SUMMARY  The Wistar Kyoto strain of spontaneously hypertensive rat (SHR) has been characterized as behaviorally hyperactive as well as hypertensive. The relationship between these two inbred traits remains uncertain, and their coexistence in the SHR has complicated studies of central nervous system mechanisms underlying the hypertensive process. A breeding program was initiated to examine the possible genetic linkage of these two traits which, if separable, would allow us to develop substrains of SHR that are hypertensive without being hyperactive, or hyperactive without being hypertensive. We crossed SHR males with Wistar Kyoto, normotensive (WKY) female rats and produced F₁ hybrids which were then randomly inbred to produce an F₂ population. When tested at 12 weeks of age, F₂ rats exhibited the expected wide range of mean systolic blood pressures (BP), from 111 to 174 mm Hg, as determined using indirect tail plethysmography. The BP in the parental rats at the time of breeding (16 weeks) was 187 ± 4.5 mm Hg (SHR males, n = 7) and 111 ± 2.4 (WKY females, n = 7). Locomotor activity was determined in an automated activity cage in F₁ and F₂ rats at 12 weeks of age. These strains exhibited a wide range of phenotypic distribution of locomotor activity scores, and the mean scores were intermediary between those of SHR rats and WKY rats of the same age. Among individual rats of both the F₁ and F₂ hybrid strains, there was no correlation between the activity score and the level of the BP at 12 weeks of age. These findings indicated that the genes responsible for the hypertensive trait and those responsible for the hyperactivity trait were not tightly linked in the hybrid populations, suggesting that different genetic factors were involved in the transmission of each of these traits. Accordingly, it should be possible to separate the two traits by further selective, recombinant inbreeding procedures. (Hypertension 5: 211-217, 1983)

KEY WORDS  • hypertension  • hyperactivity  • breeding of rats  • Wistar Kyoto rats  • blood pressure  • locomotor activity
pared to WKY rats. However, this neurochemical imbalance could be relevant to either the hyperactivity or hypertension. These considerations led us to examine the genetic coupling of the hyperactivity trait and the hypertensive trait in hybrid populations derived from a cross of SHR and WKY rats. If common, or closely linked, genetic mechanisms produce both the hypertension and the hyperactivity in the SHR, then the two traits should remain closely correlated in the hybrid populations. We now report that BP level was not correlated with locomotor activity score among individuals of either the F1 or F2 hybrid generations. These findings suggest that the genetic determinants of the hypertension are not tightly linked to those coding for the hyperactivity in these rats.

Methods

Breeding Program

Rats used in these studies are descendants of the SHR and WKY strains of Wistar-Kyoto rats developed originally by Okamoto and Aoki. They were bred, housed, and maintained at the University of Vermont (UVM) Animal Care Facility in accordance with the NIH guidelines for use of these strains. The UVM colony was started in 1974 from breeding stock supplied by the National Institutes of Health (NIH), and breeders from the NIH have periodically been introduced into the UVM colony to maintain uniformity with the national source of these strains. Rats were housed under uniform conditions of temperature (27°C), humidity (50%), and lighting (12:12, light:dark cycle). Rats received food (Purina 5001 rat chow) and water ad libitum.

The SHR × WKY cross was initiated by mating seven randomly selected, male SHR with seven randomly selected, WKY female rats at 16 weeks of age. Since SHR dams tend occasionally to devour their newborn offspring and WKY dams do not, we chose to use only WKY females in the cross breeding. When pregnancy was confirmed the females were isolated in maternity cages throughout the gestation, birth, and lactation periods. At weaning, the litters were separated by sex, and the pups were housed in groups of no more than five per cage. At 12 weeks of age, the F1 hybrids were tested for mean systolic BP by tail plethysmography, and locomotor activity scoring using an automated activity chamber.

At 16 weeks of age, five non-sibling pairs of F1 rats were randomly bred to produce the F2 population by the same procedures. At 12 weeks of age, the F2 rats were tested for BP and activity scores as described above. At least 3 days elapsed between BP recording and locomotor activity testing in individual rats.

Blood Pressure Determination

As recommended in the NIH guidelines, a noninvasive, indirect method of tail plethysmography was used to determine the systolic BP. Rats were placed in a dark, quiet, warming box (37°C) for 15 minutes prior to BP determination. The rat was then placed in a restrainer warmed to 37°C and an inflatable tail cuff was used to obtain five to eight separate recordings of systolic BP in each session. The average of these readings was taken as a measure of mean systolic BP. Testing was carried out between 900 and 1100 hours.

Locomotor Activity Measurement

Experiments in our laboratory using the automated activity chamber indicate that 15-minute activity scores in SHR peak at 10–12 weeks of age postnatally (unpublished observations). Accordingly, behavioral testing using the automated activity chamber was carried out at 12 weeks in this study. Since this instrument had not yet been available at the time the parental rats were crossbred, we used the 3-minute open field test to confirm that the SHR breeders were markedly more active than the WKY breeders used to produce the hybrid F1 generation (as shown in table 1 below).

The automated activity chamber used in this study was built by the Instrumentation and Modeling Facility of the University of Vermont. The chamber consists of a lucite cage 30 × 30 cm and 25 cm in height. Four sets of infra-red lights and photocell detectors are spaced at intervals 6 cm apart along the length of the cage, and a digitalized display recorded continuously and noiselessly the number of interruptions of light beams made by the perambulating rat. Testing was carried out in a small, quiet test room illuminated by ceiling incandescent lighting. To begin testing, a single rat was placed in the activity cage, and the cumulative score of total light beam interruptions was recorded quietly by the observer at 1-minute intervals over a 15-minute test session. Following testing the cage was cleaned with detergent, rinsed and dried before another rat was subjected to testing. Testing was carried out between 1000 and 1500 hours.

The open field test was used for measuring locomotor activity in the parental SHR and WKY rats at 16 weeks, prior to the crossbreeding. Testing was carried out in an open field consisting of a square, plywood floor, 120 cm on a side, divided into 36 squares by finely painted white lines against flat black. The floor was bound on four sides by black plywood upright panels, 30 cm high, and no ceiling covered the field which was illuminated uniformly by overhead fluorescent lights. The ambient temperature was 23°C. To

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Squares</th>
<th>Rears</th>
<th>Center squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR males (n = 7)</td>
<td>59.3±9.1</td>
<td>22.7±3.3</td>
<td>4.9±3.1</td>
</tr>
<tr>
<td>WKY females (n = 7)</td>
<td>26.6±8.8</td>
<td>5.9±1.0</td>
<td>none</td>
</tr>
<tr>
<td>Unpaired t test*</td>
<td>p = 0.02</td>
<td>p = 0.0019</td>
<td>p = 0.006</td>
</tr>
</tbody>
</table>

Values are means ± se; scores are per 3-minute test in the open field.

*SHR vs WKY, Dixon and Massey22 unpaired t test for comparing groups with unequal variances.
begin testing, the rat was placed in one corner of the field and during a 3-minute test session the observer quietly recorded the number of crossings of squares by all 4 paws (squares), the number of rearings (rears), and the number of entries into the center squares, i.e., those not bound by the walls of the field (center squares).

Activity testing was carried out between 900 and 1100 hours, and rats were undisturbed at least 3 days prior to activity testing.

Statistics
In comparing BP or activity scores between rat strains, or between males and females of a given rat strain, we made the assumption that the variances of each population in a comparison were not necessarily equal. Accordingly, an unpaired t test was used for comparing two means where the variances are unequal, as described in Dixon and Massey.

Results
The SHR × WKY cross-produced a total of 79 F1 pups, or an average of 11 per litter. Of these, 42 randomly selected, F1 rats were retained for testing BP and activity at 12 weeks of age. Among the F1 hybrids, five nonsibling pairs were randomly selected to breed the F2 generation. A total of 41 F2 pups, or an average of eight pups per litter were obtained from the F1 in-breeding. The litters of F2 pups were culled and 27 randomly selected, F2 hybrids were retained for testing BP and activity at 12 weeks of age.

Mean systolic BPs in the parental and filial populations used in this study are shown in figure 1. At 16 weeks of age the parental SHR were all hypertensive, and BP averaged 187 mm Hg. The parental WKY females were all normotensive and BP averaged 111 mm Hg. In 42 F1 rats BP was measured at 12 weeks of age and the overall mean, 136 mm Hg, was intermediate between the means of the parental rats. Male F1 rats averaged slightly higher BP than female F1 rats. This small difference was significant (p = 0.04), and similar findings have been observed by others using these breeding procedures. At 12 weeks of age, 27 F2 rats exhibited BP readings that ranged from 111 to 174 mm Hg, and the overall mean was 141 mm Hg. The F2 males averaged higher BP than the F2 females, and this difference was also statistically significant (p = 0.013). F2 rats showed the expected wide phenotypic distributions of BP, and the standard deviation of the overall mean was 21.3 mm Hg, compared with a standard deviation of 11.1 mm Hg among the F1 population.
Locomotor activity was determined in the SHR and WKY parental rats at 16 weeks of age, using the 3-minute open field test. The results, shown in Table 1, confirmed previous findings in these strains\textsuperscript{1-14} that SHR, males or females, are markedly more active than WKY rats. SHR crossed significantly more squares and made significantly more rearings in the 3-minute test than did the WKY rats. All of the SHR entered center squares of the field, whereas none of the WKY rats did.

In figure 2 the activity scores over 15 min are shown for the F\textsubscript{2} male rats at 12 weeks, as compared with nonrelated SHR and WKY rats of the same age and sex tested during the same time period as the F\textsubscript{2} rats. The SHR crossed 595 light beams during the 15-minute test period, WKY rats crossed 102 light beams/15 min ($p = 0.001$), and F\textsubscript{2} hybrids were intermediary with over 300 light beam interruptions in 15 min ($F_2$ vs WKY: $p = 3 \times 10^{-5}$; $F_2$ vs SHR: $p = 0.012$).

The development of hypertension in SHR is a multifactorial process involving a complex interaction of environmental and genetic factors, and the interplay of a number of organ systems.\textsuperscript{23-26} A similar complex interaction has also been suggested in the development of human genetic hypertensive disease.\textsuperscript{27} Of particular interest here is a comparison of the central nervous system functions, including the differences in central catecholamine systems and sympathetic nerve activity,\textsuperscript{28,29} between SHR and WKY rats. It has been assumed that these differences are associated with the inbred hypertensive process in the SHR, since the central catecholaminergic neurons are known to influence cardiovascular function. However, we and others\textsuperscript{1-14} have pointed out that the SHR are also more active in locomotor and exploratory behaviors when compared with WKY rats, and other behavioral differences have also been reported in these and other hypertensive rat strains.\textsuperscript{30,31} The presence of behavioral as well as cardiovascular differences between SHR and WKY rats is a complicating factor in those studies concerned with the role of the central nervous system in the autonomic hyperreactivity that is seen in the SHR.\textsuperscript{32,33} Furthermore, brain dopaminergic neuronal systems that are implicated in the regulation of the BP\textsuperscript{19,20} are also implicated in the regulation of locomotor activity.\textsuperscript{34}

Thus, neurotoxic or surgical destruction of mesolimbic dopaminergic neuronal systems results in locomotor hyperactivity,\textsuperscript{17,34} and electrical stimulation of the mesocortical region of the SHR brain results in a dramatic reduction of the BP and decreased firing rate of the sympathetic nerves.\textsuperscript{35}

The possibility that common neurochemical mechanism(s) may subserve both the behavioral and cardiovascular abnormalities in the SHR led us to carry out the present study designed to test whether the behavioral abnormality was tightly linked to the hypertensive trait in the highly inbred SHR.
Figure 3. Total activity counts are the cumulative scores in 15 minutes of light beam interruptions in the automated activity chamber. Horizontal bars indicate mean scores; mean ± se is noted at the bottom.

Unpaired t test: F1 males vs F1 females, p = 0.76; F2 males vs F2 females, p = 0.059; F1 males vs F2 males, p = 0.49; F1 females vs F2 females, p = 0.08. SHR males vs WKY males, p = 1 × 10^{-7}; SHR males vs F1 males, p = 1 × 10^{-4}; SHR males vs F2 males, p = 5 × 10^{-4}; WKY males vs F1 males, p = 3 × 10^{-4}; WKY males vs F2 males, p = 9.5 × 10^{-7}.

Figure 4. Mean BP and cumulative activity counts in 15 minutes in the automated activity chamber were determined in individual male (circles) and female (triangles) F1 rats at 12 weeks of age. At least 3 days elapsed between BP determination and activity measurement. Correlation coefficient (r) for all rats, males and females, was −0.12.

Figure 5. Mean BP and cumulative activity counts in 15 minutes in the automated activity chamber were determined in individual male (circles) and female (triangles) F2 rats at 12 weeks of age. At least 3 days elapsed between BP determination and activity measurement. Correlation coefficient (r) for all rats, males and females, was −0.29.
There is considerable knowledge concerning the heritability of the hypertension in Wistar Kyoto rat strains. Recombinant inbred strains derived from crosses and backcrosses of SHR and WKY rats demonstrated that genetic determinants accounted for greater than 90% of the heritability of the hypertensive trait; that the mode of the inheritance was additive; and that a relatively small number of major genes and probably a number of minor genes may be involved in the inheritance of the hypertension. The genetic linkage of the hypertension with a number of other traits has also been examined using similar breeding procedures. Judy et al. reported that the BP level was significantly correlated with the level of spontaneous sympathetic nerve activity among the recombinant inbred (F$_1$) strains. Using a stroke-prone substrain of SHR, Yamori et al. were able to correlate the severity of the hypertension inversely with the rate of cerebral blood flow to the frontal brain region in the recombinant strains. In another study, Yamori et al. crossed SHR with Wistar Mishima normotensive rats and noted significant correlations of endocrine changes with the inheritance of the hypertensive trait.

The heritability of the hyperactivity trait in SHR has not been previously examined. Consequently it is not yet known whether this trait is genetically linked to the hypertensive gene system or whether it has been fortuitously fixed in this highly inbred strain to a genetic system unrelated to the hypertensive gene system. In the present study we crossed SHR with WKY rats in order to separate the genes coding for hypertension from those coding for hyperactivity. If the genes for these traits were identical then the two traits would tend to be found in the same combinations as upon entering the cross, whereas if the genes were not identical, the two traits should be randomly assorted in the F$_2$ population. Our findings appeared to indicate the latter case, in that BP level was not correlated with locomotor activity score at 12 weeks of age in either F$_1$ or F$_2$ hybrid populations. Furthermore, female F$_2$ rats tended to be more active in locomotor behavior than male F$_2$ rats, whereas male F$_2$ rats had significantly higher BP levels than female F$_2$ rats, suggesting a further dissociation of the 2 traits by gene assortment. The data indicate that the genetic determinants of the hypertension are not identical with those coding for the hyperactivity in Wistar Kyoto rats.

More definitive conclusions concerning the heritability of the hyperactivity trait in Wistar Kyoto rat strains cannot yet be drawn based on the limited numbers of each population tested in the present pilot study. It has been noted however that the locomotor activity scores in F$_1$ and F$_2$ rats were intermediary between those of the SHR and WKY parental strains measured at the same age. This finding suggests that the mode of inheritance of the hyperactivity trait, like that of the hypertensive trait, is probably also additive, and consistent with a multigenic involvement. From the findings shown in figure 5, we can identify individual F$_2$ rats with normotensive BP (lower than 130 mm Hg) and high activity score (greater than 300 counts/15 min), as well as individuals with hypertension (BP of 150 mm Hg or more) and low activity score (less than 200 counts/15 min). By means of successive, selected, brother/sister matings through several more filial populations, it should be possible to develop substrains of SHR with the appropriate traits, i.e. hypertension without hyperactivity or hyperactivity without hypertension, as more valid subjects for research on the characteristics and treatment of these separate disorders.

Acknowledgments

We are grateful to Dr. Judith Van Houten for genetic counseling and for criticism in preparation of the manuscript, and to Janice Gellis for expert assistance.

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Hypertension. 1983;5:211-217
doi: 10.1161/01.HYP.5.2.211

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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