Ontogeny of Neural and Non-Neural Contributions to Arterial Blood Pressure in Spontaneously Hypertensive Rats

PETER G. SMITH, PH.D., CAROL WALL POSTON, B.A., AND ELLIOTT MILLS, PH.D.

SUMMARY Arterial blood pressure was measured directly by cannulation in anesthetized spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats on postnatal Days 1, 5, 9, 20, 42, and 82-86. The time course for development of the following variables was established: resting diastolic and basal (after ganglionic blockade) pressure, the neural contribution to resting pressure (resting minus basal pressure), vascular reactivity to a noradrenergic agonist, methoxamine, and to endogenous sympathetic nerve terminal norepinephrine released by tyramine (maximum pressor response and ED50) and resting and basal heart rate. Resting diastolic pressure was higher in SHR compared to WKY by 24 hours after birth. In both strains, the increase in resting diastolic pressure with age was interrupted by a plateau period (Days 5-9 in SHR; Days 9-20 in WKY). Juxtaposition of the development curves was such that the interstrain differences in pressure were statistically significant in all periods studied except Days 5 and 9. Both basal and neurally mediated components of resting diastolic blood pressure were elevated in SHR compared to WKY. The magnitude of the interstrain difference in basal pressure remained constant during development while the magnitude of the neurally mediated component showed accelerated development through 42 days of age. Reactivity to methoxamine and tyramine was higher in SHR, but the magnitude of the difference did not change with age. Cardiac sympathetic tone was higher in SHR than WKY, but did not account for the increased resting diastolic pressure in SHR. The following conclusions were reached: 1) from birth, resting diastolic blood pressure in SHR tended to be elevated relative to WKY and both neural and non-neural factors contributed to the elevated pressure; 2) development of the neural component of resting blood pressure was accelerated in SHR through 42 days of age; and 3) disturbances of growth or function of the vascular wall, which may be reflected in basal blood pressure or reactivity to noradrenergic stimulation, developed in parallel, not in series, with the enhanced sympathetic activity.

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KEY WORDS • hypertension • neonatal • sympathetic nervous system • spontaneously hypertensive rat
Methods

Experiments were performed on SHR and WKY rats born to mothers mated at Charles River Breeding Laboratories, Inc. (Wilmington, Massachusetts) or in our laboratory. Rats were housed in standard cages, fed rodent chow (Purina) ad libitum, and maintained in a 14/10-hour light/dark cycle. Pups were weaned at 24 days of age. Experiments were performed at 1, 5, 9, 20, 42, and 82–86 days of age. Body weights of experimental preparations are given in Table 1.

Rats were anesthetized with urethane (ethyl carbamate, 1–1.5 mg/g) administered intraperitoneally. Body temperature was maintained with a rectal probe in animals 20 days of age or older and in younger animals by a probe placed between the animal and dissecting board. Temperature was maintained with a regulator (Yellow Springs Instruments, Yellow Springs, Ohio) and heat lamp at 37°C (rectal probe) or 35°C (surface probe). Animals breathed room air enriched by a stream of humidified oxygen directed toward the snout.

A femoral vein was cannulated with PE tubing in rats 20 days of age or older and with a 30-gauge needle shaft connected to PE tubing in 1- to 9-day-old animals. Arterial blood pressure was measured from a femoral artery in 5- to 86-day-old rats and from a carotid artery in 1-day-old animals. Cannulas used in 1- to 20-day-old animals were made by cutting and stretching Micro-Renathane tubing (Braintree Scientific, Braintree, Massachusetts). Cannulas used in 1- and 5-day-old animals had outside diameters of approximately 180 μm and internal diameters of 90 μm. Arterial pressure was measured with a Gould 23db transducer and recorded on a Grass model 7 polygraph.

Arterial pulse pressures ranged from 20% of the diastolic pressure at Day 1 to 54% at Days 82–86. Because diameters of the arterial cannulas differed, by necessity, with the age of the preparation, we were concerned that imprecise measurements of pulse pressure might introduce errors into analysis of systolic or mean arterial pressure. Accordingly, diastolic pressures were used for analysis at all age levels.

Experimental Protocol

After the recording of resting blood pressure and resting heart rate in the anesthetized preparations, chlorisondamine (Ecolid, Ciba Pharmaceuticals, Sum-
mit, New Jersey, 2.5 mg/kg) was administered intravenously to establish ganglionic blockade. This dose of chlorisondamine was found to be supramaximum for hypotensive effect. In preliminary experiments on adult SHR and WKY of various ages (n = 4 for each strain), the extent of ganglionic blockade was assessed directly by measuring the contractile response of Muller’s muscle to electrical stimulation of preganglionic cervical sympathetic axons. Values given as percentage of maximum ± SEM. Significance: Strain difference, control response, p < 0.05. Responses after chlorisondamine not significantly different from 0 in both strains at all frequencies.

<table>
<thead>
<tr>
<th>Stimulus (Hz)</th>
<th>Control</th>
<th>Chlor</th>
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<th>Chlor</th>
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<tbody>
<tr>
<td>0.5</td>
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<td>98 ± 2</td>
<td>8 ± 5</td>
<td>100 ± 0</td>
<td>7 ± 5</td>
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Effect of chlorisondamine (Chlor) on contractile response of Muller’s muscle to electrical stimulation of preganglionic cervical sympathetic axons.

Results: Evaluation of Ganglionic Blockade in Wistar-Kyoto (WKY) Rats and Spontaneously Hypertensive Rats (SHR)

Table 2. Evaluation of Ganglionic Blockade in Wistar-Kyoto (WKY) Rats and Spontaneously Hypertensive Rats (SHR)

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Values are means ± SEM.
Statistical Analysis

Comparisons between SHR and WKY were made on five animals of each strain at each age level. Two-way analysis of variance (ANOVA: factor 1 = age, factor 2 = strain) was used to test for age- and strain-related differences and for age x strain interaction. When F values were significant for age x strain interaction, one-way ANOVA was used to test for significance of differences at individual age levels. Differences were considered significant when F ratios had p values of 0.05 or less. Where useful, comparisons between slopes of developmental curves in SHR and WKY were made by linear regression analysis.

Results

In both SHR and WKY, there was a fourfold increase in resting diastolic blood pressure between 1 and 86 days of age, and the major portion of the increase occurred during the first 42 days (Figure 1 left). The increase in pressure was interrupted by a plateau period extending from Days 5-9 in SHR and from Days 9-20 in WKY. Throughout development, pressure tended to be higher in SHR (p < 0.001, strain difference) and the interstrain difference was more pronounced at certain ages (p < 0.001, age x strain interaction). Comparisons made at individual age levels showed the resting diastolic blood pressure to be significantly elevated in SHR on Days 1 (p < 0.01), 20 (p < 0.001), 42 (p < 0.001), and 82-86 (p < 0.005).

Basal blood pressure increased about 2½ times in both strains, with the major part of the increase completed by 42 days of age (Figure 1 right). In SHR, there was a period from postnatal Days 5-9 in which basal pressure remained constant; in WKY there was a longer plateau period extending from Days 5-20. Overall, basal pressure was elevated significantly in SHR (p < 0.001), but the magnitude of the interstrain difference did not change with age (p > 0.05 age x strain interaction). The decrease in blood pressure after ganglionic blockade with chlorisondamine (Figure 2 left) was greater in SHR (p < 0.001, strain difference; p < 0.01, age x strain interaction). An age x strain interaction was attributable to large differences between SHR and WKY occurring at 20 days (p < 0.005) and 42 days of age (p < 0.001). Between 1 and 42 days of age, the rate of increase in the chlorisondamine-sensitive component of blood pressure was greater in SHR than in WKY (p < 0.001, slope difference by linear regression analysis). In SHR, the magnitude of the chlorisondamine-induced decrease in pressure attained a maximum at 42 days; in WKY, there was a further increase in the response to chlorisondamine between 42 and 82-86 days of age.

Resting heart rate in both strains increased from Day 1 to a maximum at Day 20 and then declined toward adult levels (Figure 3 left). Resting heart rate was higher in SHR (p < 0.025), but the difference remained constant with age (p > 0.05, age x strain interaction). Development of basal heart rate in the two strains (Figure 3 right) was comparable (p > 0.05 age and age x strain interaction). The effect of chlorisondamine on heart rate (Figure 2 right) was significantly different in SHR and WKY (p < 0.001), and there were age-related differences in the magnitude of the effect (p < 0.01, age x strain interaction). In 1-day-old SHR and WKY pups, ganglionic blockade resulted in a fall in heart rate (50 and 30
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The magnitude of the effect increased to a maximum at 5 days in SHR and 9 days in WKY. In WKY, chlorisondamine did not change heart rate in preparations 42–86 days of age; in SHR, heart rate did decrease (40 to 50 bpm) after ganglionic blockade at 42 and 82–86 days of age. The age x strain interaction was attributable to the significantly greater fall in rate in SHR on Days 5 (p < 0.01), 42 (p < 0.005), and 82–86 (p < 0.01).

Maximum pressor responses to the direct acting noradrenergic agonist, methoxamine, and to endogenous norepinephrine released from sympathetic nerve terminals by tyramine increased in parallel with age in SHR and WKY (Figure 4). Overall, responses to methoxamine and tyramine were larger in SHR than WKY (p < 0.005; p < 0.05), but the differences remained essentially constant with age (p > 0.05, age x strain interaction). The ED50 for the maximum pressor response
Fig. 4. Left: Ontogeny of maximum pressor response to methoxamine in SHR and WKY. Ordinate = increase in pressure (delta mm Hg). Right: Ontogeny of maximum pressor response to tyramine in SHR and WKY. Ordinate = increase in pressure (delta mm Hg).

to tyramine and to methoxamine (Table 3) decreased with age in both strains ($p < 0.001$). An age-related strain difference in ED50 was detected for methoxamine ($p < 0.01$, age X strain interaction), which was attributable predominantly to a higher value for SHR at 82–86 days of age ($p < 0.025$).

**Discussion**

Previous reports provide evidence for 89 and against10 the existence of a prehypertensive phase in neonatal rats genetically predisposed to hypertension. The present results indicate that, compared to WKY, SHR is hypertensive by 24 hours after birth. One source of disagreement among studies can arise from the fact that statistically significant differences in pressure between SHR and WKY may not be detected at all ages during the neonatal period. This inconsistency arises because the developmental curves for blood pressure in the two strains are not parallel at all ages and because the curve for SHR is shifted to the left compared to WKY. As a result, development of resting blood pressure in SHR is already in a plateau phase at 5–9 days of age while pressure in WKY continues to increase. At least during this period, then, comparisons between SHR and WKY may not reveal significant pressure differences. It should be noted that the discontinuous nature of the developmental process reflected by the presence of plateau periods is neither unique to the variable, blood pressure, nor to the WKY and SHR strains. Thus, discontinuity is also a feature of functional maturation in the nonvascular smooth muscle end organ, Muller's muscle, in Sprague-Dawley rats.5-12

In this study both neurally mediated (i.e., chlorisondamine-sensitive) and basal components of diastolic blood pressure were increased in SHR relative to WKY. However, the relative contribution of these components to the hypertension differs with age. The difference arises because the rate of development of the neurally mediated component is accelerated in SHR relative to WKY through 42 days of age and slowed thereafter. The most pronounced interstrain difference was evident at 6 weeks of age. In contrast, while basal blood pressure was also elevated in SHR, the rate of development was comparable in the two strains.

**TABLE 3.** ED50 for Methoxamine and Tyramine in Spontaneously Hypertensive Rats (SHR) and Wistar-Kyoto (WKY) Rats

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Methoxamine</th>
<th>Tyramine</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0.20 ± 0.03</td>
<td>0.29 ± 0.04</td>
</tr>
<tr>
<td>20</td>
<td>0.12 ± 0.02</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td>42</td>
<td>0.08 ± 0.01</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>82–86</td>
<td>0.17 ± 0.03</td>
<td>0.08 ± 0.02</td>
</tr>
</tbody>
</table>

ED50s for pressor responses to methoxamine and tyramine (mg/kg) for SHR and WKY. Significance: age, methoxamine and tyramine. Age X strain, methoxamine, age levels = 82–86 days.
The effect of chlorisondamine on heart rate reveals a predominance of sympathetic tone in the cardiac innervation of SHR, which accounts for the higher resting heart rate in this strain. However, the higher resting heart rate in SHR is not a major contributant to the hypertension since the difference in rate between SHR and WKY remains essentially constant while the difference in pressure shows an age-dependent increase. Both SHR and WKY share in common some features of development of the cardiac sympathetic pathway which are unusual compared to normotensive Sprague-Dawley rats. In the latter, heart rate is unaffected by chlorisondamine until after 7 days of age, and the decrease which follows ganglionic blockade reaches a maximum (−80 bpm) at 16 days of age.13 In contrast, SHR and WKY respond with a decrease in rate (−30 to −50 bpm) at 1 day of age which attains a maximum earlier (Days 5 to 9) and is larger (−130 to −140 bpm) than in Sprague-Dawley rats. SHR and WKY therefore demonstrate a precocious onset, accelerated development, and enhanced level of tonic activity in the cardiac sympathetic pathway relative to the Sprague-Dawley strain. The precocious onset of function in the cardiac sympathetic pathway may be due to early onset of sympathetic ganglionic neurotransmission in SHR and WKY. Thus, preliminary studies of the cervical sympathetic pathway (Smith and Mills, unpublished data) indicate that ganglionic neurotransmission is functional in these strains by the 1st postnatal day while it is not established in Sprague-Dawley rats until the 6th to 8th postnatal days.14,15

The enhanced neurally mediated contribution to resting diastolic blood pressure and heart rate could arise from three sources: the central nervous system, the postganglionic neuron, or the vascular smooth muscle. Thus, the level of tonic sympathetic impulse frequency could be increased, neurotransmitter release from postganglionic sympathetic nerve terminals enhanced, or reactivity of vascular smooth muscle to released neurotransmitter augmented. During the period in which we detected accelerated development of the chlorisondamine-sensitive component of resting blood pressure in SHR, there was no comparable change in either the maximum response to methoxamine or tyramine, or the ED50 for these responses. Also, the contractile responses to tyramine of a non-vascular smooth muscle (Muller's) is smaller in SHR compared to WKY during this period.15 These results indicate that the origin of the enhanced neurally mediated contribution is more apt to be an abnormally elevated sympathetic outflow from the central nervous system rather than a perturbation of either the vasomotor sympathetic nerve terminal or vascular smooth muscle reactivity to noradrenaline. This conclusion is consistent with those drawn from experiments in which nerve activity was recorded.16,17

The present experiments were performed under anesthesia, and it is known that hemodynamics are changed compared to those of the unanesthetized SHR and WKY.18 However, the agent employed, urethane, is reported to produce similar changes in the two strains, at least in combination with chloralose,18 and we found that the dose of anesthetic needed to induce and maintain surgical anesthesia in the two strains was the same. It is unlikely, then, that any interstrain difference in anesthetic action accounts for the developmental differences that we observed. The general anesthetic probably did facilitate assessment of the neurally mediated contribution to blood pressure with chlorisondamine; by suppression of compensatory mechanisms, anesthetics enhance the drop in blood pressure produced by ganglionic blockade in the rat.19 However, the tests that we made on the cervical sympathetic pathway indicate that the extent of ganglionic blockade was comparable in anesthetized SHR and WKY, and it is known that the hypotensive effect of chlorisondamine is attributable specifically to ganglionic blockade.20 Consequently, its use in the present experiments is likely to have given a reliable estimate of the neurally mediated contribution to resting blood pressure in anesthetized SHR and WKY.

It has been proposed that heightened sympathetic nerve activity may induce sustained hypertension by affecting growth of vascular smooth muscle.1 In the present study, however, the period of accelerated development of SNS activity did not affect subsequent functional maturation of vascular smooth muscle as reflected in basal blood pressure and contractile responses to noradrenergic agonists. Thus, although a significant interstrain difference in development of these variables was observed, there was no change in the magnitude of the difference with age, as might be expected if the SNS activity directly influenced development of the muscle. The results are, in fact, more compatible with the idea that basal blood pressure in SHR rises in parallel, not in series, with the development of enhanced SNS activity. In conclusion, the present study indicates that, even in the earliest stages of postnatal development in SHR, there is an elevation of resting blood pressure that is attributable to enhancement of both neural and non-neural factors.

Acknowledgments

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References

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