Genetic Association of Hypertension and Vascular Changes in Stroke-Prone Spontaneously Hypertensive Rats

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SUMMARY Isolated tail arteries from stroke-prone spontaneously hypertensive rats (SHRSP) exhibit oscillatory contractile activity in response to norepinephrine, whereas those from normotensive Wistar-Kyoto rats (WKY) do not. To determine whether the norepinephrine-induced oscillations are related to high blood pressure or to separable genetic differences between strains, the response to norepinephrine was studied in adult SHRSP, WKY, and progeny of genetic crosses of SHRSP and WKY (F1, F2, F1 x SHRSP, F1 x WKY). Helical tail artery strips were mounted in a tissue bath for isometric force recording. Rats were classified as responders if oscillatory activity in the presence of 1.8 x 10^-7 M norepinephrine exceeded 250 mg/10 min (milligrams of force amplitude during a 10-minute interval). The blood pressures (mm Hg ± SEM; tail cuff method) and percentage of rats exhibiting norepinephrine-induced oscillations were as follows: WKY: 109 ± 3, 0%; F1: 129 ± 4, 0%; F2: 150 ± 4, 38%; F1 x WKY: 137 ± 3, 9%; F1 x SHRSP: 188 ± 7, 71%; SHRSP: 207 ± 7, 100%. The distribution of the frequency of animals with oscillatory activity among the progenies was consistent with the hypothesis that a single gene locus determines the observed difference in oscillatory activity between the WKY and SHRSP strains. The allele from the SHRSP that determines the activity phenotype is recessive to the allele contributed by the normotensive WKY strain. In the segregating F2 progeny, the blood pressure of the responders was higher than that of the nonresponders (161 ± 6 vs 144 ± 4 mm Hg; p<0.05). We conclude that the genetic locus that controls the norepinephrine-induced oscillatory activity may contribute to the observed blood pressure difference between WKY and SHRSP. (Hypertension 8: 904-910, 1986)

KEY WORDS • blood pressure • norepinephrine • tail artery • Wistar-Kyoto rats

THE established phase of hypertension is characterized by normal cardiac output and elevated total peripheral resistance.1 The factors responsible for maintenance of elevated peripheral resistance may be extrinsic to the vasculature itself (i.e., neural and hormonal influences) or intrinsic to the blood vessel (i.e., structural and functional alterations). Many intrinsic vascular abnormalities have been described in hypertensive animals,2 but whether these vascular changes are a cause or consequence of elevated blood pressure is unclear. Several experimental techniques have been used to determine whether specific vascular changes are primary or secondary to hypertension development. Experiments in which vascular beds are protected from elevated blood pressure by proximal ligatures have yielded conflicting results. In some instances vascular changes persist in the protected beds,3 4 whereas other investigators have found that vascular abnormalities are absent in vessels that have been protected from elevated blood pressure.5 Vascular alterations are present in spontaneously hypertensive rats (SHR) whose blood pressure has been controlled from the time of weaning with antihypertensive drug treatment, suggesting that these vascular changes are primary events and not secondary to hypertension.6 This contention also is supported by the
observations that vascular changes appear before the
development of hypertension in deoxycorticosterone-
salt hypertensive rats\textsuperscript{4} and SHR.\textsuperscript{1,8}

Despite previous work suggesting that primary vas-
cular defects may contribute to hypertension develop-
ment, most investigators have not attempted to quanti-
fy the contribution of these abnormalities to the total
elevation in blood pressure. Therefore, the present
study was undertaken to determine if a specific vascu-
lar abnormality potentially could cause an elevation in
blood pressure and, if so, to determine the size of the
pressure increment that could be attributed to this trait.
The vascular trait examined in this regard was the
development of oscillations in generated force in re-
sponse to norepinephrine in isolated tail arteries of
stroke-prone SHR (SHRSP). This norepinephrine-
induced oscillatory activity has been described pre-
viously\textsuperscript{9,10} and has been observed only in SHRSP
under the conditions of our experiments. A genetic
analysis of blood pressure and the frequency of occur-
rence of norepinephrine-induced oscillations in tail
arteries was performed on the offspring of matings of
SHRSP and normotensive Wistar-Kyoto rats (WKY).
A genetic approach was used in order to establish
whether the oscillatory activity exhibited by SHRSP
was associated with a significant increment in blood
pressure in progenies of the cross of SHRSP and WKY
or whether oscillatory activity and blood pressure are
independent, separable traits in SHRSP.

Materials and Methods

The SHRSP and WKY used in these experiments
were obtained from a colony in the Department of
Anatomy and Cell Biology at The University of Michi-
gan, which was initially derived from stock procured
from the National Institutes of Health. The F\textsubscript{1} gen-
eration was produced by matings of SHRSP and WKY.
The F\textsubscript{2} generation resulted from brother-sister matings
of F\textsubscript{1} rats. Backcross populations were formed by the
crossing of F\textsubscript{1} rats with each parental line
(F\textsubscript{1} x SHRSP and F\textsubscript{1} x WKY). Rats were group-
housed in light-cycled (lights on 600–1800), tempera-
ture-controlled quarters with ad libitum access to food
and water. Both male and female rats were used and
were 200 to 400 days of age at the time of experimenta-
tion. Systolic blood pressure was determined in con-
scious, restrained rats by a tail cuff plethysmographic
method (pneumatic transducer).

Rats were killed by cervical dislocation, and tail
arteries were excised immediately and stored in a cold
physiological salt solution until experimentation. With
the use of a dissecting microscope, tail arteries were
dissected free of fat and connective tissue and cut into
helical strips (0.7 x 10 mm). Tail artery strips were
mounted on stainless steel hangers and suspended in a
50-mL tissue bath filled with physiological salt solu-
tion. The temperature of the tissue bath was main-
tained at 37°C throughout the experiment. Vascular
strips were allowed to equilibrate for 60 minutes under
a constant passive force of 600 mg. Isometric force
was measured using Grass FT 03 force transducers and
recorded on Grass polygraphs (Quincy, MA, USA).
The physiological salt solution was aerated with a mix-
ture of 95% O\textsubscript{2} and 5% CO\textsubscript{2}, and its composition (mM)
was as follows: NaCl, 130; KCl, 4.7; KH\textsubscript{2}PO\textsubscript{4}, 1.18;
MgSO\textsubscript{4}-7H\textsubscript{2}O, 1.17; NaHCO\textsubscript{3}, 14.9; CaCl\textsubscript{2}-2H\textsubscript{2}O,
1.6; dextrose, 5.5; and CaNa\textsubscript{2} ethylenediaminetetra-
acetic acid, 0.03.

Concentration-response curves to norepinephrine
(Levophed bitartrate) were determined in all experi-
ments. Norepinephrine (6 x 10\textsuperscript{-9} to 6 x 10\textsuperscript{-6} M) was
added to the tissue bath, and the strips were allowed to
contract for 20 minutes. Between doses, norepineph-
rine was rinsed from the tissue bath and the strips were
allowed to recover to baseline force values. Oscillatory
activity was quantitated in strips from SHRSP and
WKY and was defined as the sum of the phasic con-
tractile amplitudes for all oscillations occurring during
the final 10 minutes of norepinephrine incubation. In
preliminary experiments on parental animals, oscilla-
tory activity was found to be reproducible in separate
vascular strips from any given rat. All rats (i.e.,
SHRSP, WKY, and progeny) were categorized as ei-
ther responders or nonresponders to norepinephrine
with respect to the development of oscillatory activity.
A phasic contractile activity of 250 mg (frequency
taxilligrams of force) at 1.8 x 10\textsuperscript{-7}M norepi-
nephrine (the concentration that produced maximal oscilla-
tory activity in vessels from SHRSP) was established
as the criterion for oscillatory activity. Rats with ves-
sels showing contractile activity greater than this crite-
ron were classified as responders, whereas rats that
did not meet this criterion were considered nonre-
ponders.

A group t test was used to compare blood pressures
of responders and nonresponders within segregating
progeny. Chi-square goodness of fit was used to deter-
mine the significance of deviation of the observed fre-
quency of oscillatory activity from predictions based
on a single gene model.\textsuperscript{11} A p level of less than 0.05
was used as the criterion for statistical significance.

Results

Representative tracings depicting the oscillations in
developed force in response to norepinephrine are
shown in Figure 1. On addition of norepinephrine to
the bath, all strips responded with a rapid increase in
force. In tail artery strips from WKY, this initial in-
crease in force was followed by a sustained contraction
that was maintained until norepinephrine was rinsed
from the bath. In contrast, vessels from SHRSP dis-
played phasic contractions and relaxations in response
to norepinephrine (oscillatory activity) that began 3 to
6 minutes after addition of the agonist and continued
until removal of norepinephrine. Tail arteries from all
SHRSP examined exhibited oscillatory activity,
whereas this response was absent in tail arteries from
WKY.

The concentration-response relationship of norepi-
nephrine-induced oscillatory activity is shown in Fig-
FIGURE 1. Representative tracings of force development in response to $1.8 \times 10^{-7}$ M norepinephrine in tail arteries from WKY and SHRSP. All tail arteries from SHRSP exhibited fluctuations in generated force in the presence of norepinephrine, whereas contractile responses to norepinephrine remained constant in tail arteries from WKY.

FIGURE 2. Oscillatory responses to norepinephrine in tail arteries from SHRSP and WKY. Points and vertical bars represent means ± SEM.

FIGURE 3. Frequency histograms depicting the tail cuff pressures in each population of rats are shown. The mean systolic blood pressure (mm Hg ± SEM) for each population was as follows: WKY: 109 ± 3; $F_1$: 129 ± 4; $F_2$: 150 ± 4; $F_1 \times$ WKY: 137 ± 3; $F_1 \times$ SHRSP: 188 ± 7; SHRSP: 207 ± 7. The systolic blood pressures of the $F_1$ progeny were closer to the WKY parent than to the SHRSP parent.

Rats were classified as responders or nonresponders to norepinephrine based on the development of oscillatory activity according to the criterion described in Methods. The proportion of rats in each progeny that responded to norepinephrine with oscillatory activity is shown in Figure 4. None of the tail arteries from WKY or $F_1$ rats exhibited norepinephrine-induced oscillatory activity, whereas 100% of vessels from SHRSP displayed such activity. Figure 5 shows that there was a direct relationship between the average systolic blood pressure of a progeny and the percentage of animals that exhibited oscillatory responses to norepinephrine. To determine whether transmission of the norepinephrine-induced oscillatory activity from parents to offspring can be explained by a single gene mode of inheritance, a chi-square goodness of fit of the observed versus expected frequency of responders to norepinephrine in each progeny was performed. The data suggest that the phenotype of WKY is dominant to the phenotype of SHRSP. Assuming that the presence of oscillatory activity is a recessive trait, one would expect to observe 25% responders in the $F_2$, none in the $F_1$ or $F_1 \times$ WKY, and 50% in the $F_1 \times$ SHRSP. Table I shows that our data do not deviate significantly from those expectations; hence, the difference between the WKY and SHRSP is consistent with allelic differences at a single gene locus.

To determine if an increment in blood pressure was associated with segregation of the oscillatory activity, the average blood pressure of the responders and nonresponders in the segregating progenies was compared (Table 2). In the $F_2$ population, rats that exhibited oscillatory activity had significantly higher blood pressures than those that did not respond to norepinephrine with oscillations (161 ± 6 vs 144 ± 4 mm Hg; $p<0.05$). Although responders had a higher blood pressure than nonresponders in the $F_1 \times$ SHRSP backcross progeny, the difference between these smaller samples did not reach statistical significance.
VASCULAR CHANGES IN HYPERTENSION/Bruner et al.

Discussion

The purpose of the present experiments was to establish whether norepinephrine-induced oscillatory activity observed in tail arteries of SHRSP can be explained by a single gene difference with WKY and whether an increment in blood pressure is associated with the inheritance of this trait. As reviewed in the Introduction, several experimental approaches including antihypertensive therapy and surgical protection of vascular beds from elevated blood pressure have been used to determine whether vascular changes could possibly cause hypertension or whether they result from elevated arterial pressure. A genetic analysis not only will indicate whether the vascular change is primary but has the added advantage of providing an estimate of the increment in arterial pressure that can be attributed to the genetic locus that determines the vascular change.12

Four criteria have been suggested12 as necessary before a genetic locus can be accepted as one that causes an elevation in blood pressure: 1) a significant difference in the trait of interest must be demonstrated between two strains; 2) the pattern of inheritance of the trait must follow Mendelian genetics; 3) the genes controlling the trait must cosegregate with an increment in blood pressure significantly different from zero; and 4) some logical physiological link must exist between the trait and regulation of blood pressure. The trait of norepinephrine-induced oscillatory activity in tail arteries of SHRSP meets all of these criteria. First, a significant difference does exist between SHRSP and WKY with respect to the occurrence of oscillatory activity. Tail arteries from all SHRSP examined demonstrated this activity, whereas no oscillations were observed in tail artery strips from WKY.

The second criterion for establishing a genetic association between a specific trait and blood pressure is that the trait must follow Mendelian inheritance.

Figure 3. Distribution of blood pressures (tail cuff method) in WKY, F1, F2, F1 × WKY, F1 × SHRSP, and SHRSP. Numbers on the ordinate are the percentage of animals in each group. Arrows on the abscissa represent mean blood pressures for each group (values reported in text).

Figure 4. Frequency of occurrence of oscillatory activity in tail arteries from WKY, F1, F2, F1 × WKY, F1 × SHRSP, and SHRSP. Numbers on the ordinate are the percentage of animals in each group that exhibited norepinephrine-induced oscillations (see text for numerical values).

Figure 5. Relationship between blood pressure and the occurrence of oscillatory activity in each progeny.
The third criterion that must be satisfied for a trait to be implicated in determining blood pressure is that the genes controlling the trait must associate with an increment in blood pressure that is significantly different from zero. This criterion can be evaluated in the F2, F1 × WKY, and F1 × SHRSP progeny. These progeny have been termed segregating progeny since it is in these rats that the genes controlling blood pressure and the oscillatory activity have a chance to recombine with respect to one another. If the genetic loci controlling these two traits are closely associated in the parental strains, then the traits will remain associated in the segregating progeny and a significant difference in blood pressure between the two oscillatory phenotypes (nonresponders and responders) will be observed. On the other hand, if the genetic loci controlling blood pressure and the vascular response are separable, then they will distribute randomly with respect to one another and there will be no difference in blood pressure between responders and nonresponders in the segregating progeny. In the present experiments, the blood pressure difference between the two oscillatory phenotypes could not be evaluated in the F1 × WKY progeny because in the backcross to the dominant parent, few, if any, rats with the recessive phenotype are observed (i.e., only 1 rat exhibited oscillatory activity in this progeny). Therefore, the F2 and F1 × SHRSP progeny were evaluated for blood pressure differences between responders and nonresponders. A significant difference in blood pressure between responders and nonresponders was observed in the F2 progeny (161 vs 144 mm Hg, respectively). In the F1 × SHRSP progeny, no significant difference in blood pressure was observed between responders and nonresponders. One possible explanation for a lack of blood pressure difference between the two phenotypes in this progeny is the following: blood pressure is a polygenic trait, and in the present experiments the effect of a single locus on blood pressure was examined. This locus controls the norepinephrine-induced oscillations in tail arteries and is responsible for a relatively small but significant (17 mm Hg) elevation in blood pressure in F2 rats. The genetic composition of F2 rats as a population is derived from the parent SHRSP and WKY in the ratio of 1:1. However, in the F1 × SHRSP and F1 × WKY progeny, the genetic composition is 3:1, with the parent to which the F1 was backcrossed contributing 75%.

### Table 1. Observed and Expected Frequencies of Oscillatory Activity Based on a One Genetic Locus Model

<table>
<thead>
<tr>
<th>Progeny</th>
<th>Frequency</th>
<th>Observed</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>0/9</td>
<td>0/9</td>
<td></td>
</tr>
<tr>
<td>SHRSP</td>
<td>13/13</td>
<td>13/13</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>0/18</td>
<td>0/18</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>13/34</td>
<td>8.5/34</td>
<td></td>
</tr>
<tr>
<td>F1 × WKY</td>
<td>1/11</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>F1 × SHRSP</td>
<td>12/17</td>
<td>8.5/17</td>
<td></td>
</tr>
</tbody>
</table>

Statistical evaluation (chi-square goodness of fit) of observed versus expected frequencies of oscillatory activity was performed on the F2 and F1 × SHRSP progeny. Calculation of a chi square for the F1 × WKY progeny was not possible because of the expected frequency of zero in this group. NS = not significant.

Therefore, the frequency of occurrence of oscillatory activity was investigated in offspring of standard genetic crosses of SHRSP and WKY. In the group of rats studied, the presence of oscillatory activity was found to be a recessive trait since none of the F1 rats exhibited oscillations. On the assumptions that one genetic locus controls this vascular trait and that oscillatory activity is recessive, one may calculate the expected frequency of occurrence of oscillations in the F2, F1 × WKY, and F1 × SHRSP progeny. These expected frequencies are 25% in F2, 0% in F1 × WKY, and 50% in F1 × SHRSP. The observed frequencies of oscillatory activity in the rats studied were not significantly different from predictions based on a single genetic locus with two alleles; however, our observed frequencies were not precisely what would be expected based on this model. The deviation of our observations from the calculated expectations may be due to 1) misclassification of some rats because of the arbitrary nature of our criterion for oscillatory activity and 2) other segregating factors that may modify the effect of the primary genetic locus. The data do satisfy the first two criteria for genetic association of two traits: a vascular response has been identified that is different in SHRSP and WKY, and the mode of inheritance of this response is consistent with Mendelian inheritance at a single genetic locus.

### Table 2. Systolic Blood Pressures of Phenotypes for Oscillatory Activity in Segregating Progeny

<table>
<thead>
<tr>
<th>Variable</th>
<th>F2</th>
<th>F1 × SHRSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>aa</td>
<td>Aa</td>
</tr>
<tr>
<td>Segregation ratio</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Corresponding phenotype</td>
<td>Oscillatory activity, No oscillatory activity</td>
<td>Oscillatory activity, No oscillatory activity</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>161 ± 6</td>
<td>144 ± 4*</td>
</tr>
<tr>
<td>Number of rats</td>
<td>13</td>
<td>21</td>
</tr>
</tbody>
</table>

Blood pressure values are means ± SEM. a = allele of SHRSP; A = allele of WKY. *p < 0.05 (Student’s t test), compared with F2 rats that exhibited oscillatory activity.
of the total genome. Therefore, in these progeny, a small blood pressure difference of 17 mm Hg contributed by one genetic locus may be obscured against a genetic background that heavily favors either one of the original parental strains.

The final criterion to be met before one accepts that a candidate gene is involved in the etiology of hypertension is that the trait must make "physiological sense" regarding its potential to influence blood pressure. The altered vascular response to norepinephrine observed in SHRSP in the current experiments is congruent with many other studies that have demonstrated an alteration in sympathetic nervous system function in SHR. Sympathetic nerve activity and blood pressure increase with age over a similar time course in SHR.14 That this increase in nerve activity is important for hypertension development is supported by the findings that chemical sympathectomy15 and treatment with sympatholytic drugs16 attenuate hypertension development in SHR. Isolated, perfused hindquarters of SHR16 and perfused kidneys from SHR17 and SHRSP18 show exaggerated vasoconstrictor responses to exogenous norepinephrine, although this is not a universal finding.19 Isolated tail artery preparations from SHR also exhibit an increased postjunctional sensitivity to norepinephrine when compared with that in normotensive controls.4,20,21 In addition, an enhanced vasoconstrictor nerve influence has been demonstrated in SHR.22 Thus, there is evidence that both enhanced sympathetic nervous system function and increased sensitivity of vascular smooth muscle to norepinephrine occur in SHR. The vascular response to norepinephrine examined in the current experiments has been shown to be due to an altered calcium-stimulated potassium efflux in SHRSP.23 This alteration in membrane ionic mechanisms may lead to enhanced vascular sensitivity and to an increase in total peripheral resistance. Additional support for an important physiological role for the oscillatory activity observed in these studies comes from the work of Colantuoni et al.24 and Funk et al.25 These investigators have suggested that spontaneous arteriolar vasomotion is an important contributor to vascular resistance. Furthermore, it has been estimated that 30% of the increased hindquarter vascular resistance in renal hypertensive rats can be attributed to rhythmic arteriolar vasomotor activity.25 Precisely how the oscillatory activity examined in the present experiments relates to events in the intact vascular bed is not yet clear.

Despite the many reports of alterations in vascular sympathetic function in SHR, few attempts have been made to determine whether these alterations cosegregate with a significant increment in blood pressure. This is especially surprising in light of the fact that a genetic analysis has two major strengths: 1) traits that are associated with blood pressure can be distinguished from those that result from chance fixation of genetic differences between strains and 2) if a trait is genetically associated with elevated blood pressure, the size of the blood pressure increment caused by the specific genetic locus can be determined. To date, two studies have used genetic methods to associate altered sympathetic function with elevated blood pressure in SHR. In the first, Judy et al.26 found a significant correlation between mean arterial pressure and sympathetic nerve activity in a segregating progeny of SHR and normotensive Wistar/Lewis rats. In the second, Laher and Triggle27 demonstrated that postfunctional sensitivity to norepinephrine in isolated tail artery strips was significantly correlated with mean arterial pressure in SHR, WKY, F1, F1 × WKY, and F1 × SHR. However, this second observation does not necessarily mean that blood pressure and norepinephrine sensitivity are genetically associated, since this correlation was obtained across progeny and not within a single segregating progeny. In the present experiments, the occurrence of norepinephrine-induced oscillatory activity was associated with a significant blood pressure difference within the segregating F2 progeny. The magnitude of the blood pressure elevation associated with allelic differences at the genetic locus controlling this vascular trait is approximately 17 mm Hg.

In conclusion, the observations reported herein are consistent with the hypothesis that a single genetic locus determines the norepinephrine-induced oscillatory activity difference between the WKY and SHRSP strains. Allelic variations at this single gene are associated with a significant fraction of the difference in blood pressure between the strains. This association may be attributable to the linkage of this locus with unmeasured genetic factors involved in the determination of blood pressure. However, because the defect underlying oscillatory activity is likely to play a direct physiological role in the etiology of hypertension, this gene may be a primary cause of the observed blood pressure difference between the strains.

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