Gender-related differences in advanced glycation endproducts, oxidative stress markers and nitric oxide synthases in rats

X Wang[1], K Desai[1], BHJ Juurlink[2], J de Champlain[3] and L Wu[1]

[1]Department of Pharmacology, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada; [2]Department of Anatomy and Cell Biology, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada and [3]Department of Physiology, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada

An age- and blood pressure-associated increase in methylglyoxal (MG) and MG-induced advanced glycation endproducts (AGEs), including N^{ε}-carboxyethyl-lysine (CEL) and N^{ε}-carboxymethyl-lysine (CML), in the kidney of spontaneously hypertensive rats (SHR) has been shown. In the present study, gender-related changes in AGEs and nitric oxide synthase were investigated in Sprague–Dawley (SD) and stroke-prone SHR (SHRsp) rats. Immunohistochemical analyses were conducted on kidneys from 24-week-old male and female SD rats as well as SHRsp. The systolic blood pressure of SHRsp was significantly higher than that of SD rats. Male SD rats had more intense kidney staining for CEL than female SD rats. Both male and female SHRsp had more marked CEL and CML staining localized to kidney tubules, as opposed to SD rats. Female rats showed more staining in glomerular vessels than male rats in both SD and SHRsp. Nuclei containing nuclear factor-κB (NF-κB) p65 and activated macrophages were seen in the kidney from SHRsp, not so much in SD rats, localized to renal tubules in male and glomerular vessels in female SHRsp. A higher protein level of NF-κB p65 was found in SHRsp than in SD rats. SD rats had more intense kidney neuronal nitric oxide synthase staining than SHRsp. The intensity of inducible nitric oxide synthase staining was significantly higher in SHRsp than in SD rats, with no gender differences in either strain. SHRsp and male rats exhibited higher AGEs and oxidative stress than SD and female rats, respectively. These differences might partly account for the development of hypertension in SHRsp and the higher vulnerability of male animals to renal pathology.

Kidney International (2006) **69,** 281–287. doi:10.1038/sj.ki.5000043

KEYWORDS: gender; advanced glycation endproducts; hypertension; NOS

Correspondence: *L Wu, Department of Pharmacology, University of Saskatchewan, A120 Health Sciences Building, 107 Wiggins Road, Saskatoon, SK, Canada S7N 5E5. E-mail: liw070@duke.usask.ca*

Received 8 April 2005; revised 30 June 2005; accepted 11 August 2005

The kidney plays a major role in blood pressure homeostasis[1] and possibly the pathogenesis of hypertension in spontaneously hypertensive rats[2] (SHR) and other models of hypertension such as deoxycorticosterone acetate salt-sensitive hypertension.[3] A number of enzymes including the nitric oxide synthase (NOS) isoforms, cytochrome *P*450s, and various ion pumps are involved in the modulation of renin secretion and secretion/absorption of ions. An oxidative stress-induced alteration in the function of one or more of these enzymes/proteins can readily disturb the homeostatic mechanisms operating within the kidney and lead to increased blood pressure. Nitric oxide (NO), synthesized by NOS, is an important vasodilator[4] and renin secretion modulator[5] and can be considered as an antihypertensive mediator. The gender of the animal has been shown to have an important influence on the expression/activity of endothelial nitric oxide synthase in the blood vessels[6–8] and the kidney,[9] conferring protection to the female gender against development of hypertension, atherosclerosis, and cardiovascular mortality.[10,11] It has been shown in humans and animals that NO level is greater in females than in males because estrogens not only stimulate NO production[6,7] but also decrease inactivation of NO by oxygen radicals.[12] Post-menopausal females and males have reduced arterial NO activity, which was restored to premenopausal levels in females after 2 weeks of estrogen replacement therapy.[13]

The impact of gender on renal disease has also been observed in rats. Aging male rats show a reduction in renal NOS compared to females[14] and develop age-dependent kidney damage, proteinuria and glomerulosclerosis, whereas females and orchidectomized males are resistant to the development of renal injury.[15] Male rats have a greater tendency, an androgenic effect, than female rats, to develop proteinuria after chronic NOS inhibition.[16] Also, male stroke-prone spontaneously hypertensive rats (SHRsp) have more frequent renal lesions and more severe renal damage compared to female rats.[17] The incidence and prevalence of end-stage renal disease are higher in men than in women.[18]

The expression/activity of NOS is also altered in hypertensive animal models such as SHR. Earlier studies have shown increased reactive oxygen species, enhanced NOS expression, and NO production in SHR. These findings support the role of oxidative stress in the genesis and/or maintenance of hypertension and compensatory upregulation of the expression of endothelial nitric oxide synthase, inducible nitric oxide synthase (iNOS),[19] and neuronal nitric oxide synthase (nNOS)[20] in SHR.

There is evidence that oxidative stress is enhanced in males compared with females.[21–23] Plasma thiobarbituric acid-reactive substances and urinary 8-isoprostaglandin $F_2\alpha$ were higher in men than in women,[22] and male rats produced more superoxide anions than females.[23] We have recently shown an age- and blood pressure-associated increase in methylglyoxal (MG), a metabolite of glucose, and MG-induced irreversible advanced glycation endproducts (AGEs), N^{ε}-carboxyethyl-lysine (CEL), and N^{ε}-carboxymethyl-lysine (CML), in the plasma and the kidney of SHR.[24] However, gender differences with regard to these AGEs are not known.

There are no data as yet on gender-related changes in CEL and CML, which are precursors of oxidative stress.[25] Also, there is no study correlating gender-related changes in CEL, CML, and NOS with hypertension development. In the present study, gender-related changes in AGEs and NOS were investigated through an immunohistochemical characterization in Sprague–Dawley (SD) rats and SHRsp.

RESULTS

Basal parameters

The systolic blood pressure of SHRsp was significantly higher than that of age-matched SD rats (Table 1). There is no difference in blood pressure between male and female rats of the same strain. The body weight of males was more than that of females within each strain ($P<0.001$). In addition, SD rats weighed more than age-matched SHRsp of the same gender ($P<0.001$). The plasma glucose levels of male and female SHRsp were 5.4 ± 0.3 mM ($n=10$) and 5.1 ± 0.2 mM ($n=6$), respectively, which were comparable to those in age-matched SD rats: 4.9 ± 0.1 mM ($n=10$) of male and 5.8 ± 0.6 mM ($n=6$) of female.

Increased MG-induced CEL and CML in the kidney of SHRsp

Using the immunohistochemical staining method, we investigated whether MG-induced AGEs were increased in the kidney from SHRsp. Figure 1a shows a more intense or positive staining for CEL in the kidney from male and female SHRsp, compared to age-matched SD rats of the same gender ($n=3$ for each age group). The staining intensity in male SD rats was significantly higher than that in female SD rats (Figure 1b). There was no difference between male and female SHRsp. Both male and female SHRsp had more marked CML staining in the kidney as opposed to SD rats of the same gender (Figure 2). Most of the positive staining for CEL or CML was localized to renal tubules in both strains. Very little staining was observed in glomerular vessels of male rats, and female rats had more positive staining in glomerular vessels than male rats for both SD and SHRsp. The negative (no primary antibody) and positive (control immune globulin) control sections revealed no staining (not shown).

Table 1 | Systolic blood pressure and body weight of SHRsp and age-matched SD rats

Rats	Gender	BP (mmHg)	BW (g)
SD	Male	120 ± 3	583 ± 10
	Female	116 ± 2	$452\pm8^{\#\#}$
SHRsp	Male	$184\pm5^{***}$	$386\pm5^{***}$
	Female	$180\pm3^{***}$	$275\pm4^{***,\ \#\#}$

Values are mean$\pm$s.e.m. BP, systolic blood pressure; BW, body weight.
$^{***}P<0.0001$, SHRsp *vs.* SD rats of the same gender, $^{\#\#}P<0.001$, Female *vs.* male of the same strain. (n=6 each group).

ED-1 and nuclear factor-κB

In SHRsp, activated macrophages could be identified in the kidney, indicating an inflammatory state in its structure. Positive staining of ED-1 was only detected in the renal tubules of male SHRsp. As to female SHRsp, ED-1 staining was only observed in glomerular vessels (Figure 3a).

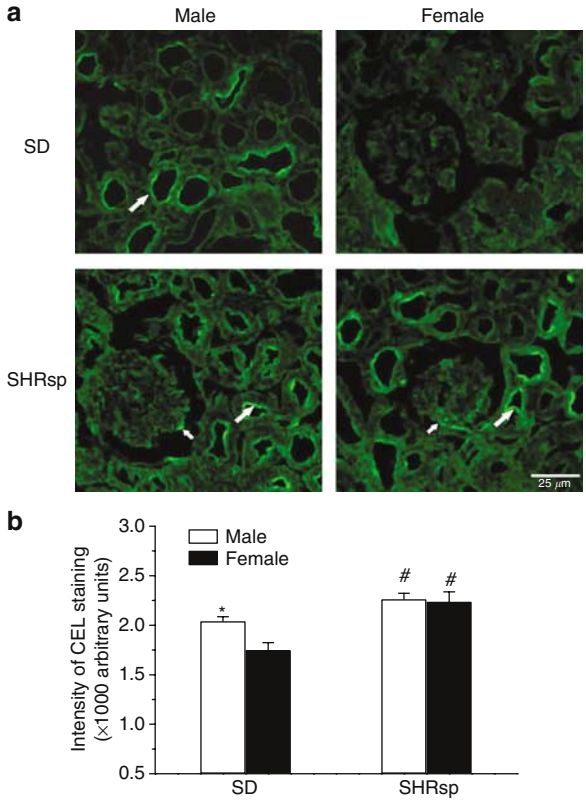

Figure 1 | Immunohistochemical detection of CEL in the kidney from SHRsp and age-matched SD rats. (**a**) CEL staining was detected in kidney sections from male (left panel) and female (right panel) SHRsp and SD rats. Large arrows indicate the representative immunoreactivity in renal tubules, and small arrows show staining in the glomerulus. (**b**) Intensity of CEL staining ($n=3$ in each group). $^{*}P<0.05$, male compared to female rats of the same strain. $^{\#}P<0.05$, SHRsp compared to SD rats of the same gender.

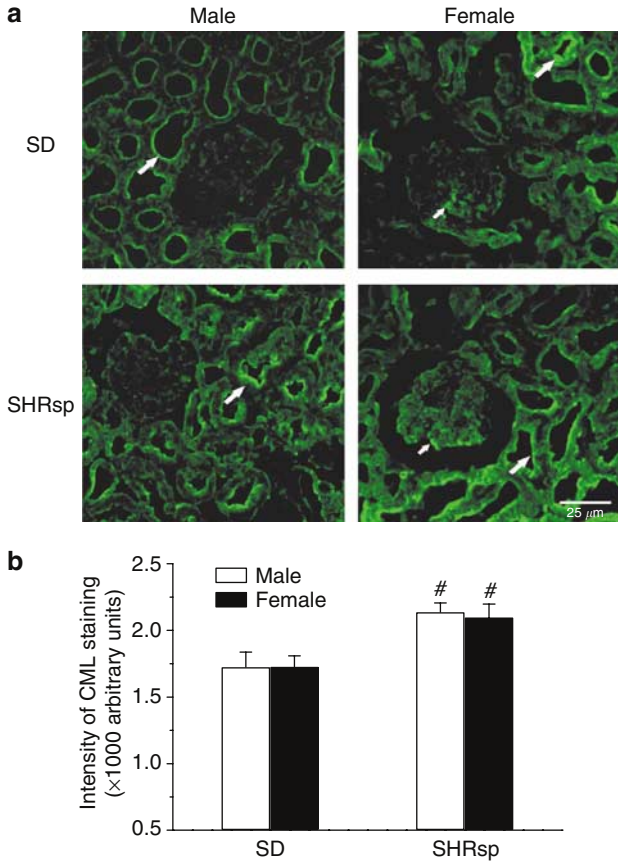

Figure 2 | Immunohistochemical detection of CML in the kidney from SHRsp and age-matched SD rats. (**a**) CML staining was detected in kidney sections from male (left panel) and female (right panel) SHRsp and SD rats. Large arrows indicate the representative immunoreactivity in renal tubules, and small arrows show staining in the glomerulus. (**b**) Intensity of CML staining ($n = 3$ in each group). $^{\#}P < 0.05$, SHRsp compared to SD rats of the same gender.

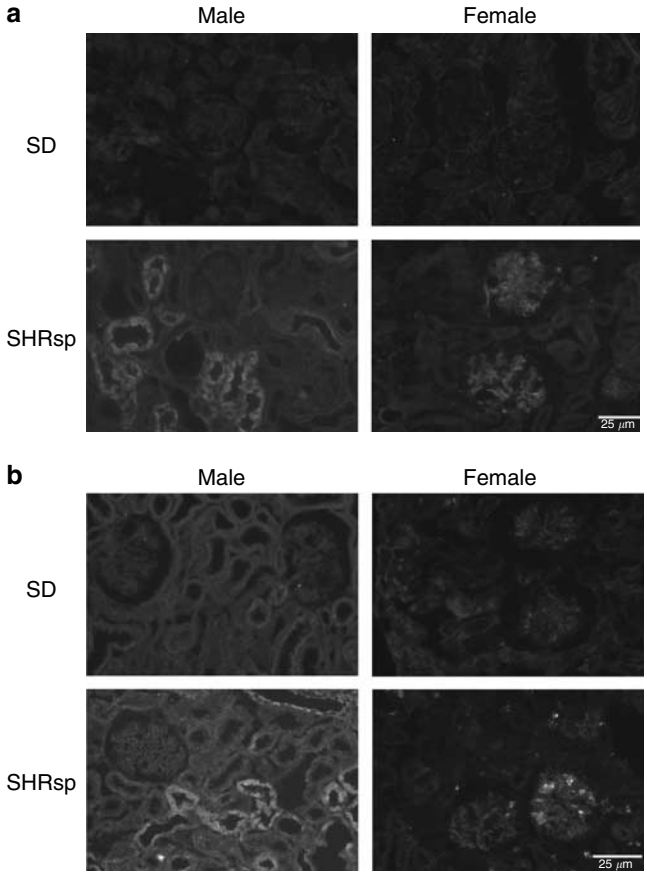

Figure 3 | Immunohistochemical detection of (a) ED-1 and (b) NF-κB in the kidney from SHRsp and age-matched SD rats. Positive staining of ED-1 was only detected in the renal tubules of male SHRsp. In female SHRsp, the staining was only observed in glomerular vessels (**a**). Micrographs show that a positive nuclear NF-κB p65 signal was observed in male and female SHRsp, and very little staining was detected in the kidney from SD rats (**b**) ($n = 3$ for each group).

Activated macrophages were not detectable in the kidney from SD rats. Immunohistochemical staining revealed nuclei containing nuclear factor-κB (NF-κB) p65 in the kidney from SHRsp, and very little staining was detected in the kidney from SD rats (Figure 3b). Positive staining was localized in the renal tubules of male SHRsp, whereas in the female SHRsp it was localized to the glomerular vessels.

Western blot analysis demonstrated that nuclearly localized NF-κB p65 was significantly higher in male SD rats and SHRsp than female rats of the same gender (Figure 4).

nNOS immunoreactivity

SD rats had more intense kidney nNOS staining than age-matched SHRsp ($n = 3$ for each age group) (Figure 5). The intensity of nNOS staining was significantly higher in female rats than in age-matched male rats of the same strain for both SD rats and SHRsp. Positive staining was localized to the glomerular vessels in female SD rats and SHRsp, whereas in male rats of both strains the staining was only observed in renal tubules. Negative and positive control sections revealed no staining (data not shown).

iNOS immunoreactivity

Both male and female SHRsp had more iNOS staining than age-matched SD rats of the same gender, but there was no gender difference within either strain (Figure 6). Most of the positive staining was localized to renal tubules in SHRsp. Very little staining was observed in glomerular vessels.

DISCUSSION

Here, we show gender differences in markers of oxidative stress and enzymes involved in blood flow regulation and renin secretion in the kidneys of male and female SD rats and SHRsp. These differences possibly contribute to the reported greater protection of the female gender against development of hypertension, atherosclerosis, renal damage, and disease and cardiovascular mortality.[10,11,15–18]

SHRsp showed marked staining for AGEs, CEL, and CML, in the kidney, despite normal blood glucose levels. AGEs are precursors of oxidative stress[25] and our findings support elevated oxidative stress levels in SHRsp and SHR.[24] More importantly, male SD rats had an elevated level of CEL

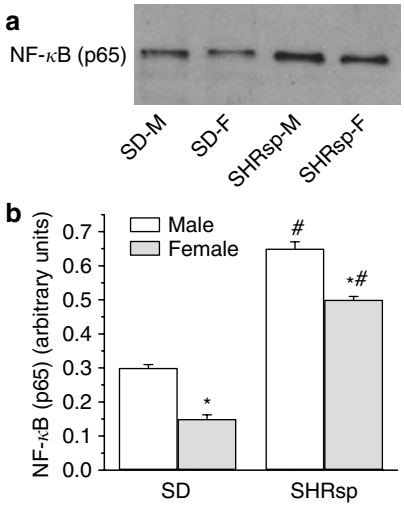

Figure 4 | Western blot showing the localization of activated NF-κB in the kidney from SHRsp and age-matched SD rats. (**a**) A representative Western blot of nuclearly localized NF-κB p65. (**b**) Quantification of NF-κB p65 relative to β-actin (the *y*-axis depicts the relative units). Data were taken from kidney tissues of 3–4 animals. $^{*}P<0.05$, male compared to female rats of the same strain. $^{\#}P<0.05$, SHRsp compared to SD rats of the same gender.

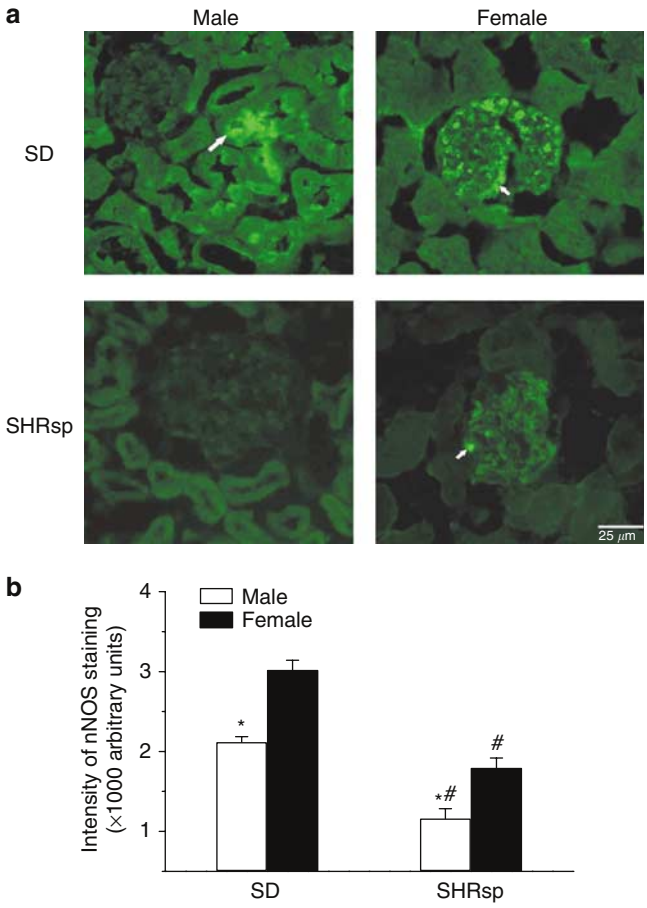

Figure 5 | Immunoreactivity of nNOS in renal tissues from male and female SHRsp and age-matched SD rats. (**a**) Immunohistochemical staining of nNOS. Female rats (right panel) show more positive nNOS staining than age-matched male rats of the same strain (left panel). Large arrows indicate representative immunoreactivity in renal tubules, and small arrows show staining in the glomerulus ($n=3$ for each group). (**b**) Intensity of nNOS staining. $^{*}P<0.05$, male compared to female rats of the same strain. $^{\#}P<0.05$, SHRsp compared to SD rats of the same gender.

compared to age-matched females. This gender difference in CEL formation has not been reported before. This also supports findings of elevated superoxide anion levels and oxidative stress in male Wistar rats,[23] and gender differences in terms of oxidative stress levels in humans, using plasma thiobarbituric acid-reactive substances and urinary 8-iso-prostaglandin $F_2\alpha$ as markers.[21,22] An elevated oxidative stress has been associated with hypertension[19,26,27] and can readily disturb the homeostatic mechanisms in the kidney, which contribute to blood pressure regulation.[1] In our study, antibodies against CEL and CML were used. The antibody against MG-induced CML is less selective as this antibody can also react with glyoxal (another dicarbonyl compound)-induced AGE.[28] In contrast, anti-CEL antibody is specifically against MG-induced CEL and has been suggested to be a good indicator of MG-induced AGE or glycoxidation product.[29] Gender difference in CEL and CML staining was lost in SHRsp with an associated severe hypertension in both male and female SHRsp (Table 1, Figures 1 and 2).

NF-κB p65, a marker of inflammation, was detected in SHRsp but not in SD rats. However, Western blot analysis demonstrated gender differences in nuclearly localized NF-κB p65, even though they were significantly lesser in SD than in SHRsp. Thus, NF-κB p65 was significantly higher in male SD rats and SHRsp than female rats of the same strain (Figure 3b), and may be one of the reasons for greater susceptibility of the male gender to cardiovascular and kidney disease, including male SHRsp.[17] Moreover, activated macrophages were detected in the kidney of SHRsp but not in SD rats. These findings are consistent with the possibility that activation of NF-κB p65 and renal interstitial infiltration of immune cells play a role in the pathophysiology of hypertension.[30] We have previously shown that oxidative stress is likely involved in MG-induced activation of NF-κB in vascular smooth muscle cells of SHR.[26] However, gender differences in this regard were not known. An association of inflammation and hypertension has been reported by elevated C-reactive protein, another marker of inflammation.[31] Lack of NF-κB p65 staining and hypertension in the SD rats supports this association. Gender differences in terms of the localization of NF-κB p65 and ED-1, a marker of activated macrophages, in the renal tubules of male SHRsp and in the glomerular vessels of female SHRsp, are notable and can be speculated to indicate a more progressed inflammation in the males, having originated from the blood vessels.

iNOS is normally associated with inflammatory states[4] and this is supported by our observation of more intense iNOS staining in SHRsp compared to SD rats. Increased expression of iNOS in the kidney of SHR has been reported.[32] Also, AGEs induce iNOS in glomerular mesangial cells.[33] An association between iNOS and hypertension is strongly supported by our results.

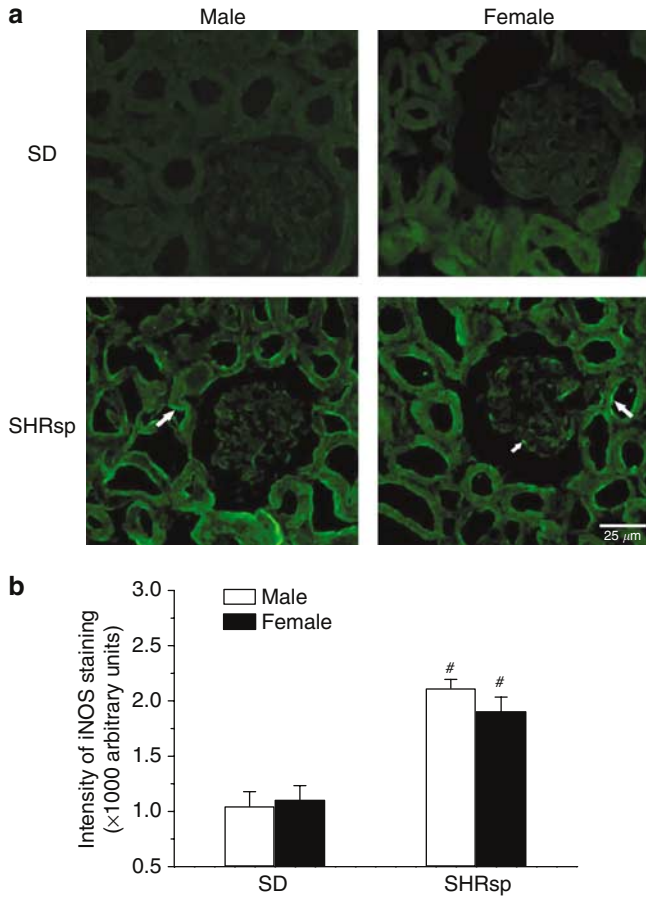

Figure 6 | Immunohistochemical (a) detection and (b) intensity of iNOS staining in renal tissues from male and female SHRsp and age-matched SD rats. Large arrows indicate the representative immunoreactivity in renal tubules, and small arrows show staining in the glomerulus. ($n = 3$ for each group). *$P < 0.05$, male compared to female rats of the same strain. #$P < 0.05$, SHRsp compared to SD rats of the same gender.

The level of nNOS was higher in SD rats compared to SHRsp. Increased[34] and decreased[35] expression of nNOS in the kidney of SHR, compared to Wistar rats, has been reported. Whereas the role of nNOS in renin secretion is controversial, a number of studies have implicated NO, derived from nNOS, stimulating renin secretion, especially after chronic sodium restriction.[5,36,37] The staining intensity of nNOS was greater in female rats compared to male rats for both SD and SHRsp. Moreover, SHRsp had lower levels of nNOS compared to SD rats for both males and females, which supports a previous report of reduced nNOS in the SHR.[35] This can be speculated as a feedback inhibition of nNOS expression due to high blood pressure in SHRsp. In the study by Kumar *et al.*[35] gender differences were not reported. The reason for a gender difference in nNOS within a strain is not clear at present. Another important difference between male and female rats, noted in our study, was localization of nNOS staining. Most of the staining in male rats was in the tubules whereas in female rats it was in the glomerular vessels. Whereas tubular expression of nNOS has been reported,[20,34,35] localization of nNOS to the glomerular vessels in female rats was unexpected, and is intriguing. The functional significance of this difference needs to be determined by separate studies. Gender differences in expression of endothelial nitric oxide synthase in the kidney and the vasculature are well established, and increased vascular NO production has been proposed as affording more protection to females against cardiovascular morbidity and mortality.[6–11]

Although differences in the NOSs have been described above, it might be worth pointing out that the age of rats used in our study, 24 weeks, is within the reproductive age and an influence of menopause, reportedly occurring after 18 months or about 72 weeks of age, can be ruled out.[38]

In conclusion, increased expression of CEL, NF-κB p65, ED-1, and iNOS in male rats compared to female rats, or in SHRsp compared to SD rats, strongly supports the contention that female gender has a better biochemical protection against cardiovascular and renal morbidity and mortality.

MATERIALS AND METHODS

Animals

Male and female SD rats and SHRsp (24 weeks old) were used to carry out this work. SD rats were used to fulfill our primary aim to investigate gender differences within a given strain with a normal blood pressure profile that was not genetically related to SHRsp. SHRsp were used instead of SHR because the SHRsp strain develops a severe form of hypertension and shows a high incidence of injuries in different vascular beds, especially in the renal vasculature and parenchyma. Such lesions are milder or even absent in the closely related SHR. Moreover, male SHRsp have more frequent lesions and more severe renal damage compared to female rats.[17] These animals were housed in a temperature-regulated animal facility and exposed to a 12 h light/dark cycle, with free access to food and water. Rats were treated in accordance with the guidelines of the Canadian Council on Animal Care, and the experimental protocols were approved by the Animal Care Committee at the University of Saskatchewan. Systolic blood pressure was determined weekly using a standard tail cuff noninvasive BP measurement system (Model 29-SSP, Harvard Apparatus, St Laurent, QC, Canada).

Tissue preparation

At the end of the study, rats were anesthetized with sodium pentobarbital (60 mg/kg body weight) intraperitoneally. Rats were perfused with heparinized phosphate-buffered saline, followed by phosphate-buffered saline containing 4% paraformaldehyde. Kidney tissues were dissected out immediately and kept in the same fixative solution overnight. Samples were then incubated in a 30% sucrose solution for 3 days at 4°C for cryoprotection. After embedding in 22-oxacalcitriol compound (Somagen Diagnostics, Edmonton, AB, Canada), 8-μm thick cross-sections were cut using a cryostat, collected on gelatin-chrome alum-coated slides and stored at −20°C until further processing.

Immunohistochemistry

CEL and CML staining. Immunostaining was performed using the method described in our previous study.[39] In brief, frozen sections were permeabilized with 0.1% Triton X-100 in phosphate-buffered saline for 5 min after three washes in 0.1 M phosphate-buffered

saline. Nonspecific antibody binding was blocked by incubation for 30 min with normal goat serum diluted at 1:30. After overnight incubation with either monoclonal anti-CML antibody or anti-CEL antibody (Novo Nordisk, A/S, Denmark) diluted at 1:100, the slides were washed 3 times and incubated with anti-mouse IgG-fluorescein isothiocyanate (Sigma, Oakville, ON, Canada) diluted at 1:200 for 1 h at room temperature, followed by another three washes. The slides were mounted in phosphate-buffered saline–glycerin (7:3 v/v), coverslipped and examined under a fluorescence microscope with the appropriate filters.

ED-1 and NF-κB. Frozen sections were immunostained by using primary antibody ED-1 (Serotec, Raleigh, NC), a marker for activated macrophages, or anti-NF-κB p65 (Transduction Laboratories, Lexington, KY, USA) that recognized the nuclear localization signal on p65. ED-1 staining was detected by Cy3-conjugated affiniPure goat antimouse IgG (Jackson ImmunoResearch, WestGrove, PA, USA), and NF-κB staining was demonstrated by the avidin–biotin–peroxidase (Vector Laboratories, Burlington, ON, Canada) detection system.

NOS staining. Kidney tissue sections (8 μm) were incubated with either monoclonal nNOS antibody or polyclonal iNOS antibody (Calbiochem, San Diego, CA, USA) diluted at 1:200. Anti-rabbit or anti-mouse IgG-FITC diluted at 1:200 was the secondary antibody.

Quantification of staining. The intensities of staining were quantified using GeneTools image analysis software (PerkinElmer®, MA). For each animal, 30 spots of each micrograph, with 10 micrographs of each section and three sections of each animal, were analyzed and the average value was used to express the intensity of staining.

Western blot analysis. Proteins were separated on polyacrylamide gels by using a minivertical electrophoresis system (Bio-Rad, Hercules, CA, USA) and transblotted onto polyvinyldifluoridene membranes, and membranes were then incubated with the appropriate primary antibodies, followed by incubation with a horseradish peroxidase-conjugated secondary antibody (Bio-Rad) as done before.[40] Proteins were visualized by using the chemiluminescence substrate kit (Amersham Biosciences), and quantified by using the UN-SCAN-IT GEL automated digitizing system (version 5.1, Silk Scientific, Orem, UT, USA). The membrane was reprobed with anti-β-actin (Sigma). Quantification was relative to the β-actin. For quantification of nuclearly localized NF-κB p65, nuclear extracts were obtained by using the NE-PER nuclear and cytoplasmic extraction kit (Pierce, Rockford, IL, USA) as described.[26]

Data analysis. Data are expressed as mean ± s.e.m., and analyzed using Student's *t*-test or one-way analysis of variance in conjunction with the Newman–Keul test where applicable. Significant difference between treatments was defined at a level of $P < 0.05$.

ACKNOWLEDGMENTS

This work was supported by an operating grant from the Canadian Institutes of Health Research (CIHR, MOP-68938) to L Wu. L Wu is supported by a New Investigator Award from CIHR. X Wang is supported by a Doctoral Research Award from Heart and Stroke Foundation of Saskatchewan.

REFERENCES

1. Cowley Jr AW, Roman RJ. The role of kidney in hypertension. *JAMA* 1996; **275**: 1581–1589.
2. Roman RJ. Altered pressure-natriuresis relationship in young spontaneously hypertensive rats. *Hypertension* 1987; **9**(Suppl III): III130–III136.
3. Hall JE, Granger JP, Smith Jr MJ, Premen AJ. Role of renal hemodynamics and arterial pressure in aldosterone 'escape'. *Hypertension* 1984; **6**(Suppl I): I183–I192.
4. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991; **43**: 109–142.
5. Sayago CM, Beierwaltes WH. Nitric oxide synthase and cGMP-mediated stimulation of renin secretion. *Am J Physiol* 2001; **281**: R1146–R1151.
6. Hayashi T, Fukuto JM, Ignarro LJ, Chaudhuri G. Basal release of nitric oxide from aortic rings is greater in female rabbits than in male rabbits: implications for atherosclerosis. *Proc Natl Acad Sci USA* 1992; **89**: 11259–11263.
7. Kauser K, Rubanyi GM. Gender difference in bioassayable endothelium-derived nitric oxide from isolated rat aortae. *Am J Physiol Heart Circ Physiol* 1994; **267**: H2311–H2317.
8. Ellison KE, Ingelfinger JR, Pivor M, Dzau VJ. Androgen regulation of rat renal angiotensinogen messenger RNA expression. *J Clin Invest* 1989; **83**: 1941–1945.
9. Neugarten J, Ding Q, Friedman A *et al.* Sex hormones and renal nitric oxide synthases. *J Am Soc Nephrol* 1997; **8**: 1240–1246.
10. Castelli WP. Cardiovascular disease in women. *Am J Obstet Gynecol* 1988; **158**: 1553–1560.
11. Celermajer DS, Sorensen KE, Spiegelhalter DJ *et al.* Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol* 1994; **24**: 471–476.
12. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 1999; **340**: 1801–1811.
13. Majmudar NG, Robson SC, Ford GA. Effects of the menopause, gender, and estrogen replacement therapy on vascular nitric oxide activity. *J Clin Endocrinol Metab* 2000; **85**: 1577–1583.
14. Erdely A, Greenfeld Z, Wagner L, Baylis C. Sexual dimorphism in the aging kidney: effects on injury and nitric oxide system. *Kidney Int* 2003; **63**: 1021–1026.
15. Baylis C. Age-dependent glomerular damage in the rat. Dissociation between glomerular injury and both glomerular hypertension and hypertrophy. Male gender as a primary risk factor. *J Clin Invest* 1994; **94**: 1823–1829.
16. Verhagen AM, Attia DM, Koomans HA, Joles JA. Male gender increases sensitivity to proteinuria induced by mild NOS inhibition in rats: role of sex hormones. *Am J Physiol Renal Physiol* 2000; **279**: F664–F670.
17. Gigante B, Rubattu S, Stanzione R *et al.* Contribution of genetic factors to renal lesions in the stroke-prone spontaneously hypertensive rat. *Hypertension* 2003; **42**: 702–706.
18. United States Renal Data System. *USRDS 1997 Annual Data Report*. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 1997.
19. Vaziri ND, Ni Z, Oveisi F, Trnavsky-Hobbs DL. Effect of antioxidant therapy on blood pressure and NO synthase expression in hypertensive rats. *Hypertension* 2000; **36**: 957–964.
20. Fernandez AP, Serrano J, Castro S *et al.* Distribution of nitric oxide synthases and nitrotyrosine in the kidney of spontaneously hypertensive rats. *J Hypertens* 2003; **21**: 2375–2388.
21. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease: the Framingham Study. *Ann Intern Med* 1976; **85**: 447–452.
22. Ide T, Tsutsui H, Ohashi N *et al.* Greater oxidative stress in healthy young men compared with premenopausal women. *Arterioscler Thromb Vasc Biol* 2002; **22**: 438–442.
23. Brandes RP, Mugge A. Sex differences in the generation of superoxide anions in the rat aorta. *Life Sci* 1997; **60**: 391–396.
24. Wang X, Desai K, Clausen JT, Wu L. Increased methylglyoxal, and advanced glycation endproducts in kidney from spontaneously hypertensive rats. *Kidney Int* 2004; **66**: 2315–2321.
25. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. *Diabetologia* 2001; **44**: 129–146.
26. Wu L, Juurlink BH. Increased methylglyoxal and oxidative stress in hypertensive rat vascular smooth muscle cells. *Hypertension* 2002; **39**: 809–814.
27. de Champlain J, Wu R, Girouard H *et al.* Oxidative stress in hypertension. *Clin Exp Hypertens* 2004; **26**: 593–601.
28. Degenhardt TP, Thorpe SR, Baynes JW. Chemical modification of proteins by methylglyoxal. *Cell Mol Biol* 1998; **44**: 1139–1145.
29. Nagai R, Araki T, Hayashi CM *et al.* Identification of N epsilon-(carboxyethyl)lysine, one of the methylglyoxal-derived AGE structures, in glucose-modified protein: mechanism for protein modification by reactive aldehydes. *J Chromatogr* 2003; **788**: 75–84.

30. Rodriguez-Iturbe B, Quiroz Y, Ferrebuz A *et al.* Evolution of renal interstitial inflammation and NF-kappaB activation in spontaneously hypertensive rats. *Am J Nephrol* 2004; **24**: 587–594.
31. Sesso HD, Buring JE, Rifai N *et al.* C-reactive protein and the risk of developing hypertension. *JAMA* 2003; **290**: 2945–2951.
32. Kumar U, Chen J, Sapoznikhov V *et al.* Overexpression of inducible nitric oxide synthase in the kidney of the spontaneously hypertensive rat. *Clin Exp Hypertens* 2005; **27**: 17–31.
33. Chang PC, Chen TH, Chang CJ *et al.* Advanced glycosylation end products induce inducible nitric oxide synthase (iNOS) expression via a p38 MAPK-dependent pathway. *Kidney Int* 2004; **65**: 1664–1675.
34. Welch WJ, Tojo A, Lee JU *et al.* Nitric oxide synthase in the JGA of the SHR: expression and role in tubuloglomerular feedback. *Am J Physiol* 1999; **277**: F130–F138.
35. Kumar U, Shin Y, Wersinger C *et al.* Diminished expression of constitutive nitric oxide synthases in the kidney of spontaneously hypertensive rat. *Clin Exp Hypertens* 2003; **25**: 271–282.
36. He XR, Greenberg SG, Briggs JP, Schnermann JB. Effect of nitric oxide on renin secretion. II. Studies in the perfused juxtaglomerular apparatus. *Am J Physiol* 1995; **268**(Part 2): F953–F959.
37. Singh I, Grams M, Wang WH *et al.* Coordinate regulation of renal expression of nitric oxide synthase, renin, and angiotensinogen mRNA by dietary salt. *Am J Physiol* 1996; **270**(Part 2): F1027–F1037.
38. Gore AC, Yeung G, Morrison JH, Oung T. Neuroendocrine aging in the female rat: the changing relationship of hypothalamic gonadotropin-releasing hormone neurons and *N*-methyl-D-aspartate receptors. *Endocrinology* 2000; **141**: 4757–4767.
39. Ndisang JF, Wu L, Zhao WM, Wang R. Induction of heme oxygenase-1 and stimulation of cGMP production by hemin in aortic tissues from hypertensive rats. *Blood* 2003; **101**: 3893–3900.
40. Wu L, Juurlink BH. The impaired glutathione system and its up-regulation by sulforaphane in vascular smooth muscle cells from spontaneously hypertensive rats. *J Hypertens* 2001; **19**: 1819–1825.