressure responses to stimulation of periarterial nerves were less than 10% of the responses in untreated rats of both the normotensive strain (NTr rats, 12 ± 5.5 mm Hg, \(n = 6\); N rats, 158 ± 20.4 mm Hg, \(n = 6\), \(P < 0.001\)) and the hypertensive strain (GHTTr rats, 10 ± 6.1 mm Hg, \(n = 8\); GH rats, 173 ± 16.2 mm Hg, \(n = 7\), \(P < 0.001\)). In renal arteries from the same animals, denervation by 6-OHDA was less ef-
effective; responses to nerve stimulation in treated rats were about 70% of control values (NTr rats, 54 ± 9.6 mm Hg; N rats, 70 ± 19.1 mm Hg; GHTr rats, 59 ± 8.3 mm Hg; GH rats, 82 ± 13.2 mm Hg), and the differences were not significant.

In the blood-perfused hindlimbs of 6-OHDA-treated rats, section of all central nervous connections caused no fall in perfusion pressure; in untreated rats, there was an immediate large fall in pressure which was sustained for the duration of the experiment. Thus 6-OHDA appears to completely eliminate nervous vasomotor control of the hindlimb vasculature.

**Electronmicroscopy of Perivascular Axons**

The morphology of sympathetic axons about the renal artery and small (3–12 layers of smooth muscle cells) mesenteric and hindlimb arteries from treated rats was consistent with a differential effectiveness of 6-OHDA treatment in these vessels.

In untreated rats, unmyelinated axon bundles containing granular and agranular synaptic vesicles were plentiful about mesenteric and skeletal muscle (gracilis) arteries. The axons usually lay between 0.1 and 0.7 μm from the smooth muscle cells; occasional axons were found 1 μm from the smooth muscle cells.

In treated rats, the number of axons about these vessels was greatly reduced. No axons were visible in mesenteric arteries from seven out of 10 treated rats, and in the remaining three there was, on average, less than one axon for each medioadventitial smooth muscle cell profile. No axons were found in one gracilis blood vessel preparation; in the remaining five preparations, less than one axon per smooth muscle cell profile was seen. In treated rats, the distance between the few axons present and the smooth muscle cells was seldom less than 1 μm; furthermore, the axons were small and contained fewer synaptic vesicles and more neurotubules than normal. In some, but not all, sections from treated rats there seemed to be an increased amount of collagen between the axons and blood vessels.

In mesenteric and gracilis arteries from treated rats, the absence of small granular vesicles from most of the axons made their adrenergic nature uncertain. In contrast, axons around renal arteries from treated rats showed normal neuromuscular relationships and contained granular and agranular synaptic vesicles.

**Perfusion Pressure in Blood-Perfused Hindlimbs and Tails**

The numbers of rats used for blood perfusion experiments, their body weights, and arterial blood pressures are shown in Table 2.

In normally innervated N and GH hindlimbs and in acutely denervated GH (GHden) hindlimbs, there were at each flow rate significant negative regressions relating perfusion pressure to body weight. These regressions were not homogeneous. There was no significant regression of perfusion pressure on body weight in other groups: thus, differences in pressure related to differences in body weight could not be corrected by covariance, and analyses of variance were performed only when the two groups compared were reasonably matched for weight. However, consideration of the results obtained in the hindlimbs of littermate controls, which were heavier than treated siblings, did not alter our conclusions in any way.

Perfusion pressure in the blood-perfused hindlimb was found to increase slowly with time. If the nerves were left intact and a series of flow rate changes repeated after 15 minutes, perfusion pressures increased similarly (by 10–25% at each flow rate) in both N (n = 4) and NTr (n = 5) rats.

**Normotensive Rats**

It was unexpected to find an elevated vascular resistance in the hindlimbs and tails of 6-OHDA-treated N rats. Perfusion pressures were significantly higher in NTr hindlimbs than in N limbs (Fig. 1, A and B, F1, m = 44, P < 0.01) and also were higher in NTr tails than in N tails.

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**Table 1** Effects of Neonatal Treatment with 6-Hydroxydopamine in N and GH Rats Aged 4–6 Months

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Treated</th>
<th>Controls</th>
<th>Treated</th>
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</thead>
<tbody>
<tr>
<td>Adrenals intact</td>
<td>125 ± 2.2 (14)</td>
<td>102 ± 3.4** (7)</td>
<td>178 ± 3.4 (10)</td>
<td>141 ± 3.1** (11)</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>130 ± 3.1 (18)</td>
<td>108 ± 2.1 *** (8)</td>
<td>164 ± 4.1 (8)</td>
<td>125 ± 2.5*** (8)</td>
</tr>
<tr>
<td>Heart weight</td>
<td>254 ± 2.0 (7)</td>
<td>253 ± 3.6 (6)</td>
<td>398 ± 17.3 (6)</td>
<td>368 ± 15.2 (7)</td>
</tr>
<tr>
<td>(mg/100 g B.W.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate</td>
<td>348 ± 5.6 (14)</td>
<td>339 ± 4.3 (7)</td>
<td>410 ± 7.1 (10)</td>
<td>397 ± 7.8 (11)</td>
</tr>
<tr>
<td>(beats/min)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Heart norepinephrine</td>
<td>614 ± 10.2 (6)</td>
<td>&lt;40 (6)</td>
<td>568 ± 48.7 (6)</td>
<td>&lt;47 (7)</td>
</tr>
<tr>
<td>(ng/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pressor response (mm Hg):</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Norepinephrine</td>
<td>58 ± 2.2 (7)</td>
<td>73 ± 4.3* (7)</td>
<td>74 ± 5.4 (10)</td>
<td>93 ± 6.6* (11)</td>
</tr>
<tr>
<td>Tyramine</td>
<td>57 ± 3.2 (7)</td>
<td>25 ± 3.7** (7)</td>
<td>67 ± 3.2 (10)</td>
<td>34 ± 2.9** (11)</td>
</tr>
</tbody>
</table>

Heart norepinephrine content was less than the sensitivity of the assay (30 ng) in five of six NTr and three of seven treated GHTr rats, and the values quoted are upper limits for the content. All other values are mean ± SEM; numbers in parentheses = numbers of rats. Asterisks indicate significance of difference between means of 6-OHDA-treated rats and corresponding untreated controls [*P < 0.05, **P < 0.01, ***P < 0.001 (Student's t-test)]. B.W. = body weight.
Genetically Hypertensive Rats

Perfusion pressures were significantly lower in GHTr rats than in GH rats in the hindlimb (Fig. 1, A and B, F1,59 = 152, P < 0.01) and tail (Fig. 1, C and D, F1,55 = 19.8, P < 0.01). It is worth noting that this reverses the relationship found between the perfusion pressures in treated and untreated N rats.

The shapes of the pressure-flow curves appear similar in GHTTr and GH rats for each vascular bed, and interaction terms were not significant.

Cutting the nerves to the perfused limbs of NTr rats caused no fall in perfusion pressure (Fig. 1B, NTr vs. NTrden); in fact, there was a slight rise in pressure. In contrast, perfusion pressures in the tail vessels of NTr rats were acutely denervated (Fig. 1C). In untreated N rats, acute denervation of hindlimb or tail blood vessels reduced perfusion pressures significantly (P < 0.01) at all flow rates and changed the shape of the pressure-flow curves (N vs. Nden) in the hindlimb (Fig. 1A, F5,40 = 12.2, P < 0.01) and the tail (Fig. 1C, F5,40 = 11.7, P < 0.01). Perfusion pressures were significantly lower (P < 0.001) at all flow rates in GHden hindlimbs and tails than in GH rats.

Comparison of Normotensive and Hypertensive Rats

Perfusion pressures in the hindlimbs and tails of chronically sympathectomized hypertensive rats and normotensive rats were similar (Fig. 1B, GHTTr vs. NTr, GHTTr vs. NTr and GHTTrden vs. NTrden). This similarity in the resistance of chronically sympathectomized vessels contrasts strikingly with the situation in untreated GH and N rats; pressures were significantly higher (P < 0.01) at each rate of blood flow in GH hindlimbs (Fig. 1A, GH vs. N and GHden vs. Nden) and tails (Fig. 1C, GH vs. N and GHden vs. Nden) whether the vascular beds were normally innervated or acutely denervated.

In untreated rats perfusion pressure increased more rapidly in GH rats than in N rats as flow rate was increased in the hindlimb (Fig. 1A, GH vs. N F1,50 = 12.2, P < 0.01; GHden vs. Nden F5,55 = 8.7, P < 0.01) or the tail (Fig. 1C, GH vs. N F5,40 = 6.0, P < 0.01; GHden vs. Nden F5,40 = 15.8, P < 0.01).

Perfusion Pressures in Locke-Perfused Hindlimbs

Perfusion pressures were significantly higher in NTr rats than in untreated N rats (Fig. 2A, F1,72 = 112, P < 0.01).

In contrast, perfusion pressures were lower in GHTTr rats than in GH rats (Fig. 2A, F1,72 = 17.6, P < 0.001), but the absolute differences in pressure at each flow rate were small.

<table>
<thead>
<tr>
<th>TABLE 2 Body Weights and Mean Intra-arterial Blood Pressure during Experimentation (Chloralose Anesthesia, 75 mg/kg) of Rats Used in Perfusion Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
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<tr>
<td>----</td>
</tr>
<tr>
<td>Tail: perfusion with blood</td>
</tr>
<tr>
<td>N rats: 6-OHDA treated</td>
</tr>
<tr>
<td>Weight-matched controls</td>
</tr>
<tr>
<td>GH rats: 6-OHDA treated</td>
</tr>
<tr>
<td>Weight-matched controls</td>
</tr>
<tr>
<td>Hindlimb: perfusion with blood</td>
</tr>
<tr>
<td>N rats: 6-OHDA treated</td>
</tr>
<tr>
<td>Littermate controls</td>
</tr>
<tr>
<td>Weight-matched controls</td>
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<tr>
<td>GH rats: 6-OHDA treated</td>
</tr>
<tr>
<td>Littermate controls</td>
</tr>
<tr>
<td>Weight-matched controls</td>
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<tr>
<td>Hindlimb: perfusion with Locke's solution</td>
</tr>
<tr>
<td>N rats: 6-OHDA treated</td>
</tr>
<tr>
<td>Weight-matched controls</td>
</tr>
<tr>
<td>GH rats: 6-OHDA treated</td>
</tr>
<tr>
<td>Weight-matched controls</td>
</tr>
</tbody>
</table>

Values are means ± SEM. B.P. = blood pressure.

(1, C and D, F1,59 = 164, P < 0.01). For each vascular bed, the pressure-flow curves of NTr rats and N rats appear substantially parallel, and this is consistent with a lack of significant interaction terms in the analyses of variance.

Cutting the nerves to the perfused limbs of NTr rats caused no fall in perfusion pressure (Fig. 1B, NTr vs. NTrden); in fact, there was a slight rise in pressure. In contrast, perfusion pressures in the tail vessels of NTr rats were acutely denervated (Fig. 1C). In untreated N rats, acute denervation of hindlimb or tail blood vessels reduced perfusion pressures significantly (P < 0.01) at all flow rates and changed the shape of the pressure-flow curves (N vs. Nden) in the hindlimb (Fig. 1A, F5,40 = 12.2, P < 0.01) and the tail (Fig. 1C, F5,40 = 11.7, P < 0.01). Perfusion pressures were significantly lower (P < 0.001) at all flow rates in GHden hindlimbs and tails than in GH rats.
Characteristics of Genetic Hypertension

Before discussing the effects of chronic sympathectomy in normotensive and hypertensive rats and the relevance of these observations to an understanding of the development of genetic hypertension, we must first outline briefly some differences between GH and N rats. Genetic hypertension has a large neurogenic component and most of the difference in blood pressure between GH and N rats is removed by ganglion blockade. In chloralose-anesthetized rats, local vascular resistance is higher in blood-perfused hindlimbs and tails of GH rats than in N rats, and an increase in the neurogenically maintained part of the resistance contributes importantly to this, especially (see Fig. 1) at low rates of blood flow. Enhanced reactivity to the natural adrenergic neurotransmitter, norepinephrine, is demonstrable in the hindlimb circulation but is not the sole cause of the heightened peripheral resistance.

The experiments in which normally innervated and acutely denervated blood-perfused hindlimbs and tails and saline-perfused hindlimbs were studied have allowed us to differentiate the neurogenic, myogenic, and structural factors affecting resistance to blood flow in untreated rats. Perfusion pressures in the innervated hindlimbs of GH and N rats diverged increasingly as flow rate was increased, and the distension of the vessels must have

In untreated rats perfusion pressures were significantly higher at each flow rate in GH rats than in N rats ($P < 0.01$). Perfusion pressures also were higher in GHTr rats than NTr rats (Fig. 2A, $F_{1, 78} = 86$, $P < 0.01$).

When the calculated relative mean internal radius of the Locke-perfused blood vessel is plotted against perfusion pressure (Fig. 2B), it is seen that the lumen is narrowest in GH rats. At a perfusion pressure of 40 mm Hg, i.e., about the middle of the experimental range, the difference between the vessel radii in N and GH rats is about 15%. In N rats, the effect of 6-OHDA treatment was to decrease the apparent vessel radius by 8%, whereas in GHTr rats there was a slight increase in radius (3%) compared with untreated GH rats.

The slopes of the lines in Figure 2B do not suggest that there are great differences between the distensibilities of the blood vessels in the four groups of rats.

**Discussion**

These experiments confirm and extend previous findings that an intact sympathetic nervous system is necessary for the full development and maintenance of genetic hypertension. In rats treated with 6-OHDA shortly after birth (GHTr and NTr rats), blood pressure averaged about 20% lower than in untreated littermates. In view of this moderately large decrease in blood pressure and the fact that genetic hypertension in its established form appears to be maintained mainly by an increase in total peripheral resistance, it was expected that a decrease in local vascular resistance, comparable to the fall in blood pressure, would be found in perfused blood vessels. In GHTTr rats, vascular resistance in the two blood-perfused preparations studied was decreased, as predicted in the hindlimb, but much less than expected in the tail. However, in NTr rats, there was a paradoxical increase in resistance in both regions.
been more vigorously opposed in GH rats by some combination of the three factors. The divergence of the pressure-flow curves was more noticeable after acute denervation, indicating that explanations of the differences between adult GH and N rats must take account of myogenic and structural factors as well as the role of neurogenically maintained tone. In fact, the data from the hindlimb experiments show that when blood flow exceeded 1 ml/min, the fraction of the total resistance maintained by nonneurogenic mechanisms increased more rapidly in GH rats than N rats.

In the Locke-perfused hindlimb, neurogenic and myogenic vasomotor tone are absent, and the structural difference most likely to account for the higher perfusion pressures in GH preparations is that the blood vessels are narrower. At equivalent pressures, the calculated difference in caliber of N and GH resistance vessels is of the order of 15%; interestingly, this figure is comparable to that found by others in Japanese spontaneously hypertensive rats.

Other factors may play a part in elevating peripheral resistance (and blood pressure) in GH rats. These include enhanced vascular reactivity, a slight increase in the viscosity of the blood, and a greater contribution of the adrenals to the maintenance of blood pressure.

Nature of the Sympathectomy Induced by 6-OHDA

Three techniques are now available for more or less completely sympathectomizing newborn rats. Immunosympathectomy is incomplete and regionally selective and, because of this, its effects on the development of hypertension in GH rats or Japanese SHR have not been easy to interpret. Sympathectomy with guanethidine is claimed to be more complete than immunosympathectomy or 6-OHDA-induced sympathectomy, but there are no reports of its effects in rats that develop hypertension spontaneously.

It does not appear that the sympathetic nervous system of GH rats is either more susceptible or more resistant to the effects of treatment with 6-OHDA at birth than that of N rats. The changes recorded in later life in blood pressure, pressor responses to norepinephrine and tyramine, and on norepinephrine concentrations in peripheral and brain tissues were roughly similar. Blood pressure is higher and pressor responses to norepinephrine and tyramine are slightly greater in GH rats than in N rats, and these differences are maintained in 6-OHDA-treated rats. Chronic sympathectomy did not seem to alter the part played by the adrenals in maintaining blood pressure in genetically hypertensive rats. The evidence for widespread if incomplete sympathectomy in treated rats is strong.

When we turn to discrete vascular regions, the extent of sympathectomy is easier to assess. In view of the suggestion that the innervation of blood vessels is particularly resistant to the effects of 6-OHDA, we looked at vasomotor nerve function in three parts of the vasculature. The responses to stimulation of the periarterial nerves of perfused mesenteric arteries are small in treated rats and suggest that very few of these nerves remained functional, whereas function was apparently only slightly diminished in the nerves around renal arteries from treated rats. This contrast is also evident when the nerves around the two arteries are compared by electronmicroscopy. Axons around renal arteries from treated rats were easily identifiable as adrenergic, whereas the very few axons detected around mesenteric arteries were of doubtful type and their distance from the blood vessels made it unlikely that any adrenergic neurotransmitter released would greatly affect the tone of the vascular smooth muscle.

The periarterial nerves in the hindlimb were as susceptible to destruction by 6-OHDA as those around mesenteric arteries. Perfusion experiments in which the nerves to the hindlimb were cut show that nervous factors play little or no part in maintaining vascular resistance in NT and GHT Tr rats. This conclusion is further supported by our previous observations that neuronal uptake of noradrenaline is virtually absent in limbs of treated rats and that there is a generalized postsynaptic supersensitivity in vasoconstrictors, including those of adrenal origin.

The large fall in blood pressure and tail perfusion pressure induced in treated rats by ganglion blockade with pentolinium is not easily explained. However, the perfused tail is not vascularly isolated from the remainder of the rat, and changes in cardiac output and systemic blood pressure due to ganglion blockade of the residual cardiovascular innervation may be transmitted to the tail vessels by the collateral circulation and lead to an overestimated magnitude of neurogenically maintained resistance in this localized region. We were not able to assess directly the extent of the denervation of tail blood vessels.

Sympathectomy and the Pathogenesis of Genetic Hypertension

The development phase of hypertension in GH rats is compressed into a brief period, and any participation of the sympathetic nervous system in the initiation, as opposed to the maintenance, of genetic hypertension must occur at a very early age. Thus, with techniques which made possible substantial destruction of the adrenergic sympathetic system from birth, there was the promise of advances in our knowledge of the processes leading to the establishment of genetic hypertension.

Chronic sympathectomy from birth, whether with 6-OHDA or, as in previous studies on GH rats and Japanese SHR with antiserum to nerve growth factor, does not lower blood pressure or peripheral resistance in adult rats as much as acute pharmacological or surgical denervation. Incomplete and regionally selective sympathectomy, the sparing of adrenal medullary function, the development of postsynaptic supersensitivity, the altered trophic relationships between nerve and vascular smooth muscle, and other long-term homeostatic adjustments combine to blunt the effects of chemical sympathectomy. The percentage fall in blood pressure induced by chronic sympathectomy with 6-OHDA is similar in GH and N rats and, thus, while GHTTr rats are no longer frankly hypertensive, it is equally true that NTTr rats are not normotensive in the usual sense. However, unlike blood pressure, local resistance in the blood-per-
fused hindlimb and tail responded quite differently to stimulus of chronic denervation in the two strains of rats. Resistance is normally much higher in the blood-perfused hindlimbs and tails of GH rats than of N rats, irrespective of whether the innervated or acutely denervated vascular beds are compared. In chronically sympathectomized rats, this difference in resistance is abolished. In GHTr and NTr rats, the quantitative similarity is achieved by the resistance shifting in opposite directions from control values. In NTr blood vessels, the perfusion pressures developed at each flow rate were greater than those in vessels from normally innervated N rats; in GHTr blood vessels (except tail vessels of rats given pentolinium), perfusion pressures were lower than in comparable vessels from normal GH rats. The high vascular resistance recorded in NTr hindlimbs confirms our previous report of this phenomenon. We were able to show previously that, in the sympathectomized limbs, the absence of neuronal uptake mechanisms enhanced the effect of circulating vasoconstrictors from the adrenals but not to an extent which would account for the compensatory increase in vascular resistance. When myogenic tone is present but vasomotor innervation is virtually absent, the developmental pathways which determine vascular resistance and its ability to accommodate to changes in blood flow in the hindlimb and tail terminate almost identically in adult GH and N rats. When myogenic tone is absent, as in the Locke-perfused hindlimb, structurally determined resistance is higher in GHTr rats than in NTr rats, and this may be added as evidence for an intrinsic difference between the blood vessels of GH and N rats. However, an alternative interpretation which describes this structural change as secondary to the residual hypertension in GHTr rats cannot be ruled out.

Genetic hypertension cannot develop fully in the absence of an intact peripheral noradrenergic nervous system. However, in sympathectomized rats, cardiovascular homeostasis is powerful, and it is necessary to be cautious in drawing conclusions from ablation experiments as to the role of the sympathetic nervous system in the etiology of genetic hypertension. A high peripheral resistance maintains genetic hypertension and, in the GH hindlimb and tail, the vascular changes that are the cause of the increased resistance are dependent on the integrity of the sympathetic nerves for their initiation. The interaction during development between vascular smooth muscle and its innervation is apparently different in GH and N rats, and the implication is that the sympathetic nervous system is a primary factor in the etiology of genetic hypertension.

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