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Review of the Y chromosome and hypertension

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Abstract

The Y chromosome from spontaneously hypertensive rats (SHR) has a locus that raises blood pressure 20-25 mmHg. Associated with the SHR Y chromosome effect is a 4-week earlier pubertal rise of testosterone and dependence upon the androgen receptor for the full blood pressure effect. Several indices of enhanced sympathetic nervous system (SNS) activity are also associated with the SHR Y chromosome. Blockade of SNS outflow reduced the blood pressure effect. Salt sensitivity was increased by the Y chromosome as was salt appetite which was SNS dependent. A strong correlation ($r = 0.57$, $P<0.001$) was demonstrable between plasma testosterone and angiotensin II. Coronary collagen increased with blood pressure and the presence of the SHR Y chromosome. A promising candidate gene for the Y effect is the *Sry* locus (testis determining factor), a transcription factor which may also have other functions.

Key words
- Sympathetic nervous system
- Testosterone
- Androgen receptor
- Collagen

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Introduction

The mammalian Y chromosome has evolved as an efficient mechanism of sex determination. There are two major components of the mammalian Y chromosome that are necessary for this process: a pseudoautosomal region (PAR) and a Y unique region. The PAR has loci that are homologous to a region on the X chromosome. These regions pair during meiosis, resulting in the homologous pairing and separation during meiosis I of the X and Y chromosomes. The unique region, which does not recombine with the X chromosome, contains the dominant testes determining locus (*Sry*). This locus is primarily responsible for male sexual phenotype. Other than the small PAR, the Y chromosome is not involved in recombination (crossing over) during meiosis. This results in the majority of the Y chromosome being inherited without variation from father to son. The classic genetic tools of linkage analysis and crossing over to locate a gene of interest are of no use for a Y chromosome locus. From a genomic view the human Y chromosome is the most thoroughly studied mammalian chromosome, with a complete physical map and overlapping YAC contig (1,2). A Y chromosome contains fewer loci than predicted by its size when compared to autosomal chromosomes, since most of its length is composed of species specific repeats. There are about 20 identified loci on the human Y chromosome but not all these are conserved on the rat Y chromosome (3). The Y chromosomes of different mammalian species contain a few conserved Y chromosome loci, such as *Sry*, but other loci are species specific. The Y chromosome of the spontaneously hypertensive rats originating from the Harlan Sprague Dawley colony (SHR/hsd) contains

a gene locus (or loci) that is instrumental in increasing blood pressure in this strain. Results from both reciprocal crosses and consomic lines indicate that the Y chromosome is responsible for about 20-25% of the total blood pressure increase in the SHR strain.

The following review will cover the following topics: 1) the Y chromosome blood pressure effect in humans and animals reported by other laboratories, 2) the development of consomic strains, 3) the sympathetic nervous system and salt sensitivity, which is modulated by the Y chromosome, 4) the Y chromosome effect on testosterone, the renin-angiotensin system, and renal norepinephrine (NE) release which is modulated by the androgen receptor, 5) a testosterone-coronary artery collagen deposition link, and 6) new molecular Y chromosome candidate genes.

Y Chromosome blood pressure effect in other laboratories - animal and human studies

It is always important to have new findings validated by similar results in independent laboratories. In 1991 Hilbert et al. (4) and Jacob et al. (5) reported results consistent with a Y chromosome effect in stroke-prone SHR (SHRSP) of about 17 mmHg in systolic pressure. Dominiczak's group (6) (Glasgow) reported a similar Y chromosome effect in SHRSP males crossed with WKY of 20 mmHg. Also male SHRSP have larger infarcts and more spontaneous strokes than females (7). The only negative report which did not show a Y chromosome effect was from Samani's laboratory (8) using the Charles River SHR (SHR/crl). It appears that the SHR/crl has a different Y chromosome. We crossed SHR/crl with our WKY rats and failed to find an SHR Y chromosome blood pressure effect (9). Recent human studies further support our hypothesis and show that a hypertensive father but not a hypertensive mother affects blood pressure in normotensive male offspring through body mass index (10). Also, Lemne (11) showed that there was increased blood pressure reactivity in children of borderline hypertensive fathers. Recently, the use of a mathematical model to analyze data from cattle that developed pulmonary hypertension at high altitude showed that those data were consistent with a Y chromosome effect (12).

Consomic line development and substrain analysis

After our original observation of the hypertensive effect of the SHR Y chromosome, we began a breeding protocol to develop consomic strains for the SHR and WKY Y chromosomes. From an original WKY ♀ x SHR ♂ cross, F_1 sons were selected and backcrossed to a WKY ♀. In each subsequent generation sons were selected and backcrossed to a WKY ♀. This strain has been designated SHR/y as it has the SHR Y chromosome in a normotensive WKY background. A second consomic was developed concurrently from an SHR♀ x WKY ♂ cross, and sons were backcrossed to an SHR♀. This strain is designated SHR/a, as it has the SHR autosomal, X chromosomal and pseudoautosomal regions of SHR with a WKY Y chromosome. These strains have been backcrossed for 19 generations. Blood pressures of the two consomic strains have been published (13), and in each the addition (or deletion) of the SHR Y chromosome adds (or subtracts) 15-20 mmHg from the parental strain systolic blood pressure. The consomic strains are maintained by backcrossing to the original female strain rather than by interbreeding of the consomic strain. This prevents random differentiation between the congenic strains and the parental strains. This also insures that the SHR and WKY strains are genetic control strains for the consomics in each generation.

The SHR Y chromosome increases blood pressure in other normotensive genetic backgrounds in addition to WKY. We crossed the

SHR Y chromosome into the normotensive King Holztman (KH) genetic background. In these hybrid animals those with the SHR Y chromosome had higher blood pressure than those with a KH Y chromosome (14). Also animals with a deficient androgen receptor (feminized male) had lower blood pressure than the siblings with normal receptors. The SHR strains from different vendors have different Y chromosomes. Our original experiments were conducted on animals from our breeding colony which had originally been purchased from Harlan Sprague Dawley (SHR/hsd and WKY/hsd). Our data are consistent with the SHR/hsd and SHR/crl strains having different Y chromosomes, most likely the result of mutation and genetic drift since the two vendor strains have been separated. Y chromosomes are more prone to these types of change than the other chromosomes because of reduced number of copies in each breeding pair. For example, for each breeding pair there are four copies of each autosomal locus (two in each parent) and three copies of each X chromosome locus (two in the female and one in the male) but only one copy of the Y chromosome.

Sympathetic nervous system

The Y blood pressure effect is associated with indices of increased sympathetic nervous system (SNS) activity including increased adrenal gland NE and chromogranin A content, increased heart and renal NE turnover, increased plasma NE response to acute stress, and reduction in blood pressure after chemical sympathectomy or clonidine treatment (13,15,16). There is a large body of evidence that suggests that the SNS is involved in both human and animal neurogenic models of hypertension, by many different mechanisms. Lee et al. (17) showed that one of the primary roles of the overactive SNS in SHR was through the trophic effects on the arteries, especially through hyperplasia of smooth muscle cells. The development of the SNS in SHR appears to be tissue specific.

A substantial portion of patients with borderline hypertension show signs of increased sympathetic and decreased parasympathetic tone. The research of Esler et al. (18) and Wallin et al. (19) has shown strong evidence for increased regional sympathetic nervous activity in human hypertension. The source of the increased SNS is unknown, but evidence is mounting that it may have a central nervous system origin. Wyss's (20) and Oparil's (21) laboratories have developed the hypothesis that inappropriate CNS regulation of SNS activity is a major contributor to NaCl-sensitive hypertension in the salt-sensitive SHR. Excessive dietary NaCl leads to a significant decrease in NE release in the anterior hypothalamic area (20), which results in decreased inhibition of SNS activity and a resultant rise in arterial pressure (21). Also direct SNS nerve recording has shown enhanced activity in SHR renal and splanchnic beds (22).

Our laboratory has also shown increased SNS in SHR on a high salt diet that under chronic stress causes increased blood pressure (23); SNS blockers prevented this salt-induced blood pressure rise (16,24). Since the NE turnover rate is an indicator of sympathetic tone, we hypothesized that the kidney and heart NE content and turnover rate would be increased in animals with the SHR Y chromosome (SHR and SHR/y) as compared to the WKY. In this study, adult male SHR/a, WKY and SHR/y (N = 8/group) kidneys and heart were removed at time 0 after saline injection and 3 h after α-methyl-DL-p-tyrosine injection (200 mg/kg, *ip*) and stored until they were homogenized and analyzed for NE by HPLC with electrochemical detection. The SHR and SHR/y kidneys showed a significant ($P = 0.0125$) elevation in NE content as compared to WKY rats. A significant ($P<0.0001$)) increase was also found in the NE turnover rate between both the SHR (27.5 ng g^{-1} h^{-1}) and SHR/y (31.7 ng

g^{-1} h^{-1}) strains when compared to the WKY strain (12.0 ng g^{-1} h^{-1}). Also there was increased heart NE turnover in SHR/y (25 ng g^{-1} h^{-1}) and SHR (8 ng g^{-1} h^{-1}) as compared to WKY (4.6 ng g^{-1} h^{-1}) (15).

These data suggest that the Y chromosome from SHR increased the NE turnover rate in the heart, since removal of the SHR Y chromosome (SHR/a strain) reduced the turnover rate and addition of the SHR Y chromosome (SHR/y strain) increased the turnover rate. The potential mechanisms for increased turnover rate are increased synthesis of the rate-limiting enzyme tyrosine hydroxylase, or increased degradation by monoamine oxidase and catechol-O methyltransferase. In conclusion, the SHR Y chromosome increased kidney and heart NE turnover rate.

To examine the effect of the Y chromosome on blood pressure, circadian rhythm, and the role of the SNS, colonies of socially interacting WKY and SHR/y and individually caged control animals were maintained on normal rat chow (25). Both caged and colony groups were also treated with clonidine to reduce SNS outflow. Blood pressure, heart rate and activity were continuously monitored using radiotelemetry. The hypertensive Y chromosome increased blood pressure in both the colony and caged groups as compared to WKY ($P<0.0001$, respectively). The WKY colony did not show a blood pressure circadian rhythm. In comparison, there was a significant nocturnal increase in blood pressure in the SHR/y colony ($P<0.0001$; light: 132 mmHg, dark: 143 mmHg). The blood pressure of the SHR/y colony on clonidine was reduced to baseline levels similar to those of the WKY colony. Although both strains showed a heart rate circadian rhythm in the colony, there was no significant difference between strains on a normal diet or receiving clonidine. Heart rate was reduced ($P<0.05$) in both strains by clonidine during the dark, but not under light conditions. The observation that clonidine reduced the blood pressure in the SHR/y strain supports the hypothesis that SNS activity is involved in the Y chromosome effect The activity profile increased in the SHR/y animals, a fact possibly due to increased SNS activity. Also the long-term indicators of SNS activity were increased, as measured by chromogranin A levels in the adrenal gland (13).

Salt sensitivity

The SHR exposed to a high sodium (3% Na) diet has salt sensitivity and increased blood pressure partly due to the SNS, and when placed in a territorial colony, its blood pressure rise is exacerbated. The objective of this experiment was to test the hypotheses that a 3% Na diet increases blood pressure in male WKY rats with the SHR Y chromosome (SHR/y) and that the presence of testosterone potentiates the SNS effect (26). A 2 x 4 factoral design was used with strain (WKY and SHR/y) and treatment [0.3% Na (NNa), 3% Na (HNa), 3% Na-clonidine (C), and 3% Na-flutamide (F)]. Blood pressure (24 h) was significantly ($P<0.0001$) higher in the SHR/y as compared to the WKY on the NNa diet. However, blood pressure rose in both strains when they were exposed to HNa ($P<0.001$). The F diet reduced blood pressure in both strains as compared to HNa ($P<0.0001$). Also, the F treatment significantly reduced the SHR/y blood pressure below the levels for the SHR/y NNa group. There was a rise in NE levels with the HNa diet as compared to NNa ($P<0.02$) which was reduced by the addition of C to the diet. In conclusion, sodium sensitivity is not mediated through the Y chromosome alone. However, our findings support the hypothesis that testosterone potentiates Na sensitivity in both strains and the hypertensive effect of the Y chromosome.

Salt appetite

The objectives of the salt appetite experi-

ments were to determine 1) if female rats have higher Na intake than males and if social stress increases Na intake, 2) if the SNS mediates the stress effect, and the gender effect, and 3) if the Y chromosome from a hypertensive father increases Na intake (27). Four rat strains (N = 10/group) of both sexes were used: 1) normotensive WKY, 2) an F_{16} backcross with a Y chromosome from a hypertensive father (SHR/y), 3) SHR, and 4) an F_{16} backcross with a Y chromosome from a normotensive father (SHR/a). Females showed greater baseline Na intake than males (hypertensive strains), intruder stress increased Na intake, and clonidine decreased Na intake, but not in WKY or SHR females. SHR/y males had higher baseline Na intake compared to WKY males. In conclusion, the higher Na intake in females during baseline and stress was partially mediated through the SNS in hypertensive strains and the SHR Y chromosome was partially responsible for the increased Na intake in SHR/y and SHR males compared to WKY.

Testosterone link

Plasma testosterone and LH levels are higher in SHR than WKY (28). Also the SHR Y chromosome when placed in a WKY background causes an earlier testosterone rise and elevated blood pressure (29). Blocking the androgen receptor or castration prevents the blood pressure rise (14,30). Testosterone influences many target organs both during development and in the adult that could have a significant impact upon blood pressure. The exact mechanisms are not known but several possibilities exist.

One potential mechanism that our laboratory has studied is that greater amounts of collagen deposition in the aorta and resistance vessels are laid down in response to testosterone. Indeed, testosterone exerts an anabolic effect on arterial connective tissue by increasing the synthesis of collagen and elastin (31,32). Testosterone may also increase collagen by causing a decrease in degradation. We have shown an increase in coronary collagen associated with testosterone and the SHR Y chromosome (33).

Testosterone-collagen link

To examine further the role of testosterone (T) upon coronary and myocardial collagen deposition the following study was conducted. The objective was to test the hypothesis that T raises blood pressure, which is associated with increased coronary adventitial collagen, whereas, the hemodynamic force of blood pressure increases the coronary media/lumen ratio. Five groups of SHR were used (N = 8-10/group): controls, hydralazine (HYZ), castration, castration + HYZ, and castration + HYZ + T + captopril. At 12 weeks of age, the castration + HYZ group was divided so that the mean blood pressure was the same in both groups (162 mmHg). Both groups continued HYZ treatment, but one group received T implants. Also, at 12 weeks of age the castration + HYZ + T + captopril group received T implants. Blood pressure in the HYZ group was reduced (192 mmHg) compared to controls (218 mmHg, $P<0.01$). Castration lowered blood pressure to 170 mmHg ($P<0.01$) compared to controls. However, T implants increased blood pressure 15 mmHg ($P<0.02$) in the castration + HYZ group, and 44 mmHg in the castration + HYZ + captopril group ($P<0.01$). Captopril in combination with HYZ significantly reduced blood pressure compared to controls, but T replacement increased blood pressure and coronary collagen deposition in spite of hydralazine and captopril treatment.

In order to examine the effect of the SHR Y chromosome on coronary and ventricular collagen deposition we examined the amount of collagen in four strains of male rats, using two different techniques. Both image analysis (Figure 1A) and hydroxyproline levels (Figure 1B) showed that addition of the SHR

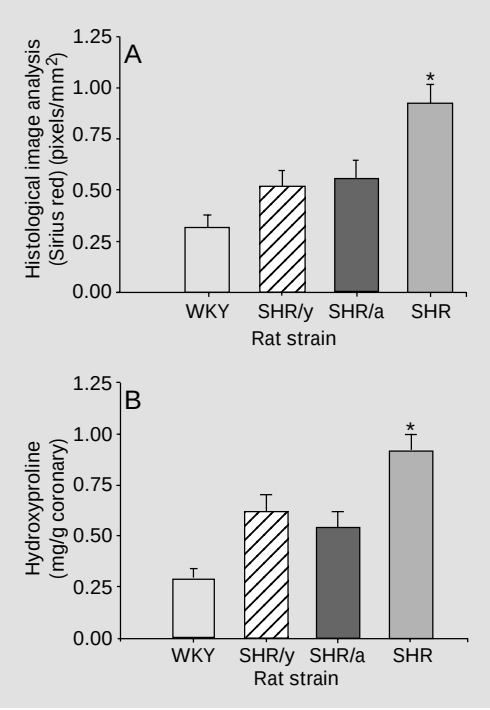

Figure 1 - *A*, Coronary adventitial collagen measurement by computer histological image analysis (pixels/mm^2) for four rat strains (means ± SEM, *P<0.05 compared to WKY group). *B*, Biochemical analysis of coronary artery hydroxyproline (mg/g coronary) by rat strain (means ± SEM, *P<0.05 compared to WKY strain).

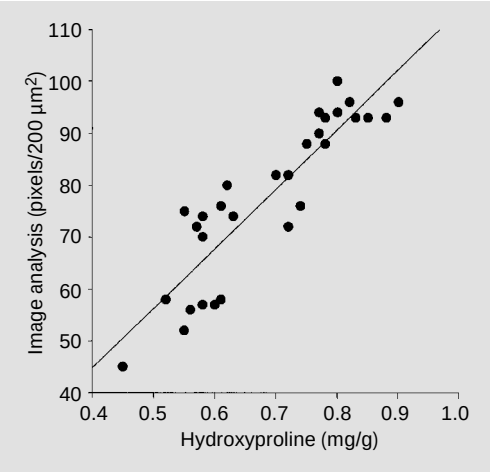

Figure 2 - Pearson's correlation of coronary artery collagen image analysis (pixels/200 µm^2) versus coronary artery hydroxyproline content (mg/g) (r = 0.88, P<0.001).

Y chromosome (SHR/y) increased collagen and removal of the SHR Y chromosome (SHR/a) decreased coronary collagen. The correlation between image analysis and biochemical analysis showed that both techniques were measuring the same thing (r = 0.88, P<0.001; Figure 2). Table 1 shows the results of telemetered blood pressure and coronary and ventricular collagen in four strains of rats with different Y chromosomes. The SHR Y chromosome placed in the WKY (SHR/y) doubled the coronary collagen measured by either of the two techniques (image analysis and hydroxyproline content) compared to WKY. Removal of the SHR Y chromosome (SHR/a) reduced coronary collagen compared to SHR. Addition or removal of the Y chromosome did not change ventricular collagen, although SHR had more collagen than WKY (Seachrist D, Dunphy G, Daneshvar H, Caplea A, Milsted A and Ely D, unpublished observations).

Testosterone and the renin-angiotensin system

The renin-angiotensin system (RAS) is an important participant in the development and maintenance of hypertension and is activated in hypertensive rats. Sexual dimorphism in the RAS is well known (34). Androgens are known to potentiate the development of hypertension. Tissue specific regulation of renin mRNA by androgens has been shown in both adrenals and brain of

Table 1 - Blood pressure and coronary and ventricular collagen by rat strain (males, 20 weeks).

*P<0.05, **P<0.01 SHR/y compared to WKY and SHR/a compared to SHR.

Rat strain	Telemetered	Coronary collagen		Ventricular collagen	
	Aortic blood pressure (20 weeks)	Image analysis (pixels/mm^2)	Hydroxyproline (mg/g)	Image analysis (pixels/200 µm^2)	Hydroxyproline (mg/g)
WKY	140 ± 3	0.26 ± 0.01	0.26 ± 0.05	66 ± 5	0.73 ± 0.06
SHR/y	172 ± 4**	0.51 ± 0.10**	0.62 ± 0.08**	64 ± 4	0.75 ± 0.06
SHR	196 ± 5	0.99 ± 0.05	0.90 ± 0.10*	89 ± 2*	0.63 ± 0.03
SHR/a	175 ± 4	0.62 ± 0.08*	0.51 ± 0.08*	60 ± 3	0.65 ± 0.03

mice (35). Chen et al. (36) showed that renal and hepatic angiotensinogen mRNA levels in SHR are dependent on androgen in both sexes. Plasma renin activity was significantly higher in gonadally intact male SHR than in females, and testosterone treatment increased plasma renin activity. Also, in some studies, genotypes of angiotensinogen codon 179 were significantly associated with variation in systolic blood pressure in men (37). We have measured plasma testosterone and angiotensin II (Ang II) simultaneously in male SHR and found a significant correlation at 15 weeks of age ($r = 0.57$, $P<0.001$; Figure 3). It appears that testosterone enhances circulating Ang II. Brilla et al. (38) showed that Ang II can increase fibroblast collagen turnover.

Both the renin and angiotensinogen genes are responsive to steroid hormones, including androgens, and glucocorticoids. Tissue- and age-related differences in angiotensinogen mRNA levels have been reported in SHR and WKY (39). Both genes can be regulated at transcriptional and post-transcriptional (mRNA stability) levels. The renin gene is on chromosome 13 and the angiotensinogen gene is on chromosome 19 in the rat. Yet these genes on different chromosomes, both originating from WKY rats, are not expressed at the same level in our WKY and SHR/y females. Differences in renin gene structure in various rat strains have been described. In the human angiotensinogen gene, several molecular variant alleles are known; in some populations the M235T polymorphism is associated with hypertension (40). We have not yet analyzed renin or angiotensinogen gene structure in our rat strains. Our hypothesis was that changes in steroid hormones occurring during the first 15 weeks of life would produce changes in renal renin and angiotensinogen mRNA that would reflect differences due to the genetic origin of each rat strain. Blood pressure in WKY was lower than in strains with SHR autosomes ($P<0.003$ *vs* SHR/a), SHR Y chromosome ($P<0.03$ *vs* SHR/y), or both SHR autosomes and SHR Y chromosome ($P<0.03$ *vs* SHR). Angiotensinogen mRNA was not detected until 5 weeks of age, and no strain differences were seen at that age. Angiotensinogen mRNA levels increased by 2-4-fold between 5 and 15 weeks of age. Renin mRNA levels were highest in all strains at 1 week and decreased by as much as 20-fold with age. At one week of age the strains containing SHR autosomes (SHR and SHR/a) had higher renin mRNA levels than WKY and SHR/y, and overall, levels of renin mRNA appeared to decrease at slightly earlier ages in SHR and SHR/a than in WKY and SHR/y (41).

In summary, our results fail to reveal consistent patterns of RAS differences that reflect the presence or the absence of the SHR Y chromosome. In male rats we find no evidence of either coordinate regulation of renin and angiotensinogen gene expression or of imprinting. In both WKY and SHR/y male rats, the X chromosome is always contributed by their WKY mothers; in SHR and SHR/a males, the X chromosome always comes from their SHR mothers.

We have also investigated the effects of estrogen removal (ovariectomy, OVX) and androgen addition (testosterone implants) in 3 groups of female SHR/y rats and the parental rat strain, WKY: group 1) intact (control), 2) ovariectomy at 3 weeks old, and 3) ova-

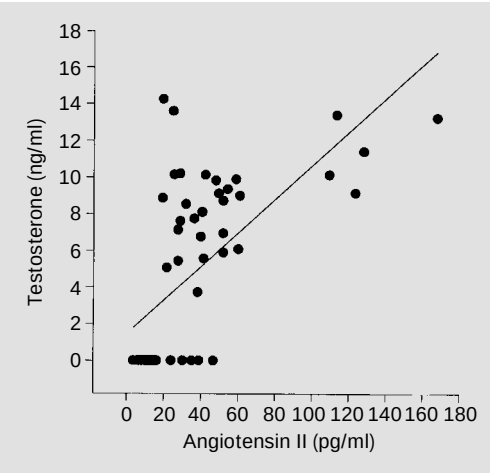

Figure 3 - Pearson's correlation of plasma testosterone (ng/ml) versus angiotensin II levels (pg/ml) in SHR males (20 weeks) ($r = 0.57$, $P<0.001$).

riectomy with testosterone implant at 3 weeks old. SHR/y females have the parental WKY autosomes and X chromosomes and have no chromosomes of SHR origin; thus they are genetically equivalent to female WKY rats. One-way ANOVA showed significant blood pressure differences between WKY and SHR/y at 10 weeks and at 16 weeks ($P<0.0001$). Treatment had a significant effect on blood pressure ($P<0.0001$). Blood pressure was highest in the 16-week SHR/y OVX + T group, reaching 204 ± 9 mmHg ($P<0.05$ *vs* all other groups). Plasma renin activity was significantly higher in WKY than in SHR/y ($P<0.01$, two-way ANOVA).

Levels of renin mRNA and angiotensinogen mRNA in the kidney followed similar patterns in each strain. There were significant strain differences in renin mRNA levels ($P<0.02$) and age differences in angiotensinogen mRNA levels ($P<0.003$). Correlation analysis indicated that renal angiotensinogen mRNA was positively correlated with renal renin mRNA in all groups of both strains ($P<0.001$, $r = 0.7515$). The combination of removing estrogen early in development and supplementing the ovariectomized females with testosterone revealed strain differences in blood pressure response.

Renin and angiotensinogen mRNA levels appear to be regulated coordinately within each strain, although actual mRNA levels differ between strains. Strain differences in regulation of RAS genes may result from epigenetic mechanisms such as genome imprinting of these genes or of another gene that functions as a common regulator of renin and angiotensinogen (42).

Strain differences to OVX and response to OVX + T were seen. Age-dependent effects on blood pressure were also found. OVX increased blood pressure in both strains in 10-week-old females but not in 16-week-old animals. The addition of T to 16-week-old SHR/y females resulted in a very large increase in blood pressure, to 204 ± 9 mmHg. In 13-week-old SHR females, Reckelhoff et al. (43) have reported no differences in blood pressure between OVX and intact females, untreated or treated with enalapril. The interactions of steroid hormones with the RAS are complex and appear to be influenced by age and genetic background.

It might be that the rat liver, like the mouse liver, is androgenized at an early stage and the hepatic angiotensinogen system might play a key role in the cascade of events involving testosterone and its effects on hypertension. Sex differences in the developmental pattern of blood pressure may also be related to organizational effects of perinatal sex steroids on the immature CNS. We have not explored this possibility. However, since female SHR also develop hypertension, it is possible that the rise in testosterone level may be responsible only for an earlier onset of hypertension in males. Several actions of testosterone have not been explained by known metabolites or receptors. Therefore, the idea has emerged that for some testosterone actions the androgen receptor may not be necessary. Also we have reported that in males lacking a functional androgen receptor there appear to be direct cell membrane testosterone-mediated effects which influence calcium flux (14). Thus, it is evident that a cell can recognize and respond to testosterone by a variety of independent mechanisms some of which remain to be defined. The androgen receptor is gaining a wider role and there is evidence that it is an important mediator of gene expression and signal transduction pathways (44).

Testosterone-renal link

Sex influences have been described in the kidney with regard to adrenergic receptors (45). We have recently described an enhanced fractional renal release of NE due to the SHR Y chromosome (46). The objective of the following study was to look at the effects of T on renal NE release and content in the isolated perfused kidney in different Y

chromosome backgrounds. The study involved male SHR, WKY and two consomic strains with different Y chromosomes (N = 5-8/group). Adult animals were castrated and implanted at the base of the neck with Silastic tubing (Dow Corning, Midland, MI, USA) containing testosterone propionate (14). Blood T levels were measured by RIA two weeks after castration. The left kidney was isolated and perfused with oxygenated Krebs solution at a constant flow and temperature with electrical stimulation of the renal nerves. Perfusate was collected and analyzed for NE by HPLC. Renal perfusate and renal tissue NE levels were significantly elevated by T. The average T value with a single T implant was 13 ng/ml, and for a double T implant 30 ng/ml. The Y chromosome from the SHR produced a significant increase in renal NE release compared to the WKY Y chromosome. Significance was shown between all groups: 1 *vs* 2 implants (P = 0.0067), 1 *vs* sham implants (P = 0.015), and 2 *vs* sham implants (P<0.001). In conclusion, T caused an enhanced renal NE release that was strain specific, with the Y chromosome raising renal NE content and release.

Follow-up research using the isolated kidney with an androgen receptor (AR)-deficient model (testicular feminized male, Tfm) or flutamide treatment has shown that the lack of a functional AR resulted in a significant reduction in renal NE release (Figure 4). Flutamide reduced renal NE release in both SHR/y and WKY. Also in the Tfm there was a large renal NE reduction. Three hybrid strains of adult male rats were used and within each strain normal AR kidneys were compared to deficient AR kidneys: SHR x KH (N = 21), KH x KH (N = 11), SHR/y x KH (N = 15). Kidneys were isolated and perfused with oxygenated Krebs-Henseleit solution and pressure and flow measured as well as NE in the effluent by HPLC under nonstimulated and electrically stimulated conditions. In each strain the lack of a functional AR resulted in a significant reduction in the release of renal NE: SHR = 23% (440 to 340 pg/ml), KH = 38% (265 to 165 pg/ml), and SHR/y = 42% (180 to 105 pg/ml), P = 0.0017. LDH values were low, suggesting minimal cell damage after perfusion. In conclusion, the AR increased renal NE release and AR blockade or mutation reduced renal NE release in the isolated kidney in 3 different strains of rats (47).

Interaction of testosterone with the sympathetic nervous system

Testosterone interacts with several hormonal systems and requires interaction with them for a full androgen response. For instance, there is evidence that testosterone influences NE metabolism, storage and release (48). Testosterone has been reported to increase α-adrenergic receptors in rat tail arteries, whereas gonadectomy attenuated the total apparent number of binding sites in SHR (49). Philippe et al. (50) have shown a more than two-fold increase in the α_1-adrenergic receptors in response to both testosterone and dihydrotestosterone. The early rise in testosterone in SHR could induce greater sensitivity to NE, which in turn could produce higher blood pressure at an early age. There may also be an early developmental testosterone interaction with tissue NE that cannot be detected by plasma measure-

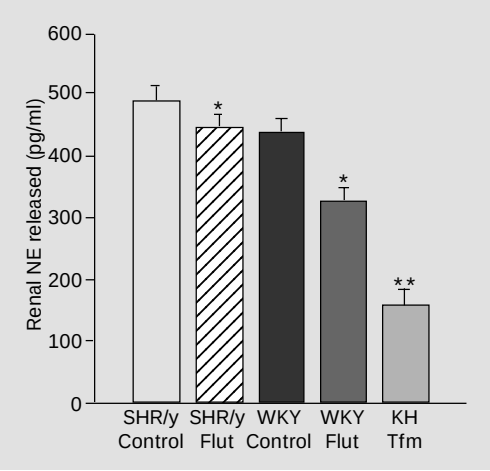

Figure 4 - Y Chromosome and androgen receptor influence on renal norepinephrine (NE) release (pg/ml) from isolated kidney by treatment group. Data are reported as means ± SEM. *P<0.05 and **P<0.001 compared to SHR/y control. Flut, Flutamine; Tfm, testicular feminized male.

ments. Indeed, Mayerhofer and Hodges (51) showed that NE regulates the number of luteinizing hormone receptors in the adult hamster testis and affects Leydig cell function, especially during acute stress. The same group further showed (52) that in immature hamster testes catecholamines can act through both α and ß adrenergic receptors and may be potent stimulators of testosterone production. Isoproterenol treatment resulted in marked Leydig cell hypertrophy in young Sprague Dawley rats (53).

Androgens also have an effect on uptake and release of NE from sympathetic fibers (49). Testosterone also increases s-adenosylmethionine, a major cellular methylator, and may influence the catecholamine pathway (54). The rate-limiting enzyme for the catecholamine pathway, tyrosine hydroxylase, and NE concentration in the abdominal aorta and mesenteric artery were decreased by castration and restored by testosterone in SHR but not in WKY (50). The mechanism for this effect could either be directly on the neurons or through tissue trophic factors. Also in hypothalamic regions steroids modulate tyrosine hydroxylase mRNA in dopaminergic neurons (55) (Table 2).

Table 2 - Summary of evidence of Y-SNS-testosterone interaction.

The table summarizes the data showing the involvement of the SHR Y chromosome with the SNS, renin-angiotensin and testosterone axes.

	SHR	SHR/y	WKY
Increased stress responsiveness			
Blood pressure - acute	++	++	++
Blood pressure - chronic	+	+	-
Increased temperature to restraint stress	++	++	+
Increased heat shock protein 27 in heart	++	++	+
Increased heat shock protein 70 in aorta	++	++	+
Increased sympathetic activity			
Stress-plasma NE	++	++	+
Stimulated renal NE release	++	++	+
Sympathectomy lowers blood pressure	+	+	-
Elevated adrenal chromogranin A	+	+	-
Increased heart and renal NE turnover	+	+	-
Increased salt appetite	+	+	-
Testosterone axis			
Early testosterone rise	++	++	-
Testosterone elevates Ang II	++	++	-
Earlier LH surge	++	++	-
Increased coronary collagen	++	++	-

Molecular probing for heat shock protein (Hsp) polymorphism

Preliminary data had shown a male specific band in WKY from Southern blots probed with rat *Hsp70*. This is consistent with the rat Y chromosome containing an *Hsp70*-like locus and/or homologous sequences. We made five sets of PCR primers that covered different conserved regions from rat *Hsp70* DNA sequences in GenBank. These primers were used to amplify DNA from SHR and WKY males and females. Reactions were run at two annealing temperatures (55 and 60°C) and a variety of $MgCl_2$ concentrations. All primers had single or multiple bands at 55°C and three of the five had bands at 60°C. None of the bands observed were male specific and no amplifications showed obvious amplification differences between males and females. With these five primer sets we were not able to identify or isolate a Y chromosome *Hsp70*-related sequence. These data are consistent with the hypothesis that the Southern results were from *Hsp70*-related partial sequences, rather than a full length potentially active *Hsp70* homolog.

We contracted with Stratagene to construct a male genomic library in lambda DASHII (average insert 9-22 kb) from our SHR strain. From this library we have isolated clones for at least three rat Y chromosome loci (*Ube 1y, Smcy*, and *Sry*). We have begun subcloning and sequencing the *Sry* clones. Clone 96s15 contains regions of at least 75 bp homologous to brain and testis transcripts that are identical to regions in rat *Hsp70-1* and *Hsp70-3,* as well as many other rat loci. It is possible that this type of DNA sequence homology could result in male specific bands from Southern blotting to restriction enzyme-digested genomic DNA, with-

out a complete *Hsp70* sequence on the rat Y chromosome.

In conclusion, the data are consistent with the Y chromosome containing DNA sequences identical with portions of rat *Hsp70*, but we do not have evidence of a full length potentially active copy of *Hsp70* on the rat Y chromosome.

The rat Y chromosome and *Sry*

The publication of the rat map has permitted a rapid exploration of candidate genes for hypertension on all chromosomes except the Y. The human and mouse Y chromosomes are the best characterized mammalian Y chromosomes. Although there are some similarities, the genetic organization of these two chromosomes is very different. The knowledge we do have about the rat Y chromosome shows similarities to the human and mouse chromosome but there are some very basic differences. The mouse, human and rat Y chromosomes all contain *Sry* and *Zfy* loci, but the mouse has 2 copies of *Zfy*, the rat and human one. The rat has multiple copies of *Sry* and humans and mice only one. A third locus identified in mouse, human and rat is the steroid sulfatase locus (*Sts*). The *Sts* structural locus is Y linked and pseudoautosomal in the mouse, Y linked but not pseudoautosomal in humans and X linked in the rat. We have identified a heat shock-related sequence on the rat Y chromosome and another laboratory has identified a mitochondrial D-loop-related sequence on the rat Y and neither of these sequences has been identified on either the human or mouse Y chromosome. Because of the observed differences between these three Y chromosomes, each may serve as a model for the others but differences may be more common than similarities.

We compared an *Sry* sequence from our genomic library to other published rat *Sry*, to study the relationships among multiple copies of this gene. As many as four copies of the *Sry* gene have been reported in rodents. We obtained the 5'-flanking sequence (5'-FLK), complete coding sequence and 3'-untranslated region (3'-UTR), including a stop codon and a polyadenylation signal, from the Y chromosome clone, 96s15. The three available rat *Sry* partial sequences in GenBank were compared to 5'-FLK and coding regions of the SHR gene, designated *Sry-1.* Within the coding region, minimal differences (0.032) were found across strains and species (sequences are from *Rattus norvegicus, R. exulans* and Brown Norway rats). Greater sequence divergence (0.105) was present in the 5'-FLK region sequences. No comparisons of 3'-UTR sequences are possible at this time, since the other rat *Sry* genes are partial sequences, not including that region. With the SHR full length rat *Sry* gene, we can now begin to evaluate the functional significance of the other *Sry* gene copies (56).

In conclusion, the SHR Y chromosome has a locus that raises blood pressure. Other autosomal loci, like genes controlling testosterone biosynthetic enzyme production and the biosynthetic catecholamine enzymes, may interact with the Y loci for maximum blood pressure expression.

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