Abstract We investigated the involvement of loci on the sex chromosomes in the hypertension of the spontaneously hypertensive rat (SHR) by studying male F1 and F2 generation rats derived from reciprocal crosses of SHR with Wistar-Kyoto (WKY) rats (cross 1: WKY female x SHR male; cross 2: SHR female x WKY male). At 16 weeks of age there was no significant difference in the blood pressures of F1 animals derived from the two crosses. Similarly, in the F2 generation there was no significant difference in either indirect blood pressures measured at 12, 16, or 20 weeks of age or in direct systolic and diastolic blood pressures measured at 25 weeks of age between animals derived from the two crosses maintained on a normal salt diet. In a second study, cohorts of F2 rats from the two crosses were given 1% salt in their drinking water for 10 weeks from 16 weeks of age with indirect blood pressure measurements at 16 (presalt), 18, and 20 weeks and direct blood pressure measurements at 26 weeks. Although overall these animals had significantly higher blood pressures at both 20 and 26 weeks than animals of the first study, again there was no difference in blood pressures of animals derived from the two crosses, apart from a marginally significantly higher blood pressure at 18 weeks in animals from cross 1 (with SHR grandfather). The findings indicate that the sex chromosomes of the SHR and WKY rat used in these crosses do not contain loci where alleles differentially influence blood pressure under the genetic milieu provided by the cross. The results are particularly at variance with a recent report suggesting a strong effect on blood pressure of the Y chromosome of the SHR in a similar cross. The reasons for the discrepant findings remain to be elucidated but may reflect genetic heterogeneity in SHR and WKY rats from different sources. (Hypertension. 1994;23:161-166.)

Key Words • hypertension, genetic • rats, inbred SHR • sex chromosomes

Males tend to have higher blood pressures than females. This is seen not only in humans1-3 but also in animals.6-8 Although the precise mechanism underlying this phenomenon remains unclear, it has been attributed to an effect of sex hormones, particularly testosterone, in much the same way as such hormones affect other quantitative traits such as height and weight — so-called sex-influenced traits. The rise in blood pressure of females after menopause1-3 and the blood pressure effects of castration or treatment with sex hormones8-13 have been used to support this concept.

In several inbred strains of genetically hypertensive rats, eg, the spontaneously hypertensive rat (SHR), and in their normotensive controls, eg, the Wistar-Kyoto (WKY) rat, male animals also generally have higher blood pressures than females.14 Although it is most likely that the same general mechanism explains such sex-related differences in these rat strains, recent studies have suggested that in some strains the sex chromosomes may also contain loci that have a direct effect on blood pressure. Specifically, a blood pressure-increasing effect has been observed for the Y chromosome of the SHR.15,16 and the stroke-prone SHR (SHRSP).17

To investigate the genetic basis of hypertension in the SHR, we have recently characterized large cohorts of F2 rats derived from reciprocal crosses of SHR with WKY rats for blood pressure and other phenotypes. Because of the important implications, not only in terms of understanding the genetic basis of hypertension in the SHR but also in terms of potentially extending the work to explain some of the sexual dimorphism seen in human hypertension,1-5 we sought to confirm the association reported with the Y chromosome in our crosses. Our studies also provided information on the involvement of the X chromosome (see "Discussion").

Methods

Animals and Crosses Ten SHR and 10 WKY animals (5 males and 5 females of each) were obtained from the breeding stocks of Charles River UK Ltd. The origin of their stocks was from Charles River USA Ltd, which had obtained their initial breeding stocks at generation F0 for SHR and generation F1 for WKY rats from the National Institutes of Health (personal communication, Mr D. Gates, Charles River UK Ltd). Full information on the lineage and blood pressures of the parents of the animals were provided by the suppliers. In addition, it was checked that the DNA fingerprints18 of all animals of each strain were identical (data not shown). Three SHR males were paired with 3 WKY females (cross 1) and 3 WKY males with 3 SHR females (cross 2) to provide the reciprocal crosses (Fig 1). The 4 remaining SHR were paired to provide an SHR colony and the 4 WKY animals paired to provide a WKY colony for contemporaneous analysis. From the F1 animals of each of the crosses, 8 males and females were randomly chosen and paired to generate F2 animals. Only male F2 rats were studied (Fig 1). All animals were housed under controlled conditions of temperature (21±1°C), humidity (60±10%), and light (12-hour light/dark cycle: 8 AM to 8 PM). Litters were weaned and sexed after 3 weeks and maintained by sibling group and sex thereafter for the duration of the studies.
**Fig 1.** Drawing shows breeding paradigm used to investigate the role of the sex chromosomes in the hypertension of the spontaneously hypertensive rat (SHR). Sex chromosome genotypes of the various rats analyzed are given. See "Methods" for more details. WKY Indicates Wistar-Kyoto rat; F1, first filial generation; F2, second filial generation; Xw, WKY X chromosome; Xs, SHR X chromosome; Yw, WKY Y chromosome; and Ys, SHR Y chromosome.

**Study 1**

In study 1 F2 animals (117 from cross 1, 116 from cross 2) were fed standard rat chow (Rat & Mouse No. 3 Breeding Diet, Special Diet Services Ltd) containing 0.25% sodium and 0.66% potassium and given free access to tap water throughout the course of the study.

**Study 2**

In study 2 F2 animals (69 from cross 1, 98 from cross 2) were given the same diet as above but had 1% NaCl added to the drinking water for 10 weeks from 16 weeks of age until the termination of the study at 26 weeks of age.

**Blood Pressure Measurements**

Indirect blood pressure was measured in conscious animals by tail sphygmomanometry (Narco Biosystems physiograph and transducer). Animals were prewarmed to 34°C for 20 minutes before measurements, which were always carried out between 9 AM and 1 PM. Measurements were made at 12, 16, and 20 weeks of age for SHR and WKY rats (normal salt) and F2 animals of study 1; at 16 weeks only for F1 animals; and at 16 (prestat), 18, and 20 weeks for F2 animals of study 2. At each age readings were taken on 2 separate days with three measurements on each occasion. The average of all readings was taken as the value for that age.

In addition, 16 male SHR and 16 male WKY rats, 193 F2 rats of study 1 (98 from cross 1, 95 from cross 2), and 155 animals of study 2 (63 from cross 1, 92 from cross 2) underwent direct blood pressure measurements at 25/26 weeks of age. This was carried out in conscious unrestrained animals as described by Su et al. Briefly, with animals under halothane anesthesia (2% in oxygen), a polyethylene catheter (PE-10, 0.28 mm internal diameter) was inserted via the femoral artery into the lower abdominal aorta, tunneled subcutaneously, and exteriorized at the neck. Animals were allowed to recover for at least 24 hours with food and water ad libitum. The arterial catheter was then connected to a blood pressure transducer (Statham P23 ID, Gould Inc) via a rotating swivel that allowed the animals to move freely. After verification of calibration, systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate were recorded beat by beat for 2 consecutive hours between 10 AM and 5 PM. Data were processed off-line following the method of Gustin et al., and mean values were calculated from the full period of recording. All procedures were carried out in accordance with our institutional guidelines.

**Determination of the Origin of the Y Chromosomes in F2 Rats**

Differentiating F2 rats that had inherited the Y chromosome from the SHR (Ys) from rats that had inherited the Y chromosome from the WKY rats (Yw) was straightforward (Fig 1). Male F2 animals from cross 1 all had to have Ys, and those from cross 2 all had to have Yw.

**Statistical Analysis**

Data were analyzed with MINITAB Release 7 (Minitab Inc). Analysis of variance and Student's t test were used to compare differences between groups.

**Results**

**Parental Strains**

Fig 2 shows the indirect blood pressures at 12, 16, and 20 weeks for the two parental strains. At each age the blood pressures of the SHR were significantly higher than those of WKY animals ($P<.001$ for each comparison); within each strain, the blood pressures of male animals were significantly higher than those of female animals ($P<.001$ for each comparison). There were significant sex and strain differences in age-related changes in blood pressures. WKY females showed no significant change in blood pressure from 12 to 20 weeks ($F=0.96, P=.388$), whereas the blood pressure of SHR females rose significantly from 12 to 16 weeks and then plateaued ($F=34.4, P<.0001$). Both WKY ($F=6.96, P=.001$) and particularly SHR males ($F=115.75, P<.0001$) showed a progressive rise in blood pressure to 20 weeks (Fig 2).

**Cosegregation Analysis of Blood Pressure With Strain-Specific Y Chromosomes**

**F1 Animals**

Table 1 shows the blood pressures of the F1 animals from the reciprocal crosses at 16 weeks of age together with their sex chromosome genotypes. The 16-week blood pressures of the parental strains are also given for comparison. There was no difference ($P>.05$) in the average blood pressures of male animals of cross 1 and
TABLE 1. Sixteen-Week Indirect Blood Pressures of F1 Rats Derived from Reciprocal Crosses of Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Genotype</th>
<th>BP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 hybrids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross 1 male</td>
<td>26</td>
<td>XwYs</td>
<td>156.3±2.8</td>
</tr>
<tr>
<td>Cross 1 female</td>
<td>27</td>
<td>XsXw</td>
<td>132.5±1.9</td>
</tr>
<tr>
<td>Cross 2 male</td>
<td>24</td>
<td>XeYw</td>
<td>158.6±2.5</td>
</tr>
<tr>
<td>Cross 2 female</td>
<td>33</td>
<td>XwXs</td>
<td>130.9±1.8</td>
</tr>
<tr>
<td>Parents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR male</td>
<td>82</td>
<td>XsXw</td>
<td>186.4±1.5</td>
</tr>
<tr>
<td>SHR female</td>
<td>48</td>
<td>XeXs</td>
<td>162.0±2.1</td>
</tr>
<tr>
<td>WKY male</td>
<td>44</td>
<td>XwYw</td>
<td>128.4±1.9</td>
</tr>
<tr>
<td>WKY female</td>
<td>31</td>
<td>XwXw</td>
<td>110.5±2.9</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto rat; cross 1, WKY female x SHR male; and cross 2, SHR female x WKY male. Sex chromosome genotypes: Xw indicates WKY X chromosome; Xs, SHR X chromosome; Yw, WKY Y chromosome; and Ys, SHR Y chromosome. Blood pressure of parental rats is also given. Values are mean±SEM.

cross 2. The blood pressures of the female animals from the two crosses were also very similar (P>.05).

F2 Animals: Study 1

The indirect blood pressure of F2 rats on the normal salt diet rose progressively from 12 to 20 weeks of age (Table 2; mean change from 12 to 20 weeks, 15 mm Hg). At all ages the F2 animals showed a broad range of blood pressures extending from those observed in age-matched WKY rats to those seen in SHR. For instance, at 25 weeks the SBP of F2 rats ranged from 151 to 224 mm Hg compared with mean values of 165.7±2.5 and 226.0±3.0 mm Hg for WKY rats and SHR, respectively. However, despite this, as can be seen from Table 2, there was no significant difference (P>.05) in the blood pressures of animals derived from the two crosses at any of the ages studied, indicating a lack of effect of either Ys or Yw.

F2 Animals: Study 2

Table 3 shows the results for the F2 animals treated with 1% salt in their drinking water for 10 weeks from 16 to 26 weeks of age. The presalt mean blood pressure (16 weeks) was very similar to that observed at the same age in F2 animals of study 1 (Table 2). Addition of salt significantly increased blood pressure compared with the normal diet (Tables 2 and 3). At 20 weeks mean blood pressure was 13.7 mm Hg higher, and at 25/26 weeks the mean values for SBP and DBP were 15.6 and 9.9 mm Hg higher, respectively (Tables 2 and 3, P<.0001 for all comparisons). However, there was again no difference in blood pressures of animals of the two crosses at any of the ages (Table 3) apart from at 18 weeks of age (ie, after 2 weeks of salt treatment) when the blood pressure of cross 1 males was significantly higher than that of cross 2 males (Table 3, P=.021).

Discussion

Cosegregation analysis using polymorphic DNA markers provides a powerful approach to identifying genetic loci that cause hypertension in rodent models of genetic hypertension. With the use of this approach several loci that influence blood pressure have now been identified in F1 recombinant inbred rats derived from crosses of hypertensive rats with normotensive controls.21-27 The ability to follow the inheritance of such DNA markers is critical to the evaluation of loci located on autosomal chromosomes and the X chromosome because of the potential of recombination at meiosis. However, because it is unpaired, such a restriction does not apply to the Y chromosome, and a classic genetic approach involving reciprocal crosses can be used to investigate the role of loci on this sex chromosome. In this study, using such an approach, we have investigated the role of the SHR Y chromosome in the strain's hypertension, including the effect of the chromosome on the response of blood pressure to a high salt diet. Large cohorts of F2 rats derived from an SHR x WKY cross had blood pressures measured longitudinally over several weeks, including final direct blood pressures in conscious unrestrained rats. Our findings indicate that, apart perhaps in relation to the acute response to salt (see below), the Y chromosomes of the SHR (Ys) and WKY rat (Yw) used in the cross do not contain loci where alleles differentially influence blood pressure under the genetic milieu provided by the cross.

A priori the findings are not surprising. The mammalian Y chromosome contains few identified loci other than the gene or genes required for male sex determination.28 Nevertheless, Ely and Turner15 using a similar approach had previously reported a highly significant effect of Ys on blood pressure. In their study, which also involved crosses of SHR and WKY rats, male F1 animals from the two crosses had significantly different indirect blood pressures from 12 to 20 weeks of age, an effect

TABLE 2. Longitudinal Blood Pressures of F2 Male Rats Derived from Reciprocal Crosses of Spontaneously Hypertensive and Wistar-Kyoto Rats Maintained on Normal Salt Diet

<table>
<thead>
<tr>
<th>Group</th>
<th>12 Weeks</th>
<th>16 Weeks</th>
<th>20 Weeks</th>
<th>25 Weeks SBP</th>
<th>25 Weeks DBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross 1 males (XsXs or XwYs)</td>
<td>(n=117)</td>
<td>150.2±1.2</td>
<td>157.7±1.3</td>
<td>183.1±1.5</td>
<td>119.0±1.1</td>
</tr>
<tr>
<td>Cross 2 males (XsYw or XwXw)</td>
<td>(n=116)</td>
<td>153.8±1.3</td>
<td>186.5±1.4</td>
<td>121.0±1.1</td>
<td></td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; cross 1, Wistar-Kyoto (WKY) female x spontaneously hypertensive rat (SHR) male; cross 2, SHR female x WKY male. Sex chromosome genotypes: Xw indicates WKY X chromosome; Xs, SHR X chromosome; Yw, WKY Y chromosome; and Ys, SHR Y chromosome. Values are mean±SEM.
that was carried through into the male animals of the F2 generation. Although X chromosome markers were not studied, the authors discounted the possibility that the effect they had found was due to the X chromosome from their F2 data and also provided cogent arguments against other possible explanations such as parental imprinting. They concluded that the effect, which amounted to almost half of the blood pressure difference between their SHR and WKY male rats, could only be accounted for by postulating a Y chromosome effect.

Discrepant results are not new to this sort of analysis. For instance, the first three studies21-22-29 that examined the role of the rat renin locus found markedly different effects. However, several methodological differences and, more importantly, the fact that the crosses analyzed in the three studies were very different (ie, the genetic background in which the effect of the locus was examined varied) more than adequately explain the dissimilar results obtained in these studies. However, this is the first time that a major discrepant result has been reported in the same cross. Although some differences are also apparent between the methods used by us and in the study by Ely and Turner15 (eg, they prewarmed their rats to 39°C before the indirect blood pressure measurements, whereas we prewarmed the animals to a lower temperature; their diet contained 25% more sodium [0.31%] than ours; and they did not measure direct blood pressures), it seems unlikely that these differences alone could account for the strikingly different results.

What other explanations are possible? A negative result in a study always raises the possibility that the study was not sufficiently powerful to detect a difference between the groups. However, a power calculation30 shows that our study 1 had a power of 0.90 to detect a difference of 5.0 mm Hg in DBP and 6.7 mm Hg in SBP at 25 weeks at a significance level of <.05 (the values at the earlier ages are even lower because of the larger number of animals studied at these ages). This is well within the 34 mm Hg Y chromosome component estimated by Turner et al.16 The alternative explanation along these lines is that Turner et al picked up a spurious association (ie, made a type I error) because of the relatively small numbers of F1 and F2 animals (between 8 and 10) that they analyzed from each cross.15 Given the broad range of blood pressures expected, particularly in the F2 population, this is possible. However, their consistent findings,15,16 including the recent development of congenic lines (see below), argue against this possibility.

Recent molecular studies have shown that SHR and particularly WKY rats from different suppliers are not genetically homogeneous.18,31-32 In the case of the WKY rat this probably reflects the fact that the strain was not inbred before dissemination.18,31 Therefore, although generally Ely and Turner15 and we have studied the same cross (SHR×WKY), the fact that the sources of animals for the two studies were different may be the crucial distinction in explaining the different findings. Two types of genetic heterogeneity between our animals and theirs that could account for the discrepant findings need to be considered: (1) heterogeneity at the putative Y chromosome locus itself and (2) heterogeneity at other genetic loci that influence the phenotypic expression of the Y chromosome locus. Apart from the studies on the renin locus cited earlier, recent findings by Deng and Rapp26 of the variable effects of the Dahl salt-sensitive rat angiotensin converting enzyme gene locus on blood pressure in different crosses involving this strain highlight the potential importance of the genetic background in determining the blood pressure-modulating effect of a locus. While genetic heterogeneity is an attractive and plausible explanation for the discrepant results between our study and that of Ely and Turner, we do not at present have direct evidence to support it, and the fact that in both studies the difference in blood pressures between the two parental strains is similar (at several ages) somewhat argues against it. Detailed molecular characterization of SHR and WKY rats from various suppliers using not only DNA fingerprinting18,31,32 but also the polymerase chain reaction–analyzed microsatellites15 would be very useful for the interpretation of both genetic and physiological experiments.

In reciprocal crosses of the SHRSP with WKY rats, Hilbert et al17 also found that male F1 animals with the SHRSP Y chromosome had higher systolic and diastolic femoral artery blood pressures at 16 weeks of age. Blood pressures of F1 animals were not measured, and interestingly, the Y chromosome effect in the F1 rats disappeared by giving the animals 1% NaCl to drink for 12 days. No explanation was given for this ameliorating effect of salt; again, it is difficult in simple terms to reconcile these findings with ours, particularly as the only time we observed an effect associated with the Y chromosome was after 2 weeks of salt loading, when F1 animals with Ys had a blood pressure approximately 6
mm Hg higher than animals with Yw (Table 3). Given the lack of effect at other ages in study 2, the marginal significance of the result, and the absence of an obvious explanation for the effect, the finding at 18 weeks needs to be interpreted with some caution.

One way of further investigating the role of Ys in hypertension would be to develop a congenic line in which Ys is bred into a genetic background made up otherwise of WKY genetic material. Because the Y chromosome does not recombine, this would in principle be a simple (although quite lengthy) procedure. After an initial SHR male × WKY female cross, matings of male animals from the F1 and subsequent generations with WKY females would lead to an increasing amount of WKY genetic material in the progeny while maintaining the SHR Y chromosome in the males. After 10 generations, more than 99.9% of the non-Y chromosome genetic material would be of WKY origin. A reciprocal initial cross with subsequent repeated matings to SHR females would yield a line with Yw in an SHR background. Turner et al.16 have recently reported the development of such congenic lines from SHR strains. Their initial results16 suggest that males from the two congenic lines indeed have different blood pressures. Further studies on these lines are awaited with interest.

A specific effect on blood pressure has also been reported for the X chromosome. In the study by Hilbert et al.,17 female F1 animals from the SHRSP male × WKY female cross (equivalent to our cross 1; possible sex chromosome genotypes XsXw or XwXw) had significantly higher basal blood pressures than females from the alternative cross of WKY male × SHRSP female (possible genotypes XsXs or XsXw). With the use of an X chromosome minisatellite marker, a significant blood pressure-raising effect of an Xw locus was confirmed in female F1 rats but was not seen in male F1 rats. NaCl loading again had a curious effect. The difference in females disappeared, while the Xw marker now showed significant linkage to SBP in males from one cross but not the other. In our study, only male F1 animals were studied, allowing no simple assessment of any contribution of Xs or Xw loci in this generation (Fig 1). Even if female F1 animals had been studied, analysis of any contribution of Xs or Xw loci, unlike that of loci on the Y chromosomes, would have been complicated by the potential of recombination during the F1 matings. However, our findings in the F1 generation are relevant to the question of a blood pressure-raising locus on Xw. If this were the case, one would have expected the male F1 animals of cross 1 to have a higher blood pressure than those of cross 2 (indeed much higher if Ys also possessed a blood pressure-elevating locus). The lack of any difference in the blood pressures of these two groups in combination with the data pertaining to the Y chromosome obtained in the F1 generation indicate that an X chromosome locus that influences blood pressure is again not operational in our cross.

Although a differential effect of the SHR Y chromosome could not be demonstrated in our cross, the data presented in Fig 2 and Table 1 serve to emphasize once again the well-known effect of sex on blood pressure.1,7 Although attributed to the effect of sex hormones,13 and thus ultimately linked to the Y chromosome, the underlying genetic determinants of this important effect and the precise mechanisms involved remain poorly understood.

In conclusion, we could not demonstrate any effects of sex chromosome loci on blood pressure in our SHR × WKY cross. The findings are at variance with those previously reported. The reasons for the discrepant findings are unclear but may reflect genetic heterogeneity in WKY rats and/or SHR from different sources.

Acknowledgments

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