The Hypertensive Y Chromosome Elevates Blood Pressure in F₁₁ Normotensive Rats

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Our laboratory has shown that the Y chromosome has a significant effect on blood pressure in the spontaneously hypertensive rat (SHR) model of hypertension and that the testes and androgen receptor contribute to the blood pressure rise. As an extension of our research, we have developed two new rat strains, SHR/a and SHR/y (F₁₁) to study the Y chromosome. The objectives of the following research were: 1) to study the blood pressure of rats with an SHR Y chromosome in a normotensive genetic background (SHR/y) or a normotensive Y chromosome in an SHR genetic background (SHR/a), 2) to determine the effect of male sex phenotype on the blood pressure of these rats, 3) to determine if testosterone replacement in castrated rats would restore blood pressure, and 4) to determine whether the Y chromosome from the SHR/y strain when crossed with a normotensive female can induce hypertension in androgen receptor-deficient male offspring. Blood pressure of male SHR/y rats was significantly higher than that of normotensive Wistar-Kyoto males (p<0.01), and SHR/a males had significantly lower blood pressure compared with that of the parent SHR strain (p=0.05). Testosterone replacement in castrated rats of both strains (SHR/a and SHR/y) restored blood pressure to control levels. Normotensive female King-Holtzman rats heterozygous for the testicular feminization gene were crossed with F₁₁ SHR/a and SHR/y males. The F₂ males (King-Holtzman female×SHR/a male) with normal androgen receptor and hypertensive autosomes had a final blood pressure of 155 mm Hg compared with 175 mm Hg (p<0.01) for their counterparts—F₁ males (King-Holtzman female×SHR/y male) with normal androgen receptor and a Y chromosome from hypertensive fathers. Testicular feminized rats that lacked the androgen receptor and females from both crosses had a similar blood pressure of 125–130 mm Hg. In conclusion, the hypertensive Y chromosome increased blood pressure after backcrossing (F₁₁) into a normotensive autosomal background and increased blood pressure by 20 mm Hg more than the hypertensive autosomes in a normotensive background. Also, the Y chromosome and autosomal effects both appear to require testosterone and the androgen receptor for maximal effect.

Key Words • Y chromosome • genetics • receptors, androgen • testicular feminization • rat

Sex differences in blood pressure (BP) have been reported in most developed societies and in many animal studies, with males having higher pressures than females.1–6 Recently, our laboratory has shown that the Y chromosome has a significant effect on BP in the spontaneously hypertensive rat (SHR) model of hypertension7,8 and in a new hybrid rat model of hypertension that also implicates an androgen receptor (AR) component.9 To study the Y chromosome effects, we have developed two new rat substrains: SHR/a and SHR/y. F₁₁ males of SHR/y have 99.9% of their autosomes and X chromosome from a Wistar-Kyoto (WKY) rat and a Y chromosome from an SHR father; F₁₁ males of SHR/a have 99.9% of their autosomes and X chromosome from SHR and a Y chromosome from a WKY father. As an extension of this research, we examined whether the Y chromosome effect continued after 11–12 generations and whether it was dependent on testosterone and ARs.

Methods

The parental WKY and SHR strains were obtained from Harlan Sprague Dawley, Inc., Indianapolis, Ind., which obtained the initial breeding stock from the National Institutes of Health (NIH). According to the most current Harlan Sprague Dawley genetic monitoring report, this strain is comparable with most genetically authentic hypertensive strains in the United States that were derived from the NIH colonies (personal communication, Harlan Sprague Dawley, 1988). The parental strain of King-Holtzman testicular feminized (Tfm) rats was obtained as a generous gift from Dr. Richard Krieg Jr. at the Medical College of Virginia. Their colony, in turn, was derived from a colony at the University of Oklahoma and is often referred to as the Stanley-Grumbach strain, named for its developers.

The animals deficient in AR are referred to as Tfm males, and they show the developmental effects of androgen deficiency. The Tfm male is a genotypic XY male, but the phenotype of the adult is female; internally, there are undescended testes that secrete testosterone. The Tfm trait has been studied in rats, mice, and humans and is an X-linked trait.10–14 The male offspring of a cross between a heterozygous Tfm female and a normal male are half normal males and half Tfm males. Female offspring are all phenotypically normal, but half are carriers (heterozygous) of the Tfm allele.

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Experiment 1

The objective of experiment 1 was to study BP of rats with the Y chromosome from a hypertensive father in a normotensive autosomal background (SHR/Y substrain).

Two groups of rats were used to study BP: the SHR/Y males ($n=8$) and a group of WKY normotensive males ($n=8$) that are genetically close to the SHR/Y (except the Y chromosome is from a normotensive father in the WKY rats).

A typical breeding box (40x50x20 cm) consisted of three females and one male housed in aspen shavings (changed once per week, American Excelsior, Cleveland, Ohio) and provided with water and Purina lab chow ad libitum. Ten such breeding cages were established ($n=30$ females, 10 males). Room temperature (26–28°C) and humidity (40–50%) were maintained at slightly higher than usual levels to minimize respiratory infections. The 12-hour light/dark cycle (6 AM to 6 PM, light; 6 PM to 6 AM, dark) was kept constant. Litters were weaned and grouped by sex at 3 weeks of age and maintained by sibling group and sex for the duration of the experiments. Blood pressure was measured weekly between 5 and 15 weeks of age by tail sphygmomanometry (International Biomedical, Inc., Austin, Tex.).

Each cage of rats was placed one at a time in a preheated warming chamber (39–40°C for 30 minutes); each rat was then put into a plastic restraint and five pressures were taken, which took 2–3 minutes. This technique was performed on conscious animals and, because of the overall chamber warming, the animals were calm and vasodilated and did not struggle during the procedure.

Experiment 2

The objective of experiment 2 was to study BP of rats with autosomes from a hypertensive mother and a normotensive Y chromosome (SHR/a substrain). To study the effect of the hypertensive autosomes, we developed a new substrain by backcrossing the male offspring of an SHR female and WKY male cross for 11 generations to an SHR female. Consequently, the strain designated SHR/a has a Y chromosome from a normotensive WKY rat and 99.9% of its autosomes from an SHR. Blood pressure was measured in two groups of rats: the SHR/a males ($n=8$) and a group of SHR males ($n=8$) that are genetically close to the SHR/a (except that the Y chromosome is from a hypertensive father in the SHR strain).

Experiment 3

The objective of this experiment was to determine if testosterone replacement after castration would restore BP to precastration levels in F1 SHR/Y and SHR/a rats. Three groups ($n=6$ per group, 7–8 weeks of age) of rats for each strain were used: sham controls, castrated, and castrated with testosterone implants. The sham controls had the testes exposed but not removed and the incision sutured. Also, sham implants (empty Silastic tubes) were placed subcutaneously in the dorsal neck region to control for the implant procedure. All groups were anesthetized (Brevital, 50 mg/kg i.p.; Eli Lilly and Co., Indianapolis, Ind.). For the castrated groups, the abdomen was shaved and scrubbed, and the testes were ligated and removed using sterile techniques. The rats were observed in a recovery cage until their mobility was normal. The testosterone implant group was castrated as above, and testosterone implants were made from Silastic tubing (length, 19 mm; i.d., 0.062 mm; o.d., 0.125 mm). After packing with testosterone propionate (10 mg) (Steroloids Inc.), the ends were sealed with Silastic medical-grade silicone adhesive (Type A, Dow Corning). The implants were cured overnight and soaked in 70% ethanol for 2 hours before being implanted. The implants were placed in the neck region subcutaneously and were replaced with new implants every 3 weeks under ether anesthesia. All groups were maintained in the same type of cages and environmental conditions as in experiment 1. BP was measured weekly from 10 to 16 weeks as in experiment 1.

Experiment 4

The objective of this experiment was to determine the interaction of the AR with the two types of Y chromosomes on BP. First, to study the effect of the Y chromosome from a hypertensive father, we crossed F1 SHR/Y males with King-Holtzman females that carry the Tfm trait, producing a deficient AR in 50% of the male offspring. The F1 offspring from this cross had BP measured biweekly from 7 to 29 weeks of age: females ($n=11$), normal AR males ($n=11$), and AR-deficient males (Tfm, $n=11$). The Tfm rats were determined phenotypically at 5 weeks of age by physical examination. They appear phenotypically like a female in the urogenital region, but retracted testes can be palpated. Second, to study the effect of the normotensive Y chromosome from a WKY rat, the F1 SHR/a males were crossed with King-Holtzman females carrying the Tfm trait. The F1 offspring from this cross had BP measured biweekly as in experiment 1 from 7 to 29 weeks of age: females ($n=11$), normal AR males ($n=11$), and AR-deficient males (Tfm, $n=11$) (normal in this study refers to ARs, not BP). Statistical analysis was performed by analysis of variance (ANOVA). The animals were maintained in a humane way according to NIH guidelines.

Results

The results of experiment 1 (Figure 1A) show that BP of the male SHR/Y rats was significantly higher than that of the WKY males (ANOVA, $F=13.52$, $p<0.01$). The results of experiment 2 (Figure 1B) show that the SHR/a males had significantly lower BP over time compared with that of the SHR (ANOVA, $F=5.04$, $p<0.05$). Figure 2 shows the results of experiment 3. Castration significantly reduced BP in both SHR/Y (Figure 2A) and SHR/a groups (Figure 2B). The testosterone implants increased BP back to sham control levels in both groups. The results of the first part of experiment 4 (Figure 3A) show that the F1 hybrid males that had a Y chromosome from a hypertensive father (SHR/Y) and a normal AR had a significantly higher BP than either sibling Tfm males (AR deficiency) or sibling females (ANOVA, $F=25.7$, $p<0.001$). BP in Tfm males was similar to that of the females. The second part of experiment 4 is shown in Figure 3B. F1 hybrid males with a Y chromosome from a normotensive father (SHR/a) and autosomes from a hypertensive mother and a normal AR had a significantly higher BP than either sibling Tfm males or females (ANOVA, $F=20.3$, $p<0.01$).
Figure 1. Line graphs show systolic blood pressure from 5 to 15 weeks of age in experimental rats. Panel A: F11 male rats with a spontaneously hypertensive rat (SHR) Y chromosome in a normotensive genetic background (SHR/y) (n=8, □) compared with male Wistar-Kyoto (WKY) rats (n=8, ○). Panel B: F11 male rats with a normotensive Y chromosome in an SHR genetic background (SHR/a) (n=8, ○) compared with male SHR (n=8, ●). Mean±SEM; ANOVA: F=13.52, p<0.01 and F=5.04, p<0.05, respectively.

Figure 2. Line graphs show systolic blood pressure from 10 to 16 weeks of age in F11 generation rats. Panel A: Male rats with a spontaneously hypertensive rat (SHR) Y chromosome in a normotensive genetic background (SHR/y): sham control, castrated rats, and castrated rats with testosterone implant. Panel B: Male rats with a normotensive Y chromosome in an SHR genetic background (SHR/a): sham control, castrated rats, and castrated rats with testosterone implant. Mean±SEM; ANOVA: F=10.4, p<0.01 and F=24.9, p<0.001, respectively.

Discussion

In our previous work we showed that the Y chromosome interacted with an autosomal component to increase BP7-8 and that the AR was necessary for the full expression of the SHR Y chromosome BP effect as well as a possible androgen effect that did not function through the AR.8 The present research continues to support these findings and extends this work to show that the Y chromosome from a hypertensive father continues to elevate BP after 11 generations of backcrossing to normotensive mothers. The pressure difference between the pure WKY males and F11 SHR/y males averaged 12 mm Hg, which can be thought of as the hypertensive Y effect against a normotensive autosomal background. Also, the SHR/a males averaged approximately 14 mm Hg lower than the pure SHR males, suggesting that this is the effect of the absence of the hypertensive Y chromosome. From these first two experiments, we cannot say what the Y effect may be due to, but the evidence supports a Y effect (approximately 12–14 mm Hg) acting with normotensive autosomes that is consistent through 11 generations. The F11 generation BP data for the SHR/y males (170 mm Hg) and F1 generation (170 mm Hg) from our previous studies.9 Likewise, the F11 generation BP data for the SHR/a males (165 mm Hg) was very similar to that at 15 weeks for the F1 generation (165 mm Hg) and the F1 generation (157 mm Hg) of SHR/a males from our previous studies,9 even though the pressures were taken by different individuals separated by 4 years for F1 to F11. Also, testosterone could be the main steroid responsible for the pressure effect, because testosterone replacement in castrated groups restored BP to control levels. The data are thus consistent with the F1 data and appear to hold true over 11 generations.

In our previous work, the parental strain of SHR had BP at 15 weeks of approximately 212 mm Hg, and in this study the pure SHR strain had a pressure of approximately 185 mm Hg at 15 weeks. This is not an unreasonable difference when dealing with SHR, and it does not change the fact that the BP of the SHR/a and SHR/y substrains have remained constant through 11 generations.

The present research also supports our hypothesis that ARs are necessary for the maximum BP effect of the Y chromosome. The F1 male offspring of the SHR/a and SHR/y males crossed with the King-Holtzman female demonstrated that the ARs were responsible for a 40

\[ p<0.001 \]. Tfm males also had pressures similar to those of the females.
mm Hg increase in BP in the SHR/Y cross and 20 mm Hg increase in the SHR/a cross. These data support previous work in which prepubertal castration or AR blockade in WKY rats and SHR resulted in a reduction in BP.15-19 Furthermore, the data suggest that combination of a Y chromosome and AR interaction.8 One of these mechanisms, the possibility that central nervous system organizational effects due to perinatal male- female steroid differences could strongly influence BP development, cannot be eliminated and should be further examined. The adult BP effects of high levels of testosterone present in infancy are still not known in humans. In the Rhesus monkey, it is thought that the postnatal period might be a critical one, during which the central nervous system may be organized by androgens for control of pubertal timing and sexual behavior.20 In the rat, the pubertal period and adulthood are characterized by testosterone dominance in peripheral plasma, whereas the prepubertal period (3-5 weeks) is characterized by 17 OH-progesterone dominance, which might represent a prepubertal activation of the adrenal equivalent to the human adrenarche.21 Therefore, gonadal steroids may be important for potential autosomal or Y chromosome components that could alter central nervous system or prepubertal mechanisms influencing BP.

It is also possible that the BP-lowering effects of castration and AR deficiency could be explained by a nonspecific mechanism that does not involve the Y chromosome. For instance, if testosterone increases collagen deposition in blood vessels and produces a structural change that increases blood pressure, then the Y chromosome effect may be independent of the androgen effect. Further research should try to resolve this possibility.

A recent study by Harrap's group22 has examined the possibility of a Y-linked effect on blood pressure operating through the kidney. The replacement of SHR female kidneys by SHR male kidneys and vice versa was not associated with any change in blood pressure, suggesting that the hypertensive effects of SHR kidneys is probably not through a Y chromosome mechanism.

With regard to the human Y chromosome, not much is known about it except for the sex-determining region. More than 175 genes have been assigned to the X chromosome, and because the Y is about one third the size of the X, it might be expected that 50-60 genes could be mapped on the Y. Only about 14 loci have been mapped on the human Y chromosome.23 However, very little is known about the rat Y chromosome. Most of the rodent data on the Y chromosome are derived from mouse studies, which show the following Y linkage: chemo- sensory identity,24 two loci influencing androgen metabolism,25 renal papilla length and adrenal weight and epistatic interaction of the Y chromosome with autosome 4 for kidney weight,26 and testis weight and aggression.27,28 These phenotypes may be useful markers in identifying the gene or genes on the Y chromosome that may influence BP in the rat.

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