Genetic Analysis of Blood Pressure and Sodium Balance in Spontaneously Hypertensive Rats

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SUMMARY

Blood pressure and parameters of sodium balance were measured during the first 16 weeks of life in male Okamoto spontaneously hypertensive rats (SHR, n = 22), Wistar-Kyoto rats (WKY, n = 25), and the F₁ (n = 27) and F₂ (n = 81) hybrids of the SHR and WKY. Genetic analysis revealed that blood pressure in SHR was controlled by approximately four independent genetic loci and the degree of genetic determination was 64.5%. No difference in blood pressure was discernible before 12 weeks of age between those F₂ rats that at 16 weeks had blood pressures either higher or lower than one standard deviation from the mean. Exchangeable sodium was measured sequentially in individual rats of all populations by determining their whole-body radioactivity while receiving 37.5 mM ²²Na/²³NaCl drinking fluid of constant specific activity as their sole source of sodium. The SHR had consistently higher exchangeable sodium levels than WKY and showed evidence of relative sodium retention during the early developmental phase of hypertension. Sodium intake was higher in SHR than WKY from 4 to 16 weeks of age, although saline preference was the same in both strains. None of these parameters of sodium balance were found to correlate with blood pressure in the F₂ population. It is concluded that the hereditable abnormalities of sodium balance in SHR appear to represent coincidental inbred characteristics controlled by genetic loci that are unrelated to those loci responsible for the expression of hypertension in this model. (Hypertension 8: 572-582, 1986)

KEY WORDS • spontaneously hypertensive rats • heredity • hypertension • sodium • blood pressure • Wistar-Kyoto rats • saline preference

The relationship between sodium and the development of hypertension in Okamoto spontaneously hypertensive rats (SHR) has been investigated intensively. Diets of varying sodium content have been reported to affect the development of hypertension,1-3 and abnormalities of sodium intake,4 total body sodium,4 and renal sodium handling5 have all been suggested as having relevance to the pathogenesis of hypertension in SHR. One recurring problem in these and other studies of SHR, however, is the choice of control rats. The ideal control should be genetically identical to the SHR except at those genetic loci responsible for the determination of blood pressure. Most investigators have used either normotensive Wistar-Kyoto rats (WKY) or Wistar rats, both of which are dissimilar in many respects5-6 and neither of which fulfills the criteria of an ideal control. This problem is complicated by the fact that inbred SHR may express genetically determined characteristics that bear no causative relationship to hypertension. If such characteristics are quantitatively different from those measured in the currently available controls, then they may be erroneously interpreted as of pathogenetic importance to the development of hypertension in SHR.

One approach to this dilemma has been to study various parameters of interest in a longitudinal fashion4 during the development of hypertension and in effect use the prehypertensive SHR as one form of control. However, this method does not successfully exclude the effects of growth on these parameters.

The known genetic effects7-8 of hybridization of inbred strains can be used to define which of the myriad biochemical or physiological differences between SHR and (in this case) WKY are either genetically linked or genetically coincidental to the hereditable abnormality causing hypertension. The production of the F₁ hybrid of SHR and WKY (SHR-WKY F₁), by cross-breeding SHR and WKY, and subsequently an F₂ population (SHR-WKY F₂), by crossing F₁ × F₁, allows identification of those traits that either cosegregate or are independent of those genes responsible for hypertension in SHR.4 Therefore, if a particular characteristic is shown to be quantitatively "abnormal" in
SHR compared with WKY but does not correlate with blood pressure in the SHR-WKY F$_2$, then it can be confidently stated that the genetic difference responsible for this observed "abnormality" is not the cause of hypertension in this model.\(^9\)

In the present study this powerful genetic method was combined with a sequential study of changes in blood pressure, exchangeable sodium, and sodium intake in SHR, WKY, SHR-WKY F$_1$, and SHR-WKY F$_2$ from 1 to 16 weeks of age inclusive. In addition, detailed cross-sectional examinations were performed in all groups at 16 weeks of age and in SHR-WKY F$_2$ at 6 weeks of age to test for correlation of blood pressure with a number of quantitatively defined physiological traits in the SHR-WKY F$_2$.

**Materials and Methods**

**Longitudinal Studies**

**Breeding**

Eight male and eight female rats from each strain (SHR and WKY) were randomly selected from our inbred colonies, which have been derived from the original inbred stock of SHR and WKY\(^10\) obtained from Dr. Yamori in Kyoto in 1977. Male and female breeding pairs from each strain were mated at 9 weeks of age after equilibration on radioactive NaCl. The litters from these pairs were checked twice weekly during the first 3 weeks, and any runts were removed. Individual male rats were marked for identification with indelible ink during the first 2 weeks of life, and ear tags were applied at 3 weeks of age. Weaning took place at 4 weeks of age, and the weanlings were housed four or less to a box and provided a diet identical to that of their parents. Twenty-two male SHR and 25 male WKY were reared from these parents and observed until 16 weeks of age.

To produce the F$_1$ hybrid of the SHR and WKY (SHR-WKY F$_1$), five male SHR and five female WKY were randomly selected from litters produced as described and mated at 9 weeks of age. From this cross-breeding, a total of 27 male SHR-WKY F$_1$ were reared and studied from 1 to 16 weeks of age. Eight male and eight female SHR-WKY F$_1$ then were chosen at random and mated at 9 weeks to provide an F$_2$ population (SHR-WKY F$_2$). From the litters of these matings, 81 male SHR-WKY F$_2$ were used in this experiment.

All rats were housed in conditions providing an ambient temperature of 22°C and humidity of 50%. A 12-hour day/night cycle was used throughout all studies.

**Exchangeable Sodium**

Sequential measurements of exchangeable sodium were made weekly in rats from 1 to 16 weeks of age inclusive using previously described methods.\(^4\) In brief, both breeding and weaned rats received sodium exclusively as 37.5 mM $^{23}$Na/$^2$NaCl drinking fluid maintained at constant specific activity throughout the experiment. Water was also provided ad libitum. A sodium-free purified diet, which has been used successfully in this laboratory for over eight consecutive generations, was given to all rats. This diet contains (by weight) sodium, less than 45 mg/kg; carbohydrate, 75%; protein, 15%; fat, 7%; calcium, 0.5%; and phosphate, 0.4%. The combination of this diet and the NaCl solution satisfies the nutritional growth, breeding, and lactation criteria of Rogers.\(^11\) Breeding rats were commenced on this regimen 3 weeks before mating to allow equilibration of $^{23}$NaCl and $^2$NaCl. Pups conceived after this time, even at 1 day of age, have been shown to have the same specific activity in their plasma and urine as that of the maternal drinking fluid.\(^12\)

The exchangeable sodium can be measured noninvasively and repeatedly from the first week of age by measuring the whole-body radioactivity in a Packard Armac whole-animal gamma-scintillation spectrometer (Downers Grove, IL, USA). The total body sodium (in millimoles) was determined by dividing the counts obtained for a rat by the specific activity of the drinking fluid. After correcting for differences in weight by dividing the total body sodium by the mass (in kilograms), one derives the so-called exchangeable sodium (expressed as mmol/kg). Between 1 and 16 weeks of age the values obtained by this method correlate closely with those obtained directly by nitric acid digestion of the carcass.\(^4\)

**Blood Pressure**

From 4 weeks of age, blood pressure was determined weekly by indirect tail-cuff plethysmography in unanesthetized, preheated rats.\(^13\) Recordings were obtained between 0900 and 1100 daily, and each estimation was the average of three measurements taken over a 1-minute period.

**Sodium Intake**

To obtain mean weekly estimates of sodium intake, daily recordings of saline ingestion for each cage of rats (obtained by determining the difference in weight of the saline-containing bottles of each cage over a 24-hour period: 0900-0900) were made on 5 consecutive days of each week. For an individual cage these values were divided by the average combined weight (in kilograms) of the rats for the week. After correcting for the sodium concentration (37.5 mM) a mean sodium intake per kilogram of body weight was obtained for each strain from weeks 4 to 15 inclusive.

**SHR-WKY F$_2$**

The sequential individual studies of blood pressure and sodium balance provided a unique opportunity to examine these parameters in the male SHR-WKY F$_2$. Two groups of SHR-WKY F$_2$ were arbitrarily defined on the basis of the final average blood pressure at 16 weeks of age. The first group, hypertensive F$_2$ (HF$_2$, $n = 12$), had a final blood pressure of greater than 172 mm Hg (i.e., mean blood pressure of SHR-WKY F$_2$ + 1 SD), and the second group, normotensive F$_2$ (LF$_2$, $n = 11$), had a blood pressure of less than 142 mm Hg (i.e., mean blood pressure of SHR-WKY F$_2$).
F₂ (± 1 SD). The blood pressure and exchangeable sodium of these two groups were compared during the first 16 weeks of life.

Cross-sectional Studies

The cross-sectional studies were designed to provide more accurate estimations of the parameters observed in the longitudinal experiments as well as to allow correlation with several other physiological measurements.

Exchangeable Sodium

Exchangeable sodium was measured in 16-week-old SHR, WKY, SHR-WKY F₁, and SHR-WKY F₂ daily for 5 consecutive days to provide an average value for comparison with concomitantly recorded blood pressure and sodium intake. In addition, exchangeable sodium was recorded for 5 days in 16 randomly selected male SHR-WKY F₂ at 6 weeks of age to detect any correlation with blood pressure at this age.

Blood Pressure

In the 16-week-old SHR, WKY, SHR-WKY F₁, and SHR-WKY F₂, a final blood pressure recording for each rat was taken as the average of three indirect measurements obtained on 3 consecutive days and one direct blood pressure estimation. The direct recording was obtained from conscious, unrestrained, resting rats at least 12 hours after insertion of a PE-50 polyethylene catheter into the left carotid artery during brief methohexitone anesthesia (40 mg/kg i.p.). Measurements were made using Gould Statham P23 pressure transducers (Oxnard, CA, USA) and recorded on a Grass polygraph (Model 7C; Quincy, MA, USA). The blood pressure of the 16 randomly chosen 6-week-old SHR-WKY F₂ was measured in an identical fashion. An arterial blood sample was withdrawn at the time of direct blood pressure recording in each strain and centrifuged in heparinized microhematocrit capillary tubes for estimation of hematocrit. After the blood pressure recording, the animals were killed and hearts were removed and immediately weighed.

Sodium and Fluid Intake

During the 16th week of life, all male SHR, WKY, and SHR-WKY F₁, and a similar number of randomly chosen male SHR-WKY F₂ were housed individually and estimates of saline and water intakes and body weight made daily for 5 days. The values obtained were averaged for each rat, and this representative value was used for correlation with blood pressure and exchangeable sodium. The proportion of saline ingested per total fluid intake (saline preference) also was determined in each rat.

All experimental procedures were performed in accordance with the guidelines of the National Health and Medical Research Council of Australia.

Statistical Analysis

Differences between groups at each week were analyzed using Student’s t test for independent data and over the whole experimental period using analysis of variance for fixed effects including repeated measures from the BMDP statistical software package. Relationships between groups were determined using standard parametric regression techniques.

Results

The blood pressure of SHR was significantly higher than that of WKY from the first measurement at 4 weeks of age (108 ± 2.2 [SEM] vs 94 ± 2.8 mm Hg; p < 0.001) and throughout the study (F₁,₁₈₈ = 101.9, p < 10⁻²², Figure 1). The pattern of increasing blood pressure in the SHR-WKY F₁ and SHR-WKY F₂ followed that predicted from previous genetic studies of SHR and WKY. From 6 to 16 weeks of age, the blood pressure of SHR-WKY F₁ and SHR-WKY F₂ fell a little below the calculated midparental value ((SHR + WKY)/2). Blood pressure distributions of 16-week-old SHR, WKY, SHR-WKY F₁, and SHR-WKY F₂ are depicted in Figure 2.

Because of presumed genetic uniformity, the observed variances of blood pressure in SHR and WKY theoretically are due to environmental factors. Although not homozygous at all genetic loci, the SHR-WKY F₂ should be genetically uniform, as they must be homozygous at every locus at which the parents are identical and heterozygous at every locus at which the parents are different. Accordingly, an estimate of variance of blood pressure due to environmental factors (Vₑ) in this experiment can be derived by averaging the variances in the genetically uniform populations: Vₑ = (88.4 + 67.2 + 74.0)/3 = 76.5. The larger variance of blood pressure observed in SHR-WKY F₂ must be attributed to segregation of those loci at which the SHR-WKY F₁ are heterozygous (see Figure 2). Assuming that the environmental and genetic factors are independent of one another, the variance of blood pressure (V₇) in SHR-WKY F₂ can be represented as V₇ = Vₑ + Vₒ, where Vₒ is the variance due to genetic segregation. In this experiment, Vₒ can be calculated as Vₒ = V₇ - Vₑ = 215.5 - 76.5 = 139.0.

The relative contribution of genetic factors to the total variance of blood pressure of SHR-WKY F₂ can be represented by the degree of genetic determination (DGD), which is the ratio: Vₒ/(Vₑ + Vₒ) = 64.5%.

The observation that mean blood pressure of SHR-WKY F₁ fell slightly below the midparental value is evidence that the combination of genes controlling blood pressure from SHR and WKY is not purely additive. It can be inferred that various alleles in heterozygotes exert specific dominant effects. Therefore, Vₒ can be subdivided into additive (Vₐ) and dominance (Vₐ) variances such that Vₒ = Vₐ + Vₐ.

An estimate of the number (n) of independently segregating factors (presumed to be the number of genetic loci) that influence a polygenic trait such as hypertension in SHR can be estimated as n = R²/Vₑ, where R is the range or difference between the mean blood pressure of SHR and WKY. This formula yields an estimate of the order of magnitude of n and tends to underestimate the true value. If, in view of the relatively small dominance effect on the blood pressure of...
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SHR-WKY F₁, one approximates $V_A$ by substituting $V_w$, then $n = (64)^2/(8 \times 139) = 4$.

The values for DGD and $n$ are similar to those reported in the early studies of the genetic transmission of hypertension in SHR. In general, the DGD for SHR has been reported as between 46 and 96% and controlled by one to six independently segregating genetic factors.

The blood pressure increases in HF₁ and LF₂ are of interest. At 16 weeks of age the cardiac mass of HF₁ was significantly higher than that of LF₂ ($3.52 \pm 0.08$ vs $3.18 \pm 0.11$ g/100 g; $df = 21, p < 0.01$; Table 1). There was, however, no discernible between-group difference in blood pressure until 12 weeks of age (Figure 3). Before this time the blood pressures of HF₂ and LF₂ rose together, but subsequently the blood pressure of LF₂ plateaued while that of HF₂ continued to rise.

The exchangeable sodium level of SHR was consistently higher ($F_{1,735} = 140.3, p < 10^{-29}$) and the body mass ($F_{1,735} = 8.18, p < 0.004$) consistently lower than those of WKY throughout the longitudinal study (Figures 4 and 5; see Table 1). However, the difference in exchangeable sodium became greater after 5 weeks of age (Weeks 1-4, 2.8 ± 0.4 mmol/kg; Weeks 5-16, 4.0 ± 0.2 mmol/kg) despite a concurrent reduction in the percentage difference in weight (Weeks 1-4, 20.9 ± 3.7%; Weeks 5-16, 11.5 ± 0.6%). This finding is consistent with our previous observations of relative sodium retention in the SHR compared with WKY during the early developmental phase of hypertension.

Although the longitudinal studies of exchangeable sodium revealed that values in SHR-WKY F₁ and SHR-WKY F₂ tended to fall midway between those of SHR and WKY (see Figure 4), no correlation was found between exchangeable sodium and blood pressure in SHR-WKY F₂. This was so from 4 to 15 weeks inclusive in the longitudinal study as well as in the detailed cross-sectional studies at 6 ($df = 18$, $r = -0.21, F = 0.785, p = 0.387$; Figure 6) and 16 weeks of age ($df = 63$, $r = -0.09, F = 0.51, p = 0.478$; Figure 7). The genetic analysis of exchangeable sodium in 16-week-old rats revealed that the DGD was low (27%) and that approximately two independently segregating genetic factors ($n$) affected the level of exchangeable sodium. Additionally, the exchangeable

**FIGURE 1.** Blood pressure (BP) from 4 to 16 weeks of age in male SHR, WKY, SHR-WKY F₁, and SHR-WKY F₂ (SHR vs WKY: $F_{1,588} = 101.9, p < 10^{-21}$).

**FIGURE 2.** Distribution and mean values of blood pressure (BP) in 16-week-old male SHR, WKY, SHR-WKY F₁, and SHR-WKY F₂.
sodium concentration of HF$_2$ and LF$_2$, plotted from 4 to 16 weeks of age, showed no tendency toward relative sodium retention at any time (Figure 8). In fact there was a small but significant fall in the exchangeable sodium concentration of HF$_2$ around the time that hypertension was becoming obvious in this group.

Also of importance is the finding that the exchangeable sodium concentration was strongly and negatively correlated with body mass in 16-week-old SHR-WKY F$_2$ ($df = 64$, $r = -0.48$, $F = 18.95$, $p < 0.0001$). This was not observed in either SHR or WKY, in which exchangeable sodium at 16 weeks was positively correlated with sodium intake in both strains (SHR: $df = 17$, $r = 0.534$, $F = 6.79$, $p < 0.02$; WKY: $df = 23$, $r = 0.451$, $F = 5.872$, $p < 0.02$).

Although the sodium intake of SHR was consistent-
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**Figure 5.** Body mass of male SHR, WKY, SHR-WKY F₁, and SHR-WKY F₂ from 1 to 16 weeks of age (SHR vs WKY: F₆, 375 = 8.18, p < 0.004).

**Table 1.** Characteristics of 16-Week-Old Male SHR, WKY, SHR-WKY F₁, SHR-WKY F₂, and Hypertensive and Normotensive SHR-WKY F₂

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SHR (n = 19)</th>
<th>WKY (n = 25)</th>
<th>F₁ (n = 23)</th>
<th>F₂ (n = 66)</th>
<th>HF₂ (n = 12)</th>
<th>LF₂ (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (g)</td>
<td>323 ± 28.9*</td>
<td>362 ± 31.5</td>
<td>374 ± 29.1</td>
<td>391 ± 38.9</td>
<td>386 ± 29.5</td>
<td>383 ± 53.4</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>193 ± 9.4*</td>
<td>129 ± 8.2</td>
<td>154 ± 8.6</td>
<td>158 ± 14.7</td>
<td>180 ± 5.2</td>
<td>138 ± 2.7†</td>
</tr>
<tr>
<td>Exchangeable Na (mmol/kg)</td>
<td>47.4 ± 1.6*</td>
<td>43.5 ± 1.6</td>
<td>45.3 ± 1.1</td>
<td>44.5 ± 1.7</td>
<td>44.3 ± 2.1</td>
<td>44.9 ± 2.0</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>46.5 ± 2.1†</td>
<td>44.2 ± 2.9</td>
<td>45.4 ± 2.8</td>
<td>45.2 ± 3.4</td>
<td>44.8 ± 1.8</td>
<td>43.3 ± 3.3</td>
</tr>
<tr>
<td>Cardiac mass (g/100 g)</td>
<td>4.35 ± 0.17*</td>
<td>3.46 ± 0.20</td>
<td>3.56 ± 0.21</td>
<td>3.32 ± 0.59</td>
<td>3.52 ± 0.28</td>
<td>3.18 ± 0.37§</td>
</tr>
<tr>
<td>Sodium intake (mmol/kg/day)</td>
<td>3.8 ± 0.8*</td>
<td>2.6 ± 1.1</td>
<td>3.7 ± 1.1</td>
<td>4.3 ± 1.2</td>
<td>3.8 ± 1.1</td>
<td>4.7 ± 2.6</td>
</tr>
<tr>
<td>Total fluid intake (ml/100 g/day)</td>
<td>11.6 ± 2.9</td>
<td>7.6 ± 2.9</td>
<td>10.8 ± 3.0</td>
<td>12.1 ± 0.8</td>
<td>10.9 ± 2.8</td>
<td>13.6 ± 7.0</td>
</tr>
<tr>
<td>Saline preference (%)</td>
<td>88.4 ± 9.0</td>
<td>90.9 ± 6.2</td>
<td>90.6 ± 5.3</td>
<td>94.1 ± 4.8</td>
<td>91.5 ± 5.7</td>
<td>94.6 ± 7.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. F₁ = SHR-WKY F₁; F₂ = SHR-WKY F₂; HF₂ = hypertensive F₂; LF₂ = normotensive F₂. *p < 0.001, †p < 0.01, compared with values in WKY; §p < 0.0001, §§ p < 0.05, compared with values in HF₂.

ly higher than that of WKY from 4 to 16 weeks of age (F₁, 479 = 80.8, p < 10⁻¹¹; Figure 9), the values observed in SHR-WKY F₁ and SHR-WKY F₂ resembled those of SHR rather than the midparental values, as seen in blood pressure and exchangeable sodium. This finding suggests a strong dominance effect of alleles that are responsible for high sodium intake in heterozygous SHR-WKY F₁ and SHR-WKY F₂. No correlation was observed between blood pressure and sodium intake in the 16-week-old SHR-WKY F₂ (df = 16, r = -0.26, F = 1.18, p = 0.29). In addition, no differences were recorded in the saline preference (ratio of saline to total fluid intake) in SHR, WKY, SHR-WKY F₁, or SHR-WKY F₂ (see Table 1).

Of interest is the significantly higher hematocrit of SHR compared with that of WKY at 16 weeks of age (see Table 1). Although hematocrits of SHR-WKY F₁ and SHR-WKY F₂ fell between the values seen in SHR and WKY, no correlation was observed between hematocrit and blood pressure in 16-week-old SHR-WKY F₂ (df = 27, r = 0.24, F = 1.67, p = 0.21).

**Discussion**

The difficulty that arises with any study of genetic hypertension is the choice of appropriate control strains. Generally, these have been selected on the basis of normotension with little regard to other genetic characteristics. As a result, hereditable differences in a wide range of characteristics, totally unrelated to the genetic cause of hypertension, may be found between SHR and various controls. Most importantly, pathogenetic significance may be attributed erroneously to these differences. To exclude such coincidental genetic variations between SHR and control animals, one must produce segregation of alleles in the F₂ popula-
tion and determine whether the alleles controlling the trait (or traits) of interest cosegregate with the alleles responsible for hypertension. If this is the case, then blood pressure and the quantitatively defined characteristic under investigation will be correlated in the F2 population. The genetic methods used in these experiments to define the relevance of sodium balance to blood pressure are based on the paradigm detailed by Rapp. To identify a particular biochemical or physiological trait as a primary genetic cause of hypertension, it must 1) be different in the two parental strains, 2) follow Mendelian inheritance, 3) correlate with blood pressure in the F2 population, and 4) bear some logical physiological or biochemical relationship to blood pressure. If these criteria are satisfied, a strong argument can be made that this characteristic plays a role in the pathogenesis of hypertension. If, on the other hand, they are not satisfied, then the hypothesis that strain differences in a particular trait are responsible for the strain differences in blood pressure must be
Figure 8. Exchangeable sodium (ENa) from 1 to 16 weeks of age in male SHR-WKY F2 found to be hypertensive (HIGH) or normotensive (LOW) at 16 weeks of age (see text for details). Asterisk indicates significant difference between groups (p<0.05).

Figure 9. Daily sodium intake of male SHR, WKY, SHR-WKY F1, and SHR-WKY F2 from 1 to 16 weeks of age (SHR vs WKY: F_t = 80.81, p<10^-7).

rejected. This was the case in a recent report of dissociation of genetic hyperactivity and hypertension in SHR. In that study (in which the genetic methods were identical to those used in the present study) no correlation was found between locomotor activity and blood pressure in the F2 population.

At least three genetically determined traits have been linked to hypertension in SHR by the use of experiments similar to those used in the present study. The first, a locus designated esterase-4 (Es-4), controls the genetic expression of a renal esterase hydrolyzing α-naphthyl acetate that has no known relation-
ship to hypertension. The second locus, designated hypertension-2 (Hyp-2), controls the vascular smooth muscle response to cobalt, which could logically be regarded as being one factor responsible for hypertension and appears to be independent of the secondary effects of increased vessel wall stress. The third, hypertension (ht) locus, was revealed by crossing SHR with inbred Donryu rats and demonstrating that blood pressure was controlled by this single major locus, although the biochemical-physiological pathways through which this effect is mediated are unknown.

The demonstration of this single dominant gene does not negate the importance of the Hyp-2 locus or any of the three or more as yet undefined loci that influence blood pressure in SHR. It does indicate, however, that the results of genetic experiments are sensitive to the particular strains selected for cross-breeding.

In this study, the blood pressure of SHR showed typical genetic characteristics of a polygenic trait dependent on approximately four separate genetic loci. Of particular interest was the longitudinal retrospective analysis of blood pressure in SHR-WKY F2 (see Figure 3). The delayed expression of hypertension in the SHR-WKY F2 contrasts with the difference in blood pressure between SHR and WKY that has been demonstrated to exist from birth. This is apparently the first time that such an observation has been made in SHR-WKY F2. The development of hypertension appears to occur just after the time of sexual maturation in these rats. It also is clear from these data that before approximately 12 weeks of age the final adult blood pressure of an individual rat from this population cannot be accurately predicted. Careful epidemiological studies in humans have also revealed that blood pressure during childhood and adolescence is not necessarily predictive of the blood pressure level in adulthood. This has been attributed to the greater observed variance of blood pressure in the young, which results in part from methodological problems, including the critical nature of the cuff size, the ambient temperature, and the diurnal variation of blood pressure. Even after these factors have been accounted for, growth and maturational events, which vary in timing among individuals, have a strong influence on blood pressure in childhood and adolescence. Thus, it is not surprising that the consistent tracking of blood pressure seen in adult humans was also not obvious until after sexual maturation in the male F2 rats.

Confirmation of blood pressure segregation in SHR-WKY F2 allowed analysis of the relationship between sodium balance and the development of hypertension in longitudinal and cross-sectional studies of SHR, WKY, SHR-WKY F1, and SHR-WKY F2. Sodium is of particular interest in the SHR, as differences in sodium handling have been noted between SHR and WKY in a variety of settings. Exchangeable sodium and extracellular fluid volume have been reported to be elevated in adult SHR, and even at 12 days of age the extracellular fluid volume of SHR is greater than that of WKY. Metabolic studies and previous longitudinal studies of exchangeable sodium have shown a period of relative sodium retention in the SHR at a time corresponding to the early developmental phase of hypertension. Similar findings have also been reported in the Milan strain of spontaneously hypertensive rat. Detailed examination of the control mechanisms of sodium handling in the SHR have revealed numerous differences between SHR and control strains. These include elevated plasma renin activity, plasma aldosterone levels, renal Na+,K+-ATPase and efferent renal sympathetic nervous activity as well as increases in renal vascular resistance and reduction in glomerular filtration rate and renal blood flow in young SHR. Each of these is potentially responsible for the observed differences in sodium balance. The occurrence of such abnormalities in immature SHR (i.e., before the development of marked hypertension) has suggested that sodium retention plays a pivotal role in the pathogenesis of hypertension in this strain. In addition, it has been found that a reduction or a supplementation of dietary sodium from a young age can lower or exacerbate, respectively, the final blood pressure in SHR. However, the reduction of sodium intake necessary to inhibit the genetic expression of hypertension was so severe as to inhibit normal growth.

Several differences in sodium handling between SHR and WKY were confirmed in the present study, but it appears from the genetic analysis that these differences in exchangeable sodium and sodium intake cannot be considered to cause the differences in blood pressure between these two strains. This conclusion is primarily based on the lack of correlation between blood pressure and each of the parameters of sodium balance in the F2 population. Of the four criteria for identifying the genetic causes of hypertension described by Rapp, this test of cosegregation is by far the most powerful. Although other independently segregating loci may influence blood pressure in SHR and WKY, they should not interfere with the comparison of a particular trait and blood pressure within a segregating population because of random segregation of the other independent loci, whose net effect should balance and approach zero. The absence of correlation between a particular trait or traits and blood pressure in the segregating F2 population is strong evidence that such a trait or traits (in this case, exchangeable sodium and sodium intake) are not responsible for the difference in blood pressure between SHR and WKY.

Supporting evidence for this conclusion comes from the longitudinal analysis of HF2 and LF2. The exchangeable sodium concentration in these groups was similar between 4 and 16 weeks of age, and the hypertensive group showed no tendency toward sodium retention. These findings contrast with the striking differences observed in the hypertensive (SHR) and normotensive (WKY) parental strains. Interestingly, the hypertensive group (HF2) had less severe hypertension than the SHR, yet there was considerable overlap of the two groups (see Figure 2). Although the relative cardiac mass of HF2 was less than that in SHR, this finding may be influenced by the fact that the HF2 were heavier than SHR (see Table 1). In addition, the rela-
tive cardiac mass of the normotensive $F_1$ (LF$_2$) was lower than that observed in WKY, yet the average blood pressure of LF$_2$ was higher than that in WKY.

In the absence of spontaneous genetic mutation, the factors responsible for hypertension in HF$_1$ must be the same as those causing hypertension in SHR. The slightly lower average blood pressure of HF$_1$ compared with that of SHR suggests that either the HF$_1$ do not possess the full complement of genes responsible for hypertension or there are separate environmental or primary genetic factors affecting the expression of these genes in the $F_2$ rats. Nevertheless, the considerable overlap of blood pressure in HF$_1$ and SHR indicates that HF$_1$ probably possess the majority of those genetic factors (derived from parental SHR) responsible for hypertension. On the other hand, it might imply that HF$_1$ possess a gene that has a marked effect on blood pressure, independent of sodium balance. It is more likely though, that their higher blood pressure results from the combined effect of several of the genes that are responsible for elevation of blood pressure. If a particular gene exerted its hypertensive effect through alterations of sodium balance (and assuming random selection of the hypertensive genes during segregation), at least some difference in sodium balance would be expected in HF$_1$ compared with the normotensive LF$_2$. Such was not the case. These data from HF$_1$ and LF$_2$ provide corroborative rather than conclusive evidence supporting the conclusion that the strain differences in sodium balance are not responsible for the difference in blood pressure between SHR and WKY. Nevertheless, these data indicate that in those HF$_1$ possessing the genes responsible for the majority of the hypertension in SHR (see Table 1 and Figure 2), abnormalities of sodium balance are not apparent.

It appears from the data on SHR-WKY $F_2$ that body weight is an important determinant of exchangeable sodium. The body mass of the hybrid animals was higher than either of the inbred strains and is consistent with the so-called hybrid vigor of SHR-WKY $F_1$ and SHR-WKY $F_2$. The SHR are characteristically smaller at a given age than WKY and have been reported to have proportionally less body fat than WKY. Obviously the higher proportion of lean body mass could influence the exchangeable sodium when expressed per total body mass. This difference in body mass does not satisfactorily explain the most important derangement of sodium balance (i.e., relative sodium retention observed in immature SHR), for the exaggeration of the difference in exchangeable sodium occurs at a time when the percentage difference in body mass is falling. Yet there is no hint from the patterns of exchangeable sodium of HF$_1$ and LF$_1$ that this mechanism plays an important part in the development of hypertension.

Of the other factors affecting sodium balance, sodium intake also was found to be consistently higher in SHR than in WKY. The rats in this study were provided with free access to both $37.5$ mM NaCl drinking fluid and water, and saline preference was about $90\%$ in each of the strains. Other studies have found that, under circumstances of sodium repletion, adult SHR offered $150$ to $300$ mM NaCl fluid drink more saline than required to maintain normal sodium balance. The intake of extra “desired” rather than “required” sodium is reflected in an increased preference for saline over water when the two are offered together to rats fed a normal sodium diet. These differences in saline preference have been linked to abnormalities of the action of angiotensin II in the central nervous system of SHR. The total sodium intake under such circumstances was far higher than in the present study, in which levels closely approximated the values obtained in metabolic experiments in which SHR and WKY were fed laboratory chow and water. Despite the differences in sodium intake of SHR and WKY, the sodium intake of SHR-WKY $F_2$ did not correlate with the blood pressure in these animals.

It is often tempting to relate differences between SHR and normotensive controls to the hypertensive process. If such differences exist before the development of hypertension, they appear to assume even greater pathogenetic significance. Based on the present results, differences in sodium balance between SHR and WKY appear not to be of any primary importance to those pathological, genetic mechanisms responsible for the development of hypertension in SHR. Rather, the characteristics of sodium handling in the SHR probably represent the expression of genes coselected with blood pressure by chance in the common (SHR) ancestors and subsequently conserved by inbreeding.

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