A Genetic Locus (Hyp-2) Controlling Vascular Smooth Muscle Response in Spontaneously Hypertensive Rats

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Summary: Aortic vascular smooth muscle from spontaneously hypertensive rats (SHR) is known to respond in vitro to nonphysiologic cations with greater contraction than vascular smooth muscle from certain normotensive control stocks. The pattern of inheritance of the response of aortic rings to cobalt (Co²⁺) in vitro was determined. The test characteristic utilized was the cobalt response ratio (CRR) defined as the contractile response to 0.6 μM Co²⁺ divided by the response given by maximal stimulation with 10 μM Co²⁺. The SHR were crossed with Dahl salt-resistant rats that had been inbred for 21 generations (R/JR strain) to produce F₁, F₂, and backcross populations. The CRR was 0.90 ± 0.011 in SHR, 0.74 ± 0.016 in F₁, and 0.38 ± 0.031 in R/JR. The F₁ value was significantly higher than the midparental value indicating partial SHR dominance. The F₁ males from reciprocal crosses had similar CRR indicating no sex-linked effect. In the backcross to the R/JR, the CRR showed a bimodal distribution segregating one intermediate type: one R/JR type. In the backcross to the SHR the CRR showed a unimodal distribution. In F₂ rats there was a bimodal distribution segregating three intermediate type: one R/JR type. In F₂ rats there was a significant (p < 0.005) blood pressure difference of 9.7 mm Hg between phenotypes. It is concluded that there is an autosomal locus (named “Hyp-2” for hypertension locus number 2) controlling vascular smooth muscle response to Co²⁺, which exhibits partially dominant inheritance. Alleles at Hyp-2 segregate with an increment in blood pressure. (Hypertension 4:459-467, 1982)

Key Words: genetics, vascular smooth muscle, spontaneously hypertensive rats

A myriad of properties intrinsic to the vascular smooth muscle cell have been found to be different between spontaneously hypertensive rats (SHR) and various normotensive control stocks. Representative abnormalities reported in SHR vascular smooth muscle include altered responses to norepinephrine and potassium, 1, 2 contractions in response to nonphysiologic cations (lanthanum, strontium, manganese, cobalt), I, 3 contraction in response to H⁺, 4 altered electrogenesis, 5 increased (Na⁺, K⁺)-ATPase pump activity, 6 increased Ca²⁺ leakage into the cell, 7, 8 decreased Ca²⁺ accumulation by subcellular fractions, 9, 10 decreased Ca²⁺ binding by the cell membrane, 11 increased sensitivity to Ca²⁺ antagonists, 12 and increased turnover of K⁺, Na⁺, and Cl⁻ due possibly to decreased ability of the cell membrane to retain Ca²⁺ for stabilization. 13

Some attention has been paid to determining whether the vascular smooth muscle changes noted above are secondary results of hypertension or primary causes of hypertension. Four approaches have been tried and all indicated that the changes studied were primary. 1) Visceral 14 and vas deferens 15 smooth muscle as well as vascular smooth muscle showed abnormalities in SHR indicating that smooth muscle alterations in SHR were generalized and not restricted to vessel walls exposed to high blood pressure. 2) Smooth muscle from vascular beds in the leg when protected from hypertension by a proximal ligation showed similar properties to smooth muscle exposed to hypertension in SHR. 16 3) Following pharmacologic control of blood pressure in SHR from weaning, the vascular smooth muscle of SHR still showed its characteristic abnormalities. 17 4) Vascular smooth muscle from young, prehypertensive SHR has properties similar to adult hypertensive SHR. 1 From this work it seems likely that there is a primary genetic abnormality of vascular smooth muscle. This supposition can, however, be proved only by genetic analysis. A genetic approach has the added dividend of bringing powerful techniques to bear on two questions: If there is a primary genetic “defect” in SHR vascular smooth muscle, does it actually cause...
blood pressure differences or is the genetic difference due to genetic drift (chance selection of genes) during inbreeding of the SHR? If the primary genetic effect does increase blood pressure, what is the size of the increase?

For a genetic analysis to be feasible, the difference between SHR and a control strain should be as marked as possible. For this reason alone, the in vitro response to ionic cobalt (Co$^{2+}$) was chosen for genetic studies.

Materials and Methods

The SHR and their normotensive control strain, Wistar-Kyoto rats (WKY), were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts. Dahl salt-sensitive (S) and salt-resistant (R) rats were from our own colony established from stock obtained from Dr. Lewis Dahl at Brookhaven National Laboratory. At the time of these experiments, S rats had been inbred (brother-sister matings) for 13 generations and R for 21 generations. The inbred R rats used for breeding experiments here are designated “R/JR.” The R/JR inbred strain was developed from an R subline (R/C3) which lacks pituitary colloid accumulation.$^{18}$

Rats were killed by cervical dislocation. The thoracic aorta was removed, placed in ice cold medium (see below), and dissected free of fat. Rings (5 mm long, measured against a steel ruler) were cut from the thoracic aorta just above the diaphragm and mounted on stainless steel hangers in a muscle bath at 37°C under 2 g of tension. Muscles were allowed to equilibrate 1 hour before use. The medium contained 118 mM NaCl, 4.7 mM KCl, 12.5 mM NaHCO$_3$ , 1.2 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4$, 2.5 mM CaCl$_2$, 11.1 mM glucose, and was gassed with 95% O$_2$, 5% CO$_2$, pH 7.3. Force was recorded using Grass Instrument (Quincy, Massachusetts) FT.03 force displacement transducers and a four-channel Grass Model 79 polygraph.

Two 50 ml muscle chambers were used and two muscle preparations were accommodated in each chamber. The positions in the chambers of aortic rings from rats of various populations that were studied concomitantly were assigned at random. Injections of Co$^{2+}$ into the chambers were made with microliter syringes. The standard protocol developed to type rats genetically on the basis of aortic response to cobalt was as follows. After the initial equilibrium period, 125 µl of 0.25 mM CoCl$_2$ was injected into the chamber (yielding a final concentration of 0.625 µM Co$^{2+}$) and the response was recorded for 1 hour. This was followed by the addition of 200 µl of 2.5 mM CoCl$_2$ (yielding a final cumulative concentration of 10.625 µM Co$^{2+}$), and the response was recorded for an additional hour. The ratio between the two plateau contractile responses (response to 0.625 µM Co$^{2+}$/response to 10.625 µM Co$^{2+}$) was defined as the cobalt response ratio (CRR). Examples of aortic responses to this protocol will be discussed under Results.

Blood pressure was measured with the rats under ether anesthesia. The tail cuff microphonic method was used.$^{19}$ Blood pressure was measured on at least two separate occasions for each rat between 15 and 17 weeks of age, and the average of these measurements was taken as the blood pressure for the rat. Hearts were fixed in 10% formalin and weighed following fixation.

Analysis of variance and covariance was performed on an Olivetti P6060 minicomputer using programs provided by Olivetti. When contrasts were made between groups following an analysis of variance, the S method of Scheffé was used.$^{20}$ When a trait was tested for dominance, a one-way analysis of variance was first performed on the parental and F$_1$ populations. The value of the trait in F$_1$ rats was then compared to the midparental value by the contrast: 0.5P$_1$ + 0.5P$_2$ - 1F, where P$_1$ and P$_2$ are the parental values and F$_1$ refers to the value of the trait in F$_1$ rats.

Results

In preliminary studies it was found that aortic rings from SHR, WKY, S, and R/JR rats had significant differences in responses to low concentrations of Co$^{2+}$. In order to have an accurate measure of this difference the cobalt response ratio (CRR) was used. This is merely a reflection of the response to 0.625 µM Co$^{2+}$ normalized to the maximal response to Co$^{2+}$. This ratio had lower variability than using the absolute response to a single dose and had the virtue of being reasonably easy to determine on large numbers of animals. Figure 1 shows the cobalt response ratio for...
SHR, WKY, S, and R/JR rats. Analysis of variance showed that there were significant \( p < 0.001 \) differences in CRR between strains. For contrasts of CRR between strains (S method of Scheffe\(^20\)) SHR was higher than WKY \( p < 0.025 \), S \( p < 0.001 \), and R/JR \( p < 0.001 \); WKY was higher than S \( p < 0.001 \) and R/JR \( p < 0.001 \) but S and R/JR were not statistically different \( p > 0.05 \).

To determine the genetic basis for these strain differences, standard genetic crosses were made between SHR and R/JR rats, and responses to cobalt were studied in these populations. Standard genetic crosses are: SHR \( \times \) R/JR to produce \( F_1 \); backcross to the SHR, i.e., \( F_1 \times \) SHR; backcross to the R/JR, i.e., \( F_1 \times \) R/JR; and \( F_1 \times F_2 \) to produce \( F_2 \).

Figure 2 and table 1 show the responses of aortic rings from SHR, \( F_1 \), and R/JR rats to the two standard test doses of \( \text{Co}^{2+} \). The cumulative maximal response given to the 10.625 \( \mu \text{M} \) \( \text{Co}^{2+} \) dose was the same for the three populations (table 1). Differences in responses between populations occurred only at the lower dose of 0.625 \( \mu \text{M} \) \( \text{Co}^{2+} \). There were no significant differences in CRR between males and females, and the CRR data for the sexes were combined for further analysis.

In figure 2 and table 1 it is seen that aortic responses from \( F_1 \) rats are similar to those from SHR but different from R/JR rats, thus suggesting dominance of the SHR phenotype. In table 1 the CRR for males and females combined was R/JR = 0.376 ± 0.0314 (se); \( F_1 \) = 0.743 ± 0.0161; SHR = 0.895 ± 0.0110. A one-way analysis of variance showed that there were significant \( p < 0.001 \) differences among these three values. Contrasts between any two of these three values were significant (all \( p < 0.001 \)). The \( F_1 \) value of 0.743 was significantly \( p < 0.001 \) different from the midparental value of 0.636 (average of 0.895 and 0.376). Thus for the CRR the SHR allele is partially dominant to the R/JR allele.

Figure 3 gives frequency distributions of the CRR for the standard genetic crosses between SHR and R/JR. With dominance of the SHR genotype, the backcross to the recessive is the most informative cross from the genetic standpoint. This backcross (\( F_1 \times \) R/JR) shows a bimodal distribution. By inspection (fig. 3) a CRR of 0.55 was selected as the division point between the two phenotypes in the \( F_1 \times \) R/JR population. Of the 71 rats in this population, 39 were below

### Table 1. Responses to \( \text{Co}^{2+} \) of Aortic Rings from Male and Female R/JR, \( F_1 \), and Spontaneously Hypertensive Rats (SHR)

<table>
<thead>
<tr>
<th>Response to ( \text{Co}^{2+} ) (mg)</th>
<th>Females</th>
<th>Males</th>
<th>Probabilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>R 335 ± 43</td>
<td>863 ± 33</td>
<td>823 ± 60</td>
</tr>
<tr>
<td>( F_1 ) 879 ± 46</td>
<td>1096 ± 46</td>
<td>913 ± 72</td>
<td>785 ± 74</td>
</tr>
<tr>
<td>Cumulative response to ( \text{Co}^{2+} )</td>
<td>0.381 ± 0.0426</td>
<td>0.792 ± 0.0245</td>
<td>0.907 ± 0.0115</td>
</tr>
<tr>
<td>( F_1 ) 0.366 ± 0.0454</td>
<td>0.705 ± 0.0142</td>
<td>0.870 ± 0.0218</td>
<td></td>
</tr>
</tbody>
</table>

Means ± standard errors are given in the left side of the table. Probabilities are from a 2 × 3 factorial analysis of variance. NS = not significant, i.e., \( p > 0.05 \).

*A dose of 10 \( \mu \text{M} \) \( \text{Co}^{2+} \) was given on top of the 0.625 \( \mu \text{M} \) \( \text{Co}^{2+} \) dose, yielding a cumulative total of 10.625 \( \mu \text{M} \) \( \text{Co}^{2+} \); the contraction given is the cumulative total contraction.
Figure 3. Frequency distributions of cobalt response ratio (CRR) for aortic rings from rats obtained by standard genetic crosses between SHR and R/JR inbred rat strains. The data for R/JR, F₁, and SHR populations are from the same rats shown in table 1, with males and females combined. The backcross and F₂ populations were all males. All rats were 16 to 22 weeks of age at time of sacrifice for testing. Numbers of animals on the vertical axis are expressed as a percentage of the total for a given population.

Figure 4. Frequency distributions of blood pressure for populations obtained by standard genetic crosses between SHR and R/JR inbred rat strains. All rats were males 15 to 17 weeks of age at the time blood pressures were measured. Numbers of animals on the vertical axis are expressed as a percentage of the total of a given population.
0.55 and 32 were above 0.55. These numbers do not differ (0.25 < p < 0.5) from a 1:1 ratio by a chi-square test. In the F₂ population (fig. 3) there is a break in the distribution at 0.55 suggestive of a bimodal distribution, but the bimodality was not as obvious as in the backcross to the R/JR. With a CRR of 0.55 as a division point, there were 78 rats above and 20 rats below 0.55. This did not differ (0.25 < p < 0.5) from the expected ratio of 3:1 by a chi-square test. The backcross to the dominant parent, i.e., the F₂ x SHR cross, shows a unimodal distribution for the CRR in figure 3. Thus, although heterozygous (F₁) rats and homozygous SHR had slightly but significantly different CRR, this difference was not large enough to demonstrate segregation of alleles in the backcross to the dominant parental type.

The 12 F₁ males for which CRR data were obtained (table 1) were a random sample from a larger F₁ population of 66 rats produced with reciprocal crosses (i.e., SHR female x R/JR male and R/JR female x SHR male). The two types of males (X chromosome from SHR, or X chromosome from R/JR) had identical CRRs (0.710 ± 0.0153, n = 6, and 0.701 ± 0.0253, n = 6, p > 0.5 by a t test). Thus, there was no evidence for sex-linked effect on the CRR. From the data above, it is concluded that the CRR is controlled by a single autosomal genetic locus which here is designated as the "Hyp-2" locus (hypertension locus number 2). The R/JR allele will be designated by "Hyp-2°" and the SHR allele by "Hyp-2 h."

Figure 4 gives the frequency distributions of blood pressure for male rats for R/JR, SHR, and their standard-genetic-cross populations. Data on blood pressure were available from more rats in the parental and F₁ populations than were tested for CRR, as shown in figure 3. Unimodal distributions of blood pressure were shown in all the populations with the notable exception of the backcross to the SHR. In this case the data strongly suggest a bimodal distribution.

### Table 2: Blood Pressures of Phenotypes for Cobalt Response Ratio (CRR) in Segregating Populations (F₂ and F₁ x R/JR)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>F₂ population</th>
<th>F₁ x R/JR backcross population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyp-2°Hyp-2°</td>
<td>Hyp-2°Hyp-2°</td>
<td>Hyp-2°Hyp-2°</td>
</tr>
<tr>
<td>Segregation ratio</td>
<td>1</td>
<td>2*</td>
</tr>
<tr>
<td>Corresponding phenotype</td>
<td>CRR &lt; 0.55</td>
<td>CRR &lt; 0.55</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>156.7 ± 1.88</td>
<td>166.4 ± 1.86</td>
</tr>
<tr>
<td>No. of rats</td>
<td>20</td>
<td>78</td>
</tr>
</tbody>
</table>

### Figure 5. Relationship between heart weight and body weight for F₂ rats. An analysis of covariance showed that for a given body weight the hearts of rats with phenotype CRR above 0.55 averaged 74 mg heavier than the hearts of rats with phenotype CRR below 0.55.

Blood pressures of rats at 15 to 17 weeks of age were compared with segregating CRR phenotypes in F₂ rats. Table 2 gives the genotypes at the Hyp-2 locus, their corresponding CRR phenotypes, and mean blood pressures for the CRR phenotypes. In F₂ rats there was a significant (p < 0.005) difference of about 10 mm Hg between blood pressure means for the segregating CRR phenotypes. Blood pressures were also compared between CRR phenotypes in the F₁ x R/JR population. The results in table 2 show that the mean blood pressures for the two phenotypes were essentially identical. Reasons for the significant association of CRR phenotypes and blood pressure in F₂, but not in F₁ x R/JR rats, are given in the Discussion.

Heart weights between CRR phenotypes segregating in the F₂ population were compared. Because genes for growth are segregating in F₂ rats and because it was impossible to kill and type all rats for CRR at exactly the same age, there was a wide range of heart and body weights in F₂ rats. Figure 5 shows the relationship between heart and body weight for the CRR phenotypes; it suggests that, for a given body weight, rats...
with a CRR above 0.55 have heavier hearts than rats with a CRR below 0.55. This was proven by an analysis of covariance, with heart weight as the variate and body weight as the covariate. It was concluded from the analysis that: 1) the slopes of the regression lines relating heart and body weights for the two CRR phenotypes were not different ($p = 0.67$), i.e., the lines were parallel; 2) the slope of the overall regression of heart weight and body weight was not equal to zero ($p < 0.005$); and 3) the positions (slope intercepts) of the two regression lines for CRR phenotypes were significantly different ($p < 0.005$). This difference in heart weight (slope intercepts) between the two CRR phenotypes was found between these CRR phenotypes in F2 rats. This corroborates the blood pressure difference of 10 mm Hg found between these CRR phenotypes in F2 rats.

Discussion

The genetic data presented show that vascular responses to Co+ are regulated by a single, autosomal locus with inheritance by partial dominance. Since this locus may be involved in causing blood pressure differences in the rat, it has been named "Hyp-2" for hypertension locus number 2. The previously described locus controlling blood pressure in Dahl S and R rats is named "Hyp-1." The Hyp-1 locus is known to be the structural locus for the adrenal mitochondrial cytochrome P-450, which catalyzes both 11β- and 18-hydroxylation of steroids.

For a genetic locus controlling some biochemical or physiological trait to be accepted as one causing a blood pressure difference, it should meet the following four criteria: 1) a difference in a biochemical-physiological trait between two strains must be demonstrated; 2) the trait must follow Mendelian inheritance; 3) the genes identified in criterion 2 must cosegregate with an increment in blood pressure significantly different from zero; and 4) some logical biochemical-physiological link must exist between the trait and blood pressure. Criterion 4 is necessary to reduce the possibility that the genetic trait under study actually has no direct physiological connection with blood pressure but cosegregate with an increment in blood pressure because it is closely genetically linked to a locus that really does influence blood pressure.

In the following discussion, the genotypes at Hyp-2 are designated "bb" (homozygous for SHR allele), "ab" (heterozygote), and "aa" (homozygous for R/JR allele). Criterion 3 is by far the most powerful. First, if criterion 3 is not met, then it eliminates from further consideration genetic differences between strains which, even though they may be well-defined genetic polymorphisms, do not affect blood pressure. A myriad of such differences will always be found between any two inbred strains because of chance selection and fixation of genes. Second, if criterion 3 is met, even though blood pressure is under polygenic control (meaning that genes at many genetic loci influence blood pressure), criterion 3 allows estimation of the blood pressure effects of each locus independently of the others. Genes at other independently segregating loci that influence blood pressure do not interfere with the comparison of the blood pressure effects of the genotypes at a specific locus within a segregating population. For example, an animal homozygous for $bb$ at Hyp-2 may carry genes at other loci for high or low blood pressure; similarly for $ab$ or $aa$ rats at Hyp-2. Since these other genes segregate at random with respect to Hyp-2, the net effect on blood pressure of other loci on, for example, a comparison of $bb$ versus $aa$ rats at Hyp-2 within a segregating population will be zero, provided a large number of rats are studied. Third, the problem of cause and effect relationships between a biochemical-physiological trait and blood pressure always lacks definitive resolution in comparing a hypertensive and control strain. Is the trait under consideration a cause of strain blood pressure differences, or the result of such differences? If met, criterion 3 largely solves the issue (with the reservation that closely linked genes are not resolved). A priori, the genes controlling the biochemical-physiological trait must be causing any blood pressure differences that cosegregate with them. The blood pressure of a rat cannot determine what genes (and associated phenotypes) it inherited from its parents because the genes were obtained at fertilization before the rat's circulatory system was even formed. Note that the power of this genetic argument depends completely on one being able to identify discrete genotypes and associated phenotypes in segregating populations for the biochemical-physiological trait of interest, and associating these discrete phenotypes with blood pressure differences. A mere correlation between a biochemical-physiological trait and blood pressure is insufficient to complete the argument because such a correlation can arise from primary genetic causes or as secondary consequences of blood pressure.

The CRR does meet criteria 1 and 2, but how well it meets criteria 3 and 4 merits discussion. Do genes at the Hyp-2 locus cosegregate with an increment in blood pressure and if so what is the size of the increment (criterion 3)? Since in the F2 population $ab$ and $bb$ genotypes cannot be differentiated, one must compare the blood pressure of $aa$ rats with a mixture of $ab$ and $bb$ rats. This mixture of $ab$ and $bb$ of course should occur in a 2:1 ratio (since genes will segregate $aa$: $2 ab$: $1 bb$ in an F2 population). From these considerations and the result that $aa$ versus the 2:1 mixture of $ab$ and $bb$ rats differed ($p < 0.005$) in blood pressure by about 10 mm Hg in F2 rats (table 2), it is possible to calculate that one dose of the $b$-allele increases blood pressure by 7.5 mm Hg, and two doses of the $b$-allele should, therefore, increase blood pressure by 15 mm Hg. Of course the fact that the artificial probe Co+ cannot differentiate the $ab$ and $bb$ genotypes may be a property of this measure and does not necessarily mean that the blood pressure of $ab$ and $bb$ rats does not differ.
Table 2 shows that an increment in blood pressure was associated with the CRR phenotypes in F2 rats but not in the backcross to the R/JR. This phenomenon is identical to the experience with blood pressure effects of the pituitary colloid (Pc) locus described in Dahl S and R (subline R/A61) rats. In this case, accumulation of pituitary colloid correlated strongly with (low) blood pressure in F2 rats, but in a backcross to the R/A61 line no effect could be demonstrated. The reason is the genetic background of the R rat; genetic background in the present context is interpreted to mean all other genes at loci that influence blood pressure besides the locus under consideration. These "background" genes are, of course, presumably identical to the genes referred to when one speaks about polygenic inheritance. In the case of the R rat (or its derived inbred sublines), the animal is very resistant to hypertensive stimuli by virtue of its genetic makeup. For example, the blood pressures of R rats respond very little to salt, deoxycorticosterone, cortisone, adrenal regeneration, renal artery constriction, or psychological stress. Since blood pressure regulation is polygenic it is not surprising that the hypertensive "stimulus," in this case the relatively weak Hyp-2 allele, is not effective on a background of genes at several other loci that connote resistance to elevated pressure. The genetic background in the F1 × R/JR population is made up of 75% R/JR genes and 25% SHR genes, and in F2 rats the background is 50% R/JR genes and 50% SHR genes. This may be enough of a difference to allow the expression of the Hyp-2 allele in F1 but not in F2 × R/JR rats. Yamori and Okamoto have described a well-defined genetic polymorphism in a renal esterase hydrolyzing α-naphthyl acetate. The locus involved is known as "esterase-4 (Es-4)" in the genetic literature. This locus meets criteria 1, 2, and 3 discussed above, but not criterion 4. That is, since the function of the enzyme esterase 4 is unknown, one cannot know whether the Es-4 locus, or one genetically linked to it, is responsible for the observed cosegregation of alleles at Es-4 and an increment in blood pressure. Nevertheless, an interesting effect of genetic background is evident on the association of blood pressure and Es-4 genes in crosses of SHR and normotensive Wistar rats. From figure 3 of Yamori et al., it can be estimated that the blood pressure increment associated with a single dose of the SHR allele at Es-4 is ~3 mm Hg in the backcross to the Wistar, +6 mm Hg in the F1, and +15 mm Hg in the backcross to the SHR. Thus, as the proportion of SHR genes increases in the background of a given cross (25% in backcross to Wistar, 50% in F1, and 75% in backcross to SHR), the effect associated with the SHR allele at Es-4 increases.

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strains were developed for this locus. With these congeneric strains Tanase clearly demonstrated for blood pressure 1:1 bimodal distributions in backcross populations, and 1:2:1 trimodal distributions for blood pressure in F2 populations. The locus was named "ht" (for hypertension) by Tanase. The biochemical-physiological mechanism by which the ht locus affects blood pressure is unknown. Evidence was also given by Tanase, that ht was not the only locus influencing blood pressure. The key to success in Tanase's work was finding the proper genetic background with which to demonstrate the ht locus.

Figure 4 shows a reasonable bimodal distribution for blood pressure in the backcross to the SHR. This is probably a 1:1 segregation, although that is difficult to test because of overlap between the two peaks. The blood pressure distributions in the other segregating populations (F1 × R/JR and F2) in figure 4 are unimodal. It is likely that the bimodality in the F1 × SHR population results from the ht locus described by Tanase and that the unimodal distributions in the F2 and backcross to the R/JR result from dilution of the effect of the SHR allele at the ht locus by the effects of more R/JR genes at other loci.

The size of the blood pressure effect of a single SHR allele at the ht locus was estimated by Tanase to be 19 mm Hg in males on the favorable Donryu background. This is compatible with the difference of 20 mm Hg between the centers of the peaks of the bimodal distribution for blood pressure in the F1 × SHR population. This can be contrasted with a single SHR allele at Hyp-2, which was estimated above to change blood pressure by 7.5 mm Hg. This contrast is not entirely appropriate, however, because these estimates are obtained on genetic backgrounds (F1 × SHR for the ht locus, and F2 for the Hyp-2 locus), which differ in the proportions of segregating SHR and R/JR genes. One of the background loci is almost certainly the Hyp-1 locus. The R/JR should carry the R allele at Hyp-1 since alleles at this locus were fixed in the stock originally obtained from Dahl. SHR probably carry the S allele at Hyp-1, based on previous data (see fig. 8 of ref. 38). Another background locus segregating in this experiment is certainly Es-4, since SHR and R rats also carry contrasting alleles at this locus.

In light of the detailed genetic discussion above, it is worth pointing out that data on strain comparisons like that in figure 1 are usually misinterpreted. One erroneous interpretation often made is that the CRR has nothing to do with blood pressure because S and R/JR rats, with very different propensities for hypertension, have similar CRR. The correct interpretation is that the Hyp-2 locus controlling CRR was not polymorphic in the base population from which S and R were selected. All rats in the base population were probably homozygous aa; therefore, selective breeding could have no effect at this locus. Because blood pressure is under polygenic control, it is impossible to draw definitive conclusions about cause and effect relationships between biochemical-physiological traits and blood pressure from such strain comparisons. In this case the
genetic differences between S and R/JR that affect blood pressure must arise from loci other than Hyp-2. The position of the WKY strain in figure 1 may arise because it carries an allele other than alleles a and b at Hyp-2 or because it carries a or b alleles with modification of the final phenotype by other genetic loci.

We come to the question: Is criterion 4 met for the Hyp-2 locus? This requires that there be a biochemical-physiological connection between the genetically analyzed trait and blood pressure. Obviously Co\(^{2+}\) per se has nothing to do with blood pressure, but Co\(^{2+}\) probably interacts in the smooth muscle cell with molecules that also interact with Ca\(^{2+}\). It makes sense that if the SHR vascular smooth muscle cell is genetically hyper-responsive to Co\(^{2+}\) this may result from the same abnormality that apparently allows Ca\(^{2+}\) to leak into the cell and increase vascular tone.\(^{7,8}\) Criterion 4 is not, however, completely met until "the" molecule in smooth muscle coded by the Hyp-2 locus is identified and shown to be involved in regulating smooth muscle contraction.

We have noted in the Introduction that there are many abnormalities of vascular smooth muscle described in SHR. It is possible that some of these are pleiotropic effects of the Hyp-2 locus, defined here only in terms of response to Co\(^{2+}\), but there may be other loci controlling smooth muscle abnormalities in SHR. These possibilities can be differentiated by further genetic analyses. There must also be loci that increase blood pressure through other physiologic systems in SHR.

It is concluded that there is a primary genetic mechanism operating through vascular smooth muscle contractility in SHR to increase blood pressure. Folkow et al.\(^{34}\) showed, with in situ perfused vascular beds, that the secondary structural hypertrophy of the vessel wall accounts for the hypersensitivity of the perfused beds from hypertensive SHR to agonists. The present data, however, also show that a primary genetic "defect" operates through vascular smooth muscle to increase blood pressure.

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