Female Wistar–Kyoto and SHR/y rats have the same genotype but different patterns of expression of renin and angiotensinogen genes

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Objectives **To evaluate whether renin and angiotensinogen gene expression in females from two strains of rats that share the same autosomes and X chromosomes differs. Female SHR/y rats have the parental Wistar–Kyoto rat autosomes and X chromosomes and have no chromosomes of spontaneously hypertensive rat origin; thus they are genetically equivalent to female Wistar–Kyoto rats.**

Design and methods **Because these genes are regulated by steroid hormones, we investigated the effects of removal of estrogen (ovariectomy) and addition of androgen (testosterone implants) on three groups of female SHR/y rats and the parental rat strain Wistar–Kyoto rat with groups of intact (control) rats, rats subjected to ovariectomy at age 3 weeks, and rats subjected to ovariectomy with a testosterone implant at age 3 weeks.**

Results **The combination of removing estrogen early in development and supplementing the ovariectomized females with testosterone revealed strain differences in response of blood pressure. Renin and angiotensinogen messenger RNA levels appear to be regulated coordinately within each strain, although actual levels of messenger RNA differ between the strains.**

Conclusions **Similar patterns of responses of renin and angiotensinogen genes to ovariectomy and ovariectomy plus testosterone suggest that regulation of the genes is likely to be similar or coordinate. Differences in regulation of renin–angiotensin system genes between strains 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. *J Hypertens* 16:823–828 © 1998 Lippincott-Raven Publishers.**

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Introduction

The renin–angiotensin system (RAS) is an important participant in the development and maintenance of hypertension and is activated in hypertensive rats [1,2]. Sexual dimorphism in the RAS is well known [3–6]. We investigated the effects of removal of estrogen (ovariectomy) and addition of androgen (testosterone implants) on female SHR/y rats and the parental rat strain, Wistar–Kyoto (WKY). Specifically, we asked whether the RAS in female WKY and SHR/y was differently affected by testosterone treatment. Androgens potentiate the development of hypertension [3,7] and are required for full expression of the SHR Y chromosome hypertensive locus [8].

Rats designated SHR/y were bred by backcrossing male SHR with normotensive female WKY rats, and in subsequent generations backcrossing the sons with female WKY rats, to separate hypertensive SHR autosomes and the SHR Y chromosome. This results in male SHR/y with autosomes and X chromosome originating from WKY rats and Y chromosome from SHR. Female SHR/y have the same WKY rat autosomes and X chromosome as their male siblings and a second WKY rat X chromosome inherited from their SHR/y father. The genotypes of female WKY rats and SHR/y are essentially identical, because all autosomes as well as both copies of the X chromosome in the SHR/y strain originated from WKY rats [8]. However, we have observed that their phenotypes differ in several respects, including blood pressure [9].

The objective of this study was to examine whether manipulating the steroid hormone status of these rats by removal of estrogen and by testosterone treatment revealed additional differences between the strains that would be informative regarding the mechanisms respon-

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sible for the observed differences between strains. When the steroid hormone status of the rats was altered, levels of renal renin and angiotensinogen messenger RNA (mRNA) in females of the SHR/y and WKY rat strains were different. Changes in body weight and kidney weight, as well as in blood pressure, were also quantified.

Methods

Animal strains

WKY rats and SHR/y were from colonies maintained at The University of Akron. Our WKY rats and SHR had originally been obtained from Harlan Sprague Dawley (Indianapolis, Indiana, USA) in 1981. The SHR/y strain was developed at The University of Akron by backcrossing the SHR Y chromosome into a WKY rat background for 17 generations. Male SHR/y rats have WKY rat autosomes, WKY rat X chromosome, and the SHR Y chromosome. Female SHR/y have WKY rat autosomes and WKY rat X chromosomes. The SHR/y strain is not independent of WKY rats. It is not interbred against itself. In every generation SHR/y and WKY rats have the same (WKY rat) mothers. Every generation of SHR/y is the result of crossing a male SHR/y and a female WKY rat, not of brother–sister mating of male SHR/y and female SHR/y. Thus each generation is, in effect, independently derived. Genetic testing with 42 informative markers distributed over all the chromosomes verified that the genetic profile of SHR/y accords with that of its parental strain, WKY rat (unpublished data, 1996). Consequently, any heterozygosity found in WKY rats is maintained in the SHR/y strain because it is backcrossed with WKY rats each generation. All rats were treated in a humane manner according to National Institutes of Health guidelines, and all experiments were approved by The University of Akron Institutional Animal Use and Care Committee.

Experimental protocols

Female WKY rats from The University of Akron colony (WKY/UA) and SHR/y from this colony (SHR/y/UA) aged 10 and 16 weeks were divided into three groups for each strain. Rats in one group were left intact (control), rats in a second group were subjected to ovariectomy, and rats in a third group were subjected to ovariectomy with a testosterone implant. Numbers of animals varied among groups and experiments: see Tables 1–3 for actual numbers. For renin mRNA analysis (Fig. 1) group sizes varied from six to 20, and for angiotensinogen mRNA analysis (Fig. 2) they varied from four to 18. Ovariectomy was carried out in sodium brevital-anesthetized rats aged 3 weeks. Rats in the ovariectomy plus testosterone groups were administered implants of testosterone propionate (Sigma Chemical Co., St Louis, Missouri, USA) in 19 mm silastic tubing (Dow Corning, Midland, Michigan, USA) subcutaneously at the back of the neck. We verified that these implants deliver testosterone at a dose within the physiologic range for male rats (data not shown) [10].

Table 2 **Systolic blood pressure in female Wistar–Kyoto (WKY) and SHR/y rats**

	Systolic blood pressure (mmHg)	
Age and group	WKY rats	SHR/y
10 Weeks		
Intact	141 ± 4 (22)	134 ± 2 (21)
Ovariectomy	156 ± 4 (13)*	162 ± 4 (9)*
Ovariectomy plus testosterone	167 ± 2 (10)*	177 ± 5 (10)*
16 Weeks		
Intact	148 ± 3 (17)	153 ± 7 (17)
Ovariectomy	134 ± 3 (8)	141 ± 3 (8)
Ovariectomy plus testosterone	164 ± 7 (7)	204 ± 9 (7)*†

Values are expressed as means ± SEM (numbers of rats used). *$P < 0.05$, versus intact; †$P < 0.05$, versus all other groups; $P < 0.0001$, WKY rats versus SHR/y.

Table 3 **Plasma renin activity in female Wistar–Kyoto (WKY) and SHR/y rats**

	Plasma renin activity (ng angiotensin I/h per ml)	
Age and group	WKY rats	SHR/y
10 Weeks		
Intact	3.19 ± 0.44 (8)	2.08 ± 0.57 (7)
Ovariectomy	2.15 ± 0.38 (8)	3.13 ± 0.56 (9)
Ovariectomy plus testosterone	5.42 ± 0.62 (8)*†	4.30 ± 0.73 (7)
16 Weeks		
Intact	4.67 ± 0.56 (9)	2.86 ± 0.43 (7)
Ovariectomy	3.74 ± 0.65 (6)	2.10 ± 0.38 (6)
Ovariectomy plus testosterone	5.86 ± 2.02 (5)	3.66 ± 0.53 (6)

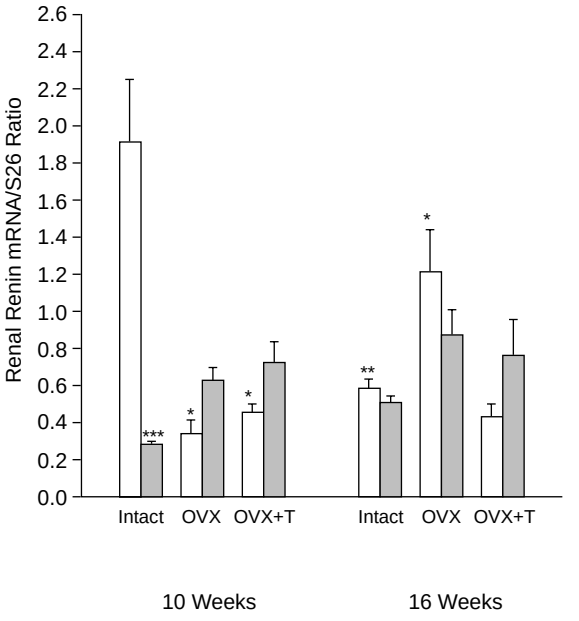
Values are expressed as means ± SEM (numbers of rats used). *$P < 0.05$, versus intact; †$P < 0.05$, versus ovariectomized, $P < 0.01$, WKY rats versus SHR/y.

Table 1 **Body weights and kidney weights of female Wistar–Kyoto (WKY) and SHR/y rats**

	Body weight (g)		Kidney weight (g)	
Age and group	WKY rats	SHR/y	WKY rats	SHR/y
10 Weeks				
Intact	149.0 ± 3.1 (22)	157.5 ± 1.8 (19)	590.7 ± 12.6 (22)	614.0 ± 12.7 (19)
Ovariectomy	183.9 ± 6.8 (13)*	193.4 ± 7.9 (9)*	676.7 ± 22.8 (13)*	696.5 ± 28.6 (9)*
Ovariectomy plus testosterone	173.5 ± 5.5 (10)	203.5 ± 7.3 (10)*	926.4 ± 36.9 (10)*†	907.7 ± 25.4 (10)*†
16 Weeks				
Intact	206.0 ± 3.7 (17)	202.4 ± 3.4 (17)	718.9 ± 19.3 (17)	751.9 ± 18.4 (17)
Ovariectomy	247.2 ± 7.7 (8)*	237.4 ± 9.4 (8)*	734.6 ± 23.2 (8)*	783.6 ± 22.2 (8)*
Ovariectomy plus testosterone	227.2 ± 10.5 (7)*	200.8 ± 9.2 (7)	1236.1 ± 61.8 (7)*†	1190.0 ± 59.9 (7)*†

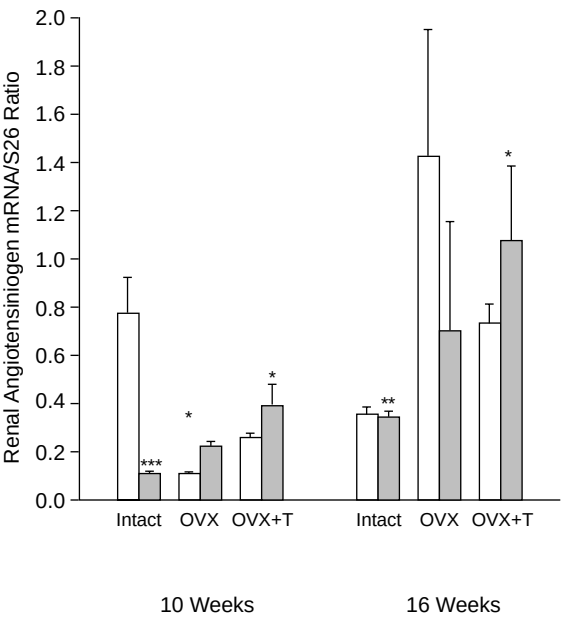
Values are expressed as means ± SEM (numbers of rats used). *$P < 0.05$, versus intact; †$P < 0.05$, versus ovariectomized. $P < 0.0001$, effects of age and treatment on body weight, effects of age and treatment on kidney weight; $P < 0.05$, WKY rat versus SHR/y body weight at age 10 weeks.

Fig. 1



Renin messenger RNA (mRNA) in female Wistar–Kyoto rat (□) and SHR/y rat kidneys. Renin mRNA levels were normalized with respect to the invariant S26 ribosomal protein mRNA. Tissues were from the same animals as those included in Table 1; the numbers of animals used are shown in Table 1. OVX, ovariectomized; T, testosterone implant. Values are expressed as means ± SEM. $*P < 0.05$, versus intact; $**P < 0.05$, versus 10-week intact; $***P < 0.05$, versus intact Wistar–Kyoto rats.

Fig. 2



Angiotensinogen messenger RNA (mRNA) in female Wistar–Kyoto rat (□) and SHR/y rat kidneys. Angiotensinogen mRNA levels were normalized with respect to the invariant S26 ribosomal protein mRNA. Numbers of animals used are shown in Table 1. OVX, ovariectomized; T, testosterone implant. Values are expressed as means ± SEM. $*P < 0.05$, versus intact; $**P < 0.05$, versus 10-week intact; $***P < 0.05$, versus intact Wistar–Kyoto rats.

Implants were replaced every 3 weeks. Values for blood pressure were obtained by taking the average of blood pressures recorded daily by a tail-cuff pulse transmitter (Narco Biosystems, Houston, Texas, USA) for 1 week before sacrifice.

Rats were decapitated and tissues rapidly removed, weighed, frozen on dry ice, and stored at −70°C until RNA was extracted using a modification of the single-step guanidinium thiocyanate procedure [11]. Northern blots were probed successively with radiolabelled complementary DNA (cDNA) encoding rat renin, rat angiotensinogen, and rat ribosomal protein S26, as described previously [12]. Direct quantitation of hybridized radiolabelled cDNA probe was carried out on a Betascope 603 blot analyzer (Betagen, Waltham, Massachusetts, USA). We expressed mRNA levels as amount of renin or angiotensinogen mRNA divided by amount of S26 mRNA [13], to normalize for variation in the mass of RNA analyzed [14].

Plasma renin activity (PRA) was measured using a kit from INCSTAR Corporation, (Stillwater, Minnesota, USA) for quantitative determination of PRA by radioimmunoassay of generated angiotensin I, as described previously [15]. Reported sensitivity of the test is 0.018 ng/tube, interassay coefficient of variation is 5.6–7.6%, and intraassay coefficient of variation is 4.6–10.0%; cross-reactivity with angiotensin I is 100%, that with tetradecapeptide 0.02%, that with angiotensin II < 0.03%, and that with angiotensin III < 0.03%, according to the kit's protocol.

Statistical analysis

Results were expressed as means ± SEM. One-way analysis of variation (ANOVA) was performed for differences in blood pressure between strains at ages 10 and 16 weeks. To detect differences between the strains in multiple comparisons, two-way ANOVA using factors of strain and age, and strain and experimental group was performed. If the two-way ANOVA revealed a significant relationship, one-way ANOVA was performed, and then a Student–Newman–Keuls test to detect significance ($P < 0.05$). The relationship between renin mRNA and angiotensinogen mRNA levels was analyzed by calculating Pearson's correlation coefficient.

Results

Evaluation of body weight and kidney weight revealed that body weight increased with age and treatment ($P < 0.0001$; Table 1). Gain of body weight by ovariectomized rats was reflected in extensive fatty deposits

visible around abdominal organs. Strain differences were significant at age 10 weeks ($P < 0.05$) but not at age 16 weeks. Kidney weights also increased with age ($P < 0.0001$) but did not differ between strains. Treatment increased kidney weights of rats at both ages for both strains ($P < 0.0001$). When ovariectomy plus testosterone groups were compared with ovariectomy groups, testosterone was found to increase kidney weights further at both ages for both strains ($P < 0.05$). Testosterone had no consistent effect on body weight when ovariectomy plus testosterone groups were compared with ovariectomy groups.

Systolic blood pressure (SBP) in rats of each group was measured (Table 2). One-way ANOVA showed that there were significant differences in blood pressure between WKY rats and SHR/y aged 10 and 16 weeks ($P < 0.0001$). Treatment had a significant effect on blood pressure ($P < 0.0001$). Blood pressure was highest in the group of SHR/y with ovariectomy plus testosterone aged 16 weeks, reaching 204 ± 9 mmHg ($P < 0.05$, versus all other groups).

Levels of renin mRNA (Fig. 1) and angiotensinogen mRNA (Fig. 2) in the kidney exhibited similar patterns for both strains. Levels both of renin and of angiotensinogen mRNA in intact female WKY rats aged 10 weeks were approximately sixfold higher than those in intact female SHR/y of the same age. There were significant differences between strains in renin mRNA levels ($P < 0.02$) and age differences in angiotensinogen mRNA levels ($P < 0.003$). Levels of both mRNAs in the intact female rats aged 16 weeks did not differ between strains. Although the difference was not statistically significant for renin mRNA, levels of angiotensinogen mRNA in SHR/y increased from age 10 to age 16 weeks ($P < 0.05$), but those in WKY rats appeared to decrease. Both ovariectomy and ovariectomy plus testosterone treatments decreased levels of renin mRNA in WKY rats aged 10 weeks ($P < 0.05$), whereas renin mRNA levels in SHR/y of the same age did not change significantly, but the level of angiotensinogen mRNA in ovariectomy plus testosterone SHR/y rats was increased ($P < 0.05$; Figs 1, 2). These treatments had, in general, similar effects on female SHR/y aged 10 and 16 weeks. The patterns of renin and angiotensinogen mRNA in WKY rats and SHR/y aged 16 weeks were different; renin mRNA levels in WKY rats in the ovariectomy group were increased ($P < 0.05$) and not different from levels in intact rats in the ovariectomy plus testosterone group. However, angiotensinogen mRNA levels increased in rats in the SHR/y ovariectomy plus testosterone aged 16 weeks group. Correlation analysis indicated that renal angiotensinogen mRNA level was positively correlated to renal renin mRNA level for all groups of both strains ($P < 0.001$, $r = 0.7515$). There were significant differences between strains in PRA ($P < 0.01$; Table 3), but no age differences. Treatment also affected PRA ($P < 0.005$). PRA was found not to be correlated to renal renin mRNA levels ($P = 0.548$, $r = 0.0384$).

Discussion

Analysis of renin and angiotensinogen mRNA levels revealed both significant differences in basal expression between strains at age 10 weeks and strain-specific patterns of response to ovariectomy and ovariectomy plus testosterone. Renin and angiotensinogen genes are known to be responsive to steroid hormones [3,6,16–19] and gonadotropins [20]. Regulation by estrogen of synthesis of angiotensinogen in hepatocytes is direct and occurs via the estrogen receptor [19,21]. Testosterone implants in Wistar rats increased weight gain by intact females, and weight increased further after ovariectomy [22]. Ovariectomy usually increases body weight, as has been shown to occur with Sprague–Dawley and renin-transgenic rats [23]. An increase in testosterone level is associated with increases in inactive plasma renin level in intact female rats [4]. Androgens also regulate the renin gene [23] and are associated with high blood pressure [8,24]. Both genes are developmentally regulated. Gomez *et al.* [25] reported that renal renin mRNA levels in newborn SHR and WKY rats are 7.9-fold higher than those in adult rats; angiotensinogen mRNA was also present in kidneys of newborn rat [25]. Immunoreactivity to angiotensinogen, as well as mRNA levels, are highest in proximal convoluted tubules of the 1-day-old rat, then decline in the adult rat [26]. For SHR but not WKY rats, angiotensinogen mRNA levels have been found to be higher in newborn rats than they are in adult rats. Tissue-related and age-related differences in angiotensinogen mRNA levels in SHR and WKY rats have been reported [27].

In our study the overall similar patterns of responses, within a strain, of renin and angiotensinogen genes to ovariectomy and ovariectomy plus testosterone suggest that regulation of the genes is likely to be similar or coordinate and that the effects measured here are secondary, due to changes in an additional regulatory gene product. Both genes can be regulated at transcriptional and post-transcriptional (mRNA stability) levels [19,28]. Differences in structure of the renin gene among various rat strains have been described [29–31]. Several molecular variant alleles of the human angiotensinogen gene are known; in some populations the M235T polymorphism is associated with hypertension [32]. We have not yet analyzed structures of renin and angiotensinogen genes in our rat strains.

Differences between strains both in renin and in angiotensinogen mRNA levels could reflect differences in numbers or binding affinities of estrogen receptors. Differences resulting from different rates of development could also account for part of the strain differences we find. Body weight is a rough index of development, and we found differences between body weights of WKY rats

and SHR/y aged 10 weeks ($P < 0.05$), but not for those aged 16 weeks. Estrus stage of the intact rats was not determined in this study. We used large sample sizes of intact rats, in experiments performed over a period of 6–12 months, and tissues were collected on different days of the estrus cycle, in an effort to minimize any effects of estrus.

PRA values differed significantly between strains, but did not reflect renin mRNA levels, indicating the lack of correspondence between circulating enzymatically active renin and renal tissue mRNA levels. Extensive data support the hypothesis that renal RAS is regulated differently than the circulating RAS [2,33–36]. Also, existence of a prorenin–angiotensin system functioning within the kidney, separate from but coordinated with the circulating system, has been proposed by Sealey and Rubattu [37]. Negative feedback by elevated PRA on tissue RAS has been suggested to occur [38]. The elevated PRA we found in the rats in ovariectomy plus testosterone groups aged 10 weeks (Table 3) was coincident with lower than normal levels of renin mRNA in WKY rats, but not in SHR/y rats and not in rats aged 16 weeks (Fig. 1).

Administration of testosterone increased kidney weights of rats in both strains at both ages, reflecting anabolic effects of androgen on growth. However, variable effects of testosterone on body weight were found (Table 1). The combination of removing estrogen by ovariectomy early in development and supplementing the ovariectomized females with testosterone revealed differences in response of blood pressure between strains ($P < 0.0001$; Table 2). The blood pressure of SHR/y in the ovariectomy plus testosterone group aged 16 weeks, averaging over 200 mmHg, is extremely high for a female rat and probably reflects the combined influences of genotype and hormonal status.

One possible outcome of this study was that expression of renin and angiotensinogen genes would be determined by the genetic origin of the autosomes, because both renin and angiotensinogen genes have been mapped onto autosomes. If this were true, then expression of renin and angiotensinogen genes in rats of these two strains would be the same. However, renin and angiotensinogen mRNA levels differed between WKY rats and SHR/y, for intact females as well as for ovariectomized females and ovariectomized plus testosterone-supplemented females, reflecting the importance of other regulatory proteins such as transcription factors in controlling expression of these genes.

A mechanism that could account for the strain-specific expression of renin and angiotensinogen mRNA that we observed is genetic imprinting. An imprinted gene is one whose expression pattern depends on whether it was inherited from the male or female parent [39]. Male WKY rats have a WKY rat Y chromosome and male SHR/y have an SHR Y chromosome. Imprinting mechanisms in a male WKY rat and in a male SHR/y may also be different. Female SHR/y have an SHR/y father, whereas female WKY rats have a WKY rat father. A paternally inherited allele may be marked in one way if it was inherited through a WKY rat father and marked in a different way if it was inherited from a SHR/y father. Differences in the paternally imprinted gene could be expressed as two different phenotypes for the daughters of the WKY rat and SHR/y fathers. The difference between female SHR/y and female WKY rats is that the female SHR/y inherited one copy of each autosome and one X chromosome from her SHR/y father, whereas the female WKY rat inherited two copies of each autosome and both X chromosomes from WKY rat parents. As an example, let us assume that a single gene on an autosome or on the X chromosome is responsible for keeping blood pressure in WKY rats low and that this gene is inactivated when it passes through gametogenesis in the male SHR/y. The female SHR/y that inherits this allele from her SHR/y father would therefore have higher blood pressure than would a female WKY rat that did not inherit an inactivated allele because both copies of the gene were inherited from WKY rat parents. Because imprinting is sex-specific, it is not surprising that different X or Y chromosomes may affect imprinted genes differently. Further studies are required to establish that genetic imprinting is responsible for the differences we find between female SHR/y and female WKY rats.

In summary, we showed that steroid hormones regulate renin and angiotensinogen mRNA coordinately but differently in female WKY rats and SHR/y with essentially identical genetic backgrounds. The higher kidney weight and lower renal renin mRNA levels of intact female SHR/y aged 10 weeks suggest that their kidneys function differently from those of WKY rats. The different responses to estrogen and testosterone of WKY rats and SHR/y may be environmentally induced rather than genetically determined. Possible explanations for the differences in mRNA levels of renin and angiotensinogen genes in rats with the same genetic background include genomic imprinting. An alternate explanation is that a mutation in renin and angiotensinogen genes has occurred in one strain but not in the other strain, or that some factor that coregulates expression both of renin and of angiotensinogen mRNA had undergone a mutation in only one strain. The similar patterns of steady-state mRNA levels of renin and angiotensinogen in rats in all treatment groups for both strains are consistent with coordinate but different regulation of expression of these two genes in the kidney.

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