Effect of Increasing Age on Hemodynamics of Spontaneously Hypertensive Rats

Juro Iruiuchijima, M.D., Yoshinobu Numao, M.D., and Hiroyuki Suga, M.D.

SUMMARY

Arterial pressure, cardiac output, and total peripheral resistance were compared between spontaneously hypertensive rats (SHR) and normotensive control rats (NCR) at 4, 7, and 12 months of age. The rats were anesthetized with pentobarbital sodium, thoracotomized and kept under positive pressure respiration. Cardiac output was measured with an electromagnetic flowmeter probe placed at the ascending aorta. On all the age groups, arterial pressure and total peripheral resistance were significantly higher in SHR than in NCR, while cardiac output per body weight was not different. However, the significant differences in pressure and resistance at each age disappeared after ganglion blockade with hexamethonium bromide. Total peripheral resistance was not higher in SHR than in NCR even after cardiac output, which had once been diminished by blockade, was restored to the pre-blockade level by dextran infusion. Phenoxybenzamine, an alpha adrenergic receptor blocker, also equalized arterial pressure and total peripheral resistance between SHR and NCR, both aged about 10 months, without decrease in cardiac output. It is concluded that, in both young and old SHR’s, the hypertensive state is maintained by an increase in total peripheral resistance ascribable to sympathetic activity.

Additional Indexing Words:
SHR  Hypertension  Aging  Sympathetic nervous system
Ganglion blockade  Alpha adrenergic receptor blockade  Total peripheral resistance

PREVIOUSLY, we measured hemodynamic parameters in relatively young (4-month-old) spontaneously hypertensive rats (SHR) and compared them with those in normotensive control rats (NCR) of the same age.1,2 In this early stage of SHR hypertension, the high level of arterial pressure was maintained by an increase in total peripheral resistance due to elevated sympathetic tone: Either ganglion blockade or alpha-receptor blockade equalized both arterial pressure and total peripheral resistance between SHR and NCR. An
increase in efferent impulse frequency in the splanchnic nerve was observed in SHR by 2 different methods.\(^3\),\(^4\) The frequency was normalized concomitantly with arterial pressure on administration of a central depressor agent, clonidine.\(^5\)

On the other hand Folkow et al\(^6\) observed, by perfusing the rat’s whole body from the aortic root with a plasma substitute, that the resistance to flow in the maximally dilated systemic vascular bed was elevated in older SHR, aged 7 months or more. This result suggests the presence of a non-nervous, structurally based increase in vascular resistance in these animals. Therefore, in the present study, the same hemodynamic measurements that we previously employed for 4-month-old rats were carried out in older rats to observe how aging affects the nature of the hypertension state. A short preliminary report of the present work has been presented already.\(^7\)

**Methods**

Spontaneously hypertensive rats (SHR, Okamoto and Aoki\(^8\)) and normotensive control rats (NCR) both at 4, 7, and 12 months of age were anesthetized with pentobarbital sodium injected intraperitoneally at a dose of 50 mg/Kg. The rat was fixed supine. An electric heating pad was placed under the animal to maintain the rectal temperature at about 38°C throughout the experiment. The left femoral artery was cannulated for measuring mean arterial pressure and the left femoral vein for intravenous injection. The trachea was intubated and positive pressure respiration was instituted with a Harvard rodent respirator. The stroke frequency and volume of the respirator were selected just enough to suppress the animal’s own respiratory movement. The thorax was opened by a median sternotomy. A Statham 2 or 2.5 mm S probe was attached around the ascending aorta for measurement of cardiac output.

The numbers of rats used were presented in Table I. Group statistical comparisons between SHR and NCR as well as among age groups were made with Student’s t-test.

After control measurements of aortic flow and arterial pressure, hexamethonium bromide, a ganglion blocking agent, was injected intravenously at a dose of

<table>
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<th>Table I. Number of Rats Used in This Study</th>
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<td><strong>Type</strong></td>
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<tr>
<td>SHR</td>
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<td>NCR</td>
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30 mg/Kg to eliminate the nervous effect on the cardiovascular system. The measurements were repeated 10 min after the injection. Since the ganglion blockade considerably reduced cardiac output, this parameter was restored by intravenous injection of a plasma substitute, dextran solution containing dextran 70.6% and glucose 5%, warmed to 38°C. This was done on 4-month-old and 12-month-old rats only. First 0.5 ml/100 Gm body weight was injected and then the same amount was injected again. After each injection arterial pressure first increased rapidly, reached a maximum and then decreased somewhat and stayed relatively stationary before a gradual decrease toward the pre-injection level. Cardiac output and arterial pressure were noted when the latter parameter was relatively stationary after the maximum. In most instances cardiac output was still lower than the original control value after the initial injection of dextran solution but exceeded it after the second injection. Therefore, the post-blockade arterial pressure when cardiac output was restored to the control value could be interpolated from the 2 values of arterial pressure, after the initial and second injections of dextran. Total peripheral resistance after ganglion blockade when cardiac output was adjusted to the pre-blockade value could be calculated as the above arterial pressure value divided by the pre-blockade cardiac output.

In a separate series of experiment, effects of alpha receptor blockade with phenoxybenzamine were studied in 6 SHR's (aged 44.0±1.0 (SD)-week-old) and 6 NCR's (aged 38.5±4.5 (SD)-week-old). Each group consisted of 3 male and 3 female rats. The drug was dissolved in 0.9% saline and administered intravenously. An initial dose of 10 µg/Kg was followed by additional 20, 70, 200, 700, 2,000, and 7,000 µg/Kg at about 10 min intervals. Flow and pressure were measured 10 min after each dosage.

Results

Changes in hemodynamic parameters with age

Fig. 1 shows changes with age (4, 7 and 12-month-old) in the mean values (±SEM) of mean arterial pressure (AP), cardiac output (CO), total peripheral resistance (TPR), and heart rate (HR). Filled circles represent the values from SHR and open circles from NCR. Triangles are the values after ganglion blockade with hexamethonium bromide.

Arterial pressure of SHR increased from the stage of 4-month-old to that of 7-month-old (P<0.005). However, there was no statistically significant difference in arterial pressure between the 7-month-old rats and the 12-month-old rats (P>0.25). Arterial pressure was similarly increased with age in NCR (P<0.025). At each age, arterial pressure was significantly higher in SHR than in NCR (P<0.001 for all the age groups). The increase in arterial pressure from 4 months of age to 7 months was observed even after ganglion blockade with hexamethonium bromide both in SHR (P<0.005) and NCR (P<0.001). After ganglion blockade the difference in arterial pressure between SHR and NCR disappeared for all the age groups.
Cardiac output per body weight was not significantly different between SHR and NCR, both before and after ganglion blockade, in all the age groups. Similar to arterial pressure, total peripheral resistance increased from 4 months of age to 7 months in SHR (P<0.001). However, the increase in total peripheral resistance with age was not significant in NCR (P>0.10). Different from arterial pressure, total peripheral resistance did not decrease with hexamethonium administration. Presumably this was due to the marked decrease in cardiac output on ganglion blockade. Before the blockade, total peripheral resistance was significantly higher in SHR than in NCR at each age (P<0.005, 0.001, and 0.005 for 4, 7, and 12-month-old groups, respectively). After ganglion blockade the significant difference between SHR and NCR disappeared, though the mean value was still slightly higher in SHR than NCR for all the age groups (P>0.10 for 4 and 7-month-old groups and P>0.50 for 12-month-old group). Total peripheral resistance after ganglion blockade increased from 4 months of age to 7 months in both SHR and NCR (each P<0.005).

After ganglion blockade, the mean total peripheral resistance of SHR was higher than that of NCR for all the groups, though the difference was insig-
significant by the group t-test. The paired t-test was also applied for the significance of difference in this parameter: 16 pairs of SHR and NCR, above 7 months of age, were matched as to both age and sex. The mean difference of total peripheral resistance from the 16 pairs was $1.38\pm5.07$ (SD) mmHg/ml/min/100 Gm body weight. Even by the paired t-test the difference in total peripheral resistance between SHR and NCR after ganglion blockade was statistically insignificant ($P>0.2$).

Heart rate was significantly higher in SHR than in NCR before ganglion blockade at each age ($P<0.05$, 0.001, and 0.005 for 4, 7, and 12-month-old groups, respectively). The difference disappeared after ganglion blockade except in the age group of 12-month-old, in which the heart rate was still significantly ($P<0.05$) higher in SHR than in NCR. On the whole, heart rate tended to decrease with age and was especially low in 12-month-old rats after ganglion blockade.

**Total peripheral resistance after ganglion blockade when cardiac output was restored by dextran infusion**

Since ganglion blocking with hexamethonium bromide greatly decreased cardiac output, considering the non-linear relationship between cardiac output and arterial pressure, the condition after this treatment might not be appropriate for assessing the vascular difference between SHR and NCR by comparing total peripheral resistance. Therefore, cardiac output was restored by intravenously infusing a plasma substitute, dextran solution. Arterial pressure corresponding to the original pre-blockade cardiac output was obtained by the procedure described under *Methods*. Total peripheral resistance after ganglion blockade with cardiac output restored to the original pre-blockade value was thus calculated.

Successive changes in total peripheral resistance by ganglion blockade and the following restoration of cardiac output are tabulated in Table II. Total

Table II. Changes in Total Peripheral Resistance (TPR) on Ganglion Blockade and Dextran Infusion (in mmHg/ml/min/100 Gm body weight; each mean±SEM)

<table>
<thead>
<tr>
<th>Type</th>
<th>Age (month-old)</th>
<th>n</th>
<th>TPR control</th>
<th>TPR after blockade</th>
<th>TPR after blockade and dextran</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>4</td>
<td>12</td>
<td>9.09±0.77</td>
<td>7.82±0.87</td>
<td>5.72±0.48</td>
</tr>
<tr>
<td>SHR</td>
<td>12</td>
<td>8</td>
<td>11.5±0.86</td>
<td>13.4±1.6</td>
<td>6.67±0.38</td>
</tr>
<tr>
<td>NCR</td>
<td>4</td>
<td>11</td>
<td>5.93±0.52</td>
<td>6.38±0.68</td>
<td>5.62±0.71</td>
</tr>
<tr>
<td>NCR</td>
<td>12</td>
<td>8</td>
<td>8.72±0.55</td>
<td>12.3±1.35</td>
<td>8.18±0.42</td>
</tr>
</tbody>
</table>
Effect of varying cumulative doses of phenoxybenzamine on hemodynamic parameters of SHR (filled circles) and NCR (open circles). Each mean from 6 rats with SEM.

Peripheral resistance after hexamethonium with restored cardiac output by dextran infusion was not different between SHR and NCR at 4 months of age, while it was even larger in NCR than in SHR at 12 months of age (P < 0.05).

Effect of alpha-receptor blockade in old rats

Effect of varying cumulative doses of phenoxybenzamine on hemodynamic parameters of SHR and NCR, both about 10-month-old, are plotted in Fig. 2. As in younger (4 to 5-month-old) rats, arterial pressure and total peripheral resistance gradually decreased with increasing dose of this drug. The decreases were more marked in SHR and the differences in these parameters between SHR and NCR were gradually diminished and finally became statistically insignificant. Cardiac output was not decreased but slightly increased on administration of phenoxybenzamine. The apparent difference in the figure in cardiac output between SHR and NCR was statistically insignificant.

DISCUSSION

In older SHR’s as in younger (4-month-old) ones, the hypertensive state is ascribable to an increase in total peripheral resistance with normal cardiac output. This finding is consistent with that by Pfeffer and Frohlich who used ether as an anesthetic. However, they observed an increase in cardiac output with normal total peripheral resistance in very young SHR’s (9 to
12-week-old). Such young animals were not examined in this study. Though cardiac output is considerably greater under ether than under pentobarbital anesthesia, relative differences of arterial pressure and total peripheral resistance between SHR and NCR are alike.

The mean value of total peripheral resistance after ganglion blockade in SHR was slightly higher than that in NCR. However, the difference was statistically insignificant for all the age groups. Even a paired t-test could not indicate a significant difference. The marked decrease in cardiac output on administration of hexamethonium might have provided an experimental condition under which mere comparison of total peripheral resistance would not reflect a difference in vascular resistance. Therefore, cardiac output was restored by intravenously infusing the dextran solution. Even after this procedure, there was no difference in total peripheral resistance between SHR and NCR at 4 months of age. At 12 months of age, the total peripheral resistance was significantly (P<0.05) higher not in SHR but in NCR. Though the reason for this unexpected result is not clear, it is certain that total peripheral resistance is no longer higher in SHR after ganglion blockade even with cardiac output restored to the pre-blockade value.

Since phenoxybenzamine did not decrease cardiac output in the older rats, this drug enabled us to compare total peripheral resistance between SHR and NCR under the condition with the vasoconstrictor influence eliminated and without a decrease in cardiac output. The result was consistent with that by ganglion blockade: alpha-adrenergic receptor blockade abolished the significant differences of arterial pressure and total peripheral resistance between SHR and NCR.

From the above 2 pieces of findings, it may be concluded that the sympathetic nature of the SHR hypertension is not confined to young animals but continues up to the age of 1 year. Though arterial pressure and total peripheral resistance increased in SHR from the stage of 4-month-old to that of 7-month-old, this held good even after ganglion blockade. Moreover, similar increases with age in arterial pressure and total peripheral resistance after blockade were present in NCR. Therefore, the increase in arterial pressure with age in SHR may be ascribable to a vascular change which develops as the rat grows older, independent from the level of arterial pressure.

In SHR older than 7 months, Folkow et al observed an increase in vascular resistance ascribable to a structural change, by perfusion with artificial solution at low perfusion pressure after guanethidine. No phenomenon which seemed to correspond to the above was observed in our study in which vascular resistance was measured more indirectly but under more natural conditions.
REFERENCES

1. Iriuchijima J: Cardiac output and total peripheral resistance in spontaneously hypertensive rats. Jap Heart J 14: 267, 1973