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Gender difference in the role of endothelium-derived relaxing factors modulating renal vascular reactivity

Rosemary Wangensteen[a], Juan Manuel Moreno[b], Juan Sainz[a], Isabel Rodríguez-Gómez[a], Virginia Chamorro[a], Juan de Dios Luna[c], Antonio Osuna[b], Félix Vargas[a,*]

[a] Departamento de Fisiología, Facultad de Medicina, Servicio de Nefrología, Unidad Experimental, E-18012 Granada, Spain
[b] Hospital Virgen de las Nieves, Granada, Spain
[c] Departamento de Bioestadística, Facultad de Medicina, Servicio de Nefrología, Unidad Experimental, Granada, Spain

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Abstract

This study analyzed the role of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) in gender differences in the renal vascular reactivity of rats. Renal responses to vasoconstrictors and vasodilators were studied in isolated kidneys from male and female rats under basal conditions, after NO and EDHF blockade or after endothelium removal. Female rat kidneys had reduced responsiveness to vasoconstrictors. The blockade of NO or of EDHF did not completely abolish the differences, but the simultaneous blockade of both factors or endothelium removal abolished gender differences. Male and female kidneys showed a similar responsiveness to endothelium-dependent and -independent vasodilators under basal conditions and after NO or EDHF blockade. In conclusion: (a) the attenuated response to vasoconstrictors in female kidneys is related to an increased production of NO and EDHF; and (b), the contributions of NO and EDHF to endothelium-dependent vasodilation are similar in the male and female renal vasculature.

Keywords: EDHF (endothelium-derived hyperpolarizing factor); Gender; NO (Nitric oxide); Vasoconstriction; Vasodilatation

1. Introduction

Gender has been associated with differences in blood pressure (Chen, 1996; Reckelhoff, 2001), vascular reactivity (Altura, 1972; Huang et al., 1997; Kauser and Rubanyi, 1994; Li and Duckles, 1994; McCulloch and Randall, 1998; Sanchez et al., 1996), and renal hemodynamics (Munger and Baylis, 1988) in rats.

Endothelial cells play a fundamental role in regulating vascular resistance through their ability to produce endothelium-derived vasodilators (Furchgott and Vanhoutte, 1989). Nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) are the main mediators of endothelium-dependent vasodilatation in the whole renal vasculature contributing to a similar extent (Vargas et al., 1994). EDHF is known to hyperpolarize and relax vascular smooth muscle via an opening of K^+ channels (Brayden et al., 1991; Vanhoutte, 1993). Although its identity remains elusive, this factor has recently attracted considerable attention (Campbell and Gauthier, 2002; Garland et al., 1995; Hecker, 2000). NO synthesis is inhibited by N^{ω}-nitro-L-arginine methyl ester (L-NAME) (Ishii et al., 1990) and tetraethylammonium (TEA), and high potassium concentrations have been used to inhibit EDHF activity (Adeagbo and Triggle, 1993; Campbell and Gauthier, 2002; Ercan et al., 1990; Rubanyi et al., 1990; Vargas et al., 1994). Endothelium-derived relaxing factors also play an important role in modulating the responsiveness to vasoconstrictors (Rubanyi et al., 1990). In this respect, endothelial denudation augmented the responsiveness to vasoconstrictors in isolated kidney (Ercan et al., 1990; Vargas and Osuna, 1996), and increased responsiveness was also produced by the inhibition of NO and EDHF (Moreno et al., 2003; Vargas and Osuna, 1996).

Estrogen increases the endothelial production of NO (Hayashi et al., 1995; Weiner et al., 1994) and it also modulates EDHF (Campbell and Gauthier, 2002; McCulloch and Randall, 1998), suggesting that estrogens have a

* Corresponding author. Tel.: +34-958-243520; fax: +34-958-246179.
E-mail address: fvargas@ugr.es (F. Vargas).

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provasodilator effect. On the other hand, testosterone increased vascular reactivity to noradrenaline (Bhargava et al., 1967). However, reports on responsiveness to vasoconstrictors (Altura, 1972; Collis and Vanhoutte, 1977; Li and Duckles, 1994; Sanchez et al., 1996; Stallone et al., 1991) and endothelium-dependent vasodilators (Li and Duckles, 1994; McCulloch and Randall, 1998; Sanchez et al., 1996; White et al., 2000) in male and female rats are contradictory. Studies on the contribution of NO and EDHF to endothelium-dependent vasodilation in male and female rat preparations also showed disparate results (Golding and Kepler, 2001; Liu et al., 2001; McCulloch and Randall, 1998; Sanchez et al., 1996; White et al., 2000). These conflicting findings raise doubts about the participation of endothelial mediators in sexually dimorphic responsiveness to vasoactive agents.

The aims of this study were: (a) to analyze the differences in renal vascular reactivity to vasoconstrictors and to endothelium-dependent and -independent vasodilators in isolated kidneys from male and female rats; (b) to determine whether the contribution of the endothelium-derived relaxing factors, NO and of EDHF, to the responsiveness to the vasoactive agents differs between the kidneys from male and female rats.

2. Material and methods

2.1. Animals

Seventy male and female age-matched Wistar rats (18–20 weeks old) born and raised in the experimental animal service of the University of Granada were used in the present study. The rats were randomly assigned to the different experimental groups ($n=7$ in each group). The experiments were performed according to the European Union guidelines for the ethical care and use of laboratory animals.

2.2. Experimental protocols

The animals were anesthetized with pentobarbital sodium (40 mg/kg, i.p.). The kidney was removed from the animal and perfused at a constant flow rate (5 ml/g of kidney weight/min) with Tyrode solution at 37 °C as previously reported (Vargas et al., 1994, 1996). The kidney was placed in a chamber, maintained at 37 °C, containing the perfusate lost from the renal vein that was not recirculated. Immediately after the kidney removal, the rats were killed with an overdose of pentobarbital sodium. Renal responses were recorded as changes in renal perfusion pressure in a pressure

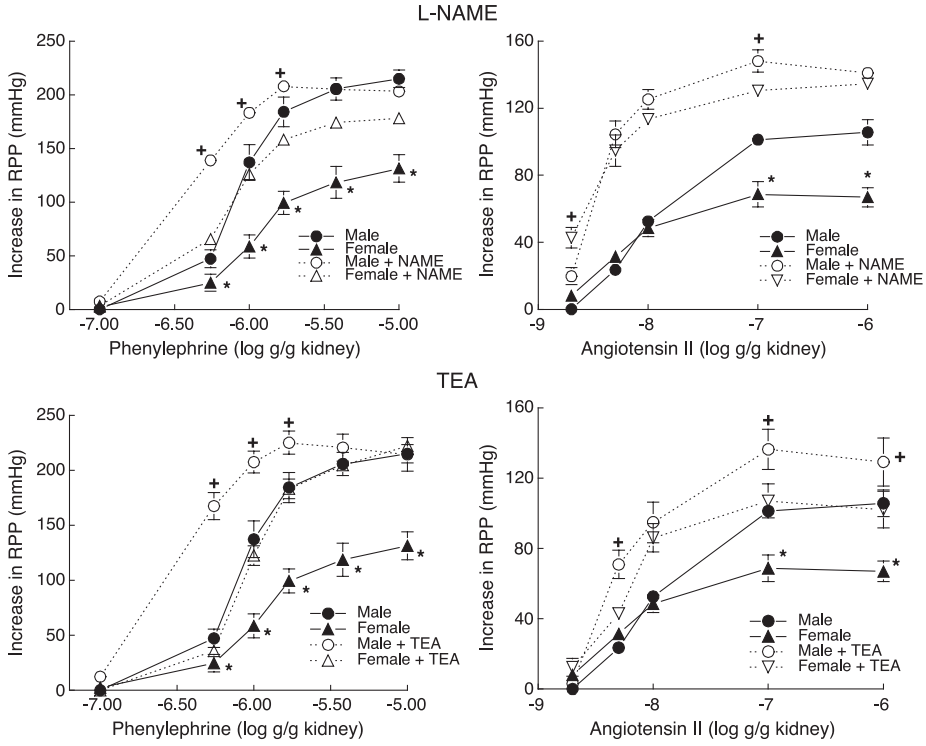

Fig. 1. Dose–response curves to phenylephrine and angiotensin II in isolated kidneys from male and female rats ($n=7$ each group) under normal conditions and after the administration of L-NAME (10^{-4} M) or TEA (3×10^{-3} M). Data are means ± S.E.M. *$P<0.01$ compared with the untreated male group; +$P<0.01$ compared with the female-treated group. For clarity, the significance of comparisons between treated and untreated preparations from the same group has been omitted.

transducer connected to the perfusion circuit downstream of the perfusion pump.

2.2.1. Experiment 1

This experiment was designed to explore the effects of NO or EDHF blockade, by the administration of L-NAME or TEA, respectively, on the renal response to vasoconstrictors in kidneys from male and female rats. Dose–response curves were made to phenylephrine and angiotensin II, in that order. These dose–response curves were performed under basal conditions or after the infusion of L-NAME (10^{-4} M), TEA (3×10^{-3} M) or L-NAME + TEA in separate preparations. The endothelial blockers were infused during the 30 min of stabilization and during the performance of dose–response curves.

2.2.2. Experiment 2

This experiment was designed to determine the influence of endothelium on gender-related differences in renal responsiveness to vasoconstrictors. Dose–response curves to phenylephrine and angiotensin II were studied in preparations with the endothelium removed. The endothelium was removed by passing air through the isolated kidney for 4.5 min. Endothelium removal was assessed by measuring the vasodilator response to a bolus dose of 10^{-6} g/g kidney acetylcholine in the vascular bed pre-constricted with phenylephrine (10^{-6} M). Preparations with a vasodilator response to acetylcholine greater than 10% were rejected.

2.2.3. Experiment 3

In this experiment, we tested whether the alterations in vascular reactivity are restricted to receptor-mediated specific agonists or they involve the nonspecific smooth muscle stimulants. To do it, we analyzed the pressor responsiveness to increasing concentrations of KCl (10–80 mM). Moreover, we also studied the effects of endothelium removal to the concentration–response curve of KCl in separate preparations. To obtain solutions containing 10, 25, 40 and 80 mM KCl, equimolar concentrations of NaCl were replaced by KCl in the Tyrode solution, to avoid increases in osmolality. The endothelium was removed as in experiment 2.

2.2.4. Experiment 4

This experiment was designed to assess the responsiveness to the endothelium-dependent vasodilator, acetylcholine, and to assess renal sensitivity to NO in the vascular bed from male and female rats. Dose–response curves were performed to the endothelium-independent vasodilator (NO donor) (Ignarro et al., 1981), nitroprusside, and to the endothelium- and NO-independent vasodilator, papaverine. Effects of NO and EDHF blockade on the response to vasodilators were also analyzed in male and female kidneys. In phenylephrine-preconstricted kidneys, acetylcholine-induced renal vasodilation is produced by NO and EDHF, whereas in 80 mM K^+-preconstricted kidneys, EDHF is blocked and acetylcholine-induced renal vasodilation is solely mediated by NO (Vargas et al., 1994). In this experiment, NO and EDHF were blocked by the administration of L-NAME (10^{-4} M) and of an increased extracellular K^+ (80 mM), respectively. The dose–response curves to the vasodilators were performed in perfused kidneys preconstricted with phenylephrine (10^{-6} M), phenylephrine plus L-NAME (10^{-4} M), or KCl (80 mM). To obtain the solution containing 80 mM of potassium, equimolar concentrations of NaCl were replaced by KCl in the Tyrode solution. Because the preparations from female rats showed a reduced responsiveness to phenylephrine, it was necessary to adjust the concentration of the vasoconstrictor to achieve a similar level of preconstriction in male and female preparations (112 ± 2 mm Hg). As the dose of phenylephrine was adjusted in each female preparation, the approximate dose given in the group was 5×10^{-6} M. The changes in renal perfusion pressure in response to vasodilators were expressed as percentages of the vasoconstriction obtained with phenylephrine or KCl.

2.3. Drugs

The following drugs were used: pentobarbital sodium (Nembutal) purchased from Serva (Heidelberg, Germany), sodium nitroprusside from Merck (Darmstadt, Germany), and acetylcholine chloride, phenylephrine hydrochloride,

Table 1
ED_{50} and maximal response in endothelium intact preparations

Groups	ED_{50} (− log *g*)	Maximal response (mm Hg)
Phenylephrine		
Male	6.10 ± 0.04	215.0 ± 8.1
Male + NAME	6.43 ± 0.02*	211.1 ± 6.3
Male + TEA	6.53 ± 0.05*	227.5 ± 10.7
Male + NAME + TEA	6.65 ± 0.03*	210.2 ± 10
Female	6.01 ± 0.05	131.4 ± 12.9$^+$
Female + NAME	6.20 ± 0.01*$^+$	179.3 ± 3.5*$^+$
Female + TEA	6.05 ± 0.04$^+$	222.0 ± 6.4*
Female + NAME + TEA	6.63 ± 0.05*	195.1 ± 7.4*
Angiotensin II		
Male	7.66 ± 0.03	110.0 ± 6.1
Male + NAME	8.60 ± 0.15*	148.9 ± 6.3*
Male + TEA	8.03 ± 0.06*	138.8 ± 12.6*
Male + NAME + TEA	8.65 ± 0.04	142.1 ± 5.5*
Female	8.09 ± 0.08$^+$	70.1 ± 5.2$^+$
Female + NAME	8.63 ± 0.13*	137.7 ± 4.4*
Female + TEA	8.17 ± 0.15	110.8 ± 10.3*$^+$
Female + NAME + TEA	8.80 ± 0.12*	135.0 ± 6.0*
KCl (mM)		
Male	35.1 ± 2.0	233 ± 5.8
Female	35.3 ± 1.5	171 ± 9.1$^+$

Concentration of phenylephrine, angiotensin II and KCl causing ED_{50} of maximal response and maximal response in isolated perfused kidneys from male and female rats. Data (− log *g*) are expressed as means ± S.E.M.

$^+$ $P < 0.05$ compared with the male group.

* $P < 0.05$ compared with its respective control group.

papaverine, angiotensin II, N^{ω}-nitro-L-arginine methyl ester, and tetraethylammonium chloride from Sigma.

2.4. Statistical analysis

Nested-design analysis of groups and doses was used to compare dose–response curves; the design had two fixed effect factors (group and dose) and one random effect factor (the kidney), with this factor nested in the group. When tests for group–dose interaction were significant, groups at different doses were compared. ED_{50} values were compared using the Wilcoxon test.

3. Results

3.1. Response to vasoconstrictors

Renal vasculature from female rats showed markedly reduced responsiveness to phenylephrine (Fig. 1, left-hand graphs, Table 1). The dose–response curve was characterized by a shift towards the right, with decreased responses to middle doses and a lower maximal response. Renal vasculature from female rats also showed an attenuated dose–response curve to angiotensin II at the highest doses.

L-NAME administration produced an increased sensitivity to phenylephrine in the preparations from both male and female rats (Fig. 1, top left graph, Table 1). In the males, dose–response curves were characterized by a greater responsiveness to threshold and middle doses, with a similar maximal response to untreated preparations; consequently, the curves were shifted to the left in a nonparallel manner. In the females, L-NAME produced a marked increase in the maximal response, but the differences between male and female kidneys were maintained. The effect of TEA administration on the dose–response curve to phenylephrine

Table 2
ED_{50} and maximal response in endothelium removed preparations

Groups	ED_{50} ($-\log g$)	Maximal response (mm Hg)
Phenylephrine		
Male	6.43 ± 0.02	211.1 ± 6.3
Female	6.35 ± 0.01	199.3 ± 5.5
Angiotensin II		
Male	7.40 ± 0.00	127.5 ± 6.4
Female	7.48 ± 0.05	125.0 ± 6.8
KCl (mM)		
Male	30 ± 1.0	235 ± 13.8
Female	31 ± 1.5	214 ± 8.4

Concentration of phenylephrine, angiotensin II and KCl causing ED_{50} of maximal response and maximal response in isolated perfused kidneys from male and female rats. Data ($-\log g$) are expressed as means ± S.E.M.

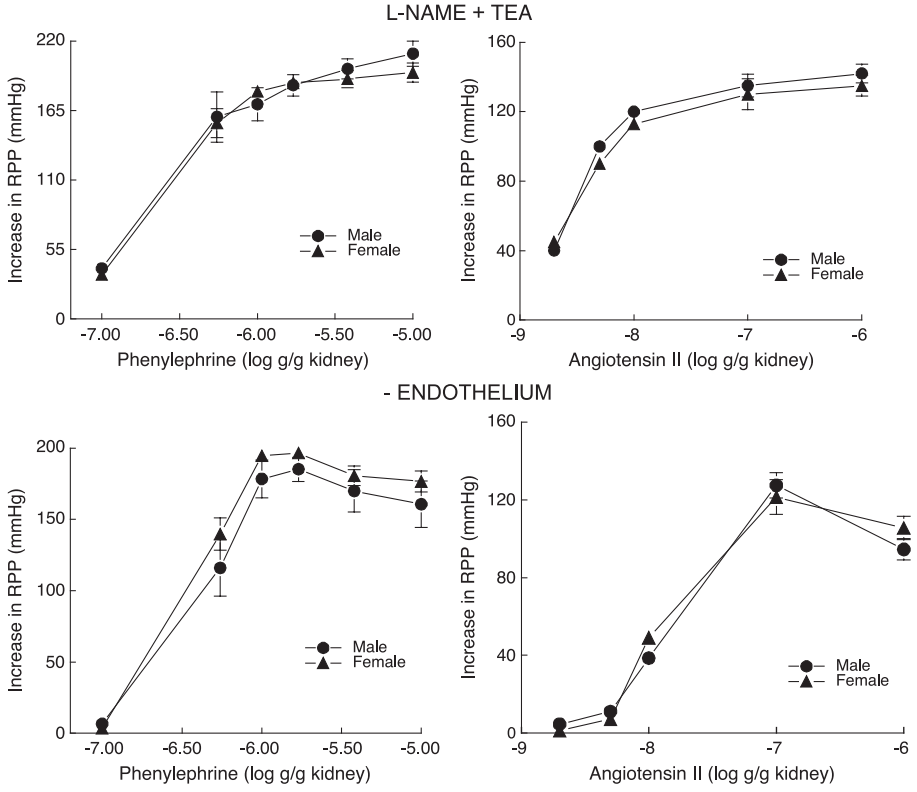

Fig. 2. Dose–response curves to phenylephrine and angiotensin II in isolated kidneys from male and female rats ($n = 7$ each group), after the simultaneous administration of L-NAME (10^{-4} M) + TEA (3×10^{-3} M) (top panels) or after removal of endothelium (−Endothelium, lower panels). Data are means ± S.E.M.

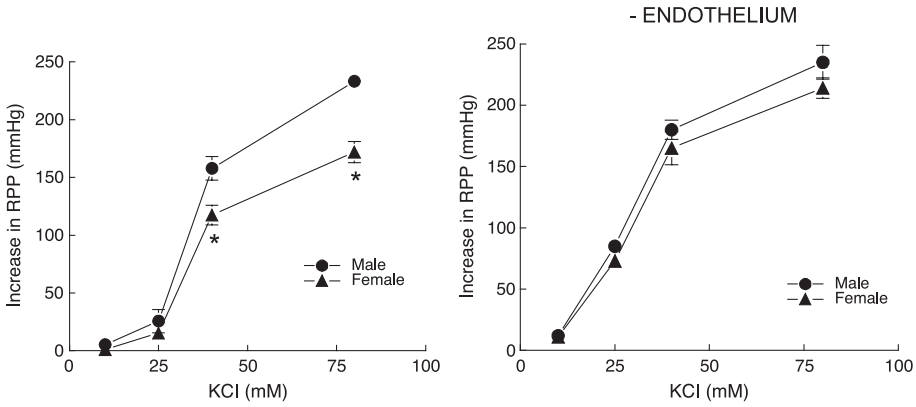

Fig. 3. Concentration–response curve to KCl in isolated kidneys from male and female rats ($n=7$ each group), under normal conditions (left panel) or after removal of endothelium (− Endothelium, right panel). Data are means ± S.E.M. *$P<0.05$ compared with the male group.

produced an increased sensitivity in male and female preparations, similar to that produced by L-NAME, and in female kidneys the maximal response was markedly increased, reaching similar values to male rat preparations (Fig. 1, bottom left graph, Table 1). Thus, greater differences between male and female preparations were observed after TEA administration than under basal conditions at the low and intermediate doses of phenylephrine, whereas no differences were observed at highest doses.

L-NAME produced a parallel shift to the left in the dose–response curve to angiotensin II in male and female preparations, with significant reductions in ED_{50} and marked increases in the maximal response. Statistical differences at two points of the dose–response curve in L-NAME treated preparations were observed, where the response was reduced in females (Fig. 1, top right graph, Table 1). TEA administration also shifted the dose–response curve to angiotensin II to the left in males and females, but this was significantly attenuated in females (Fig. 1, bottom right graph, Table 1). The simultaneous administration of L-NAME + TEA augmented the sensitivity to vasoconstrictors in both groups, without significant differences between

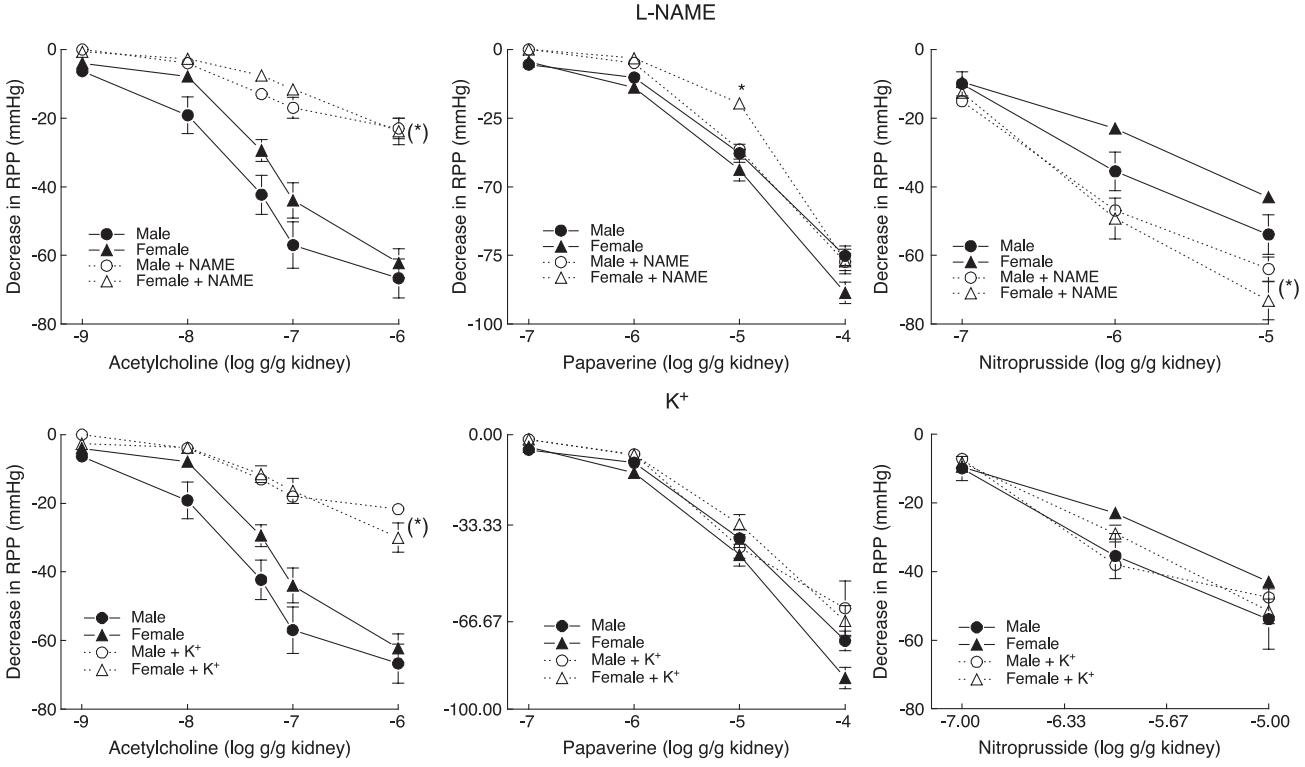

Fig. 4. Dose–response curves to increasing bolus doses of acetylcholine, sodium nitroprusside, or papaverine in isolated perfused kidneys from male and female rats preconstricted with phenylephrine at 10^{-6} M in males and around 5×10^{-6} M in females, dose required to achieve a similar vascular tone that in males, with phenylephrine plus L-NAME (10^{-4} M), or with KCl (80 mM) ($n=7$ each group). Data are means ± S.E.M. *$P<0.01$ along the dose–response curve compared with the untreated group. *$P<0.05$ compared with the untreated group.

their dose–response curves to either vasoconstrictor (Fig. 2, Table 2).

Removal of endothelium produced an increased sensitivity to vasoconstrictors in both groups, with no differences between their dose–response curves to either vasoconstrictor (Fig. 2, Table 2).

Renal vasculature from female rats showed markedly reduced responsiveness to KCl (Fig. 3, left-hand graph, Table 1). The concentration–response curve was characterized by a shift towards the right, with decreased responses to middle concentrations and a lower maximal response. Removal of endothelium produced an increased sensitivity to KCl in both groups, with no differences between their concentration–response curve (Fig. 3, Table 2).

3.2. Response to vasodilators

In isolated perfused kidneys from male and female rats preconstricted with phenylephrine, the bolus administration of acetylcholine, nitroprusside and papaverine produced a dose-related decrease in RPP (Fig. 4, top graphs). Kidneys from male and female rats showed a similar responsiveness to the three vasodilators with no significant gender differences along the dose–response curves or in the maximal vasodilatation reached.

L-NAME administration attenuated the dose–response curve to acetylcholine and increased the responsiveness to nitroprusside in both groups (Fig. 4, top graphs). After L-NAME administration, no significant differences between male and female preparations were observed in the dose–response curves to acetylcholine or nitroprusside. L-NAME did not affect the dose–response curve to papaverine in male or female kidneys.

When the preparations were perfused with the high potassium concentration (80 mM), the dose–response curve to acetylcholine was attenuated in both groups, with no differences between them (Fig. 4, bottom graphs). The dose–response curves to nitroprusside or papaverine were not significantly modified by the increased K^+ concentration in male and female kidneys (Fig. 4, bottom graphs).

4. Discussion

The present study demonstrates that renal vascular responses to agonists receptor mediated vasoconstrictors, phenylephrine and angiotensin II, and to nonspecific stimulants depolarizing solutions of KCl are lower in female versus male rats. Various mechanisms can be proposed to account for this difference. Thus, it has been shown that $alfa_1$-adrenergic receptor expression is decreased in mesenteric arteries of estradiol replaced rats compared with ovariectomized animals (Zhang and Davidge, 1999), which might participate in the attenuated responsiveness to phenylephrine in female kidneys. However, a decreased sensitivity to specific receptor-mediated agonists in female preparations should be discarded because it also happened in response to the nonspecific stimulant KCl, in consonance with the observations of Sanchez et al. (1996) in isolated aortic rings of female rabbits. A decreased reactivity of the vascular smooth muscle to vasoconstrictors in the renal tissue of female rats cannot explain this difference, because the contractile response of deendothelialized kidneys to vasoconstrictors was similar between male and female rats, therefore, indicating that the endothelium is the responsible for these differences.

Endothelial denudation and the blockade of NO and EDHF by the administration of L-NAME, TEA or L-NAME + TEA augmented the responsiveness to vasoconstrictors in the isolated kidneys from male and female rats in the present study, as previously reported in male preparations (Ercan et al., 1990; Vargas and Osuna, 1996). After NO blockade, the pressor responsiveness to phenylephrine was increased in male and female kidneys, but the differences between the groups were maintained. Therefore, these results suggest that the hyporesponsiveness to phenylephrine observed in female kidneys is not entirely due to enhanced NO production by the endothelium. This observation is consistent with the sexually dimorphic development of NO inhibition-induced hypertension in the rat (Sainz et al., 2000), in which an increased pressor responsiveness to vasoconstrictors plays an important role (Vargas et al., 1996), and suggested that a NO-independent pathway contributes to the attenuated pressor responsiveness to vasoconstrictors in female rats. However, our data contrast with a report by Sanchez et al. (1996) that the incubation of aortic rings with L-NAME abolished the phenylephrine-induced contraction differences between rings from male and female rabbits, indicating that the vascular reactivity differences were associated with a higher release of NO. These discrepancies may be due to the preparations used, because NO has been reported to play a major role in conducting arteries, and EDHF appears to be of great importance in resistance vessels (Garland et al., 1995).

Vascular reactivity to vasoconstrictors is modulated by K^+ channels. Thus, TEA, a nonspecific blocker of K^+ channels, induces a dose-dependent increase in the vascular sensitivity to noradrenaline and angiotensin II (Savineau and Marthan, 1993). However, we recently reported that the administration of TEA was unable to modify the reactivity to vasoconstrictors in endothelium-removed preparations (Moreno et al., 2003), indicating that EDHF present in the vasculature modulates the renal response to vasoconstrictors in the isolated perfused rat kidney and may, therefore, participate in the attenuated responsiveness to vasoconstrictors shown by the females in the present study.

This study clearly shows that the counterregulatory inhibitory effect of the endothelium on the responsiveness to vasoconstrictors is greater in the isolated kidneys from female respect to male rats. This gender difference in

endothelial inhibition of the contraction may be due to an augmented production/release of endothelium-derived relaxing factors in the female kidney in response to VC. In this sense, the fact that the simultaneous blockade of both factors, NO and EDHF, abolished the differences between male and female preparations, as happened after endothelium removal, indicate that NO and EDHF, and no other factors, are the responsible for the differences in the renal responsiveness to vasoconstrictors between males and females.

The present paper shows that NO and EDHF are stimulated by receptor mediated agonists and by nonspecific stimulant of vascular smooth muscle in consonance with our previous report (Vargas and Osuna, 1996). Therefore, all these data indicate that the mechanism by which vasoconstrictors induced the production of endothelium-derived relaxing factors should be the change of pressure in the vascular wall rather than endothelial membrane receptor stimulation, despite it is well known that angiotensin II induced NO production in endothelial cells via AT_1 receptors (Millatt et al., 1999).

There was no difference in the vasodilator response to acetylcholine, nitroprusside or papaverine between the male and female kidneys. Moreover, the blockade of NO or EDHF, by the administration of L-NAME or of increased extracellular potassium, respectively, produced a similar inhibition of acetylcholine-induced endothelium-dependent vasodilatation. All the above results indicate that the two endothelial components of acetylcholine-induced vasodilatation, NO and EDHF, are present in the renal vascular bed of both male and female preparations, and that the production of, or reactivity to, these factors in response to vasodilators is similar. The similarity of the vasodilator response to acetylcholine between male and female rats suggests that gender difference does not influence endothelial responsiveness to vasodilators in the resistance arteries of the rat kidney.

The present results clearly indicate that there are no differences between male and female kidneys in the relative contributions of agonist-stimulated NO and EDHF to endothelium-dependent vasodilation. These results contrast with findings that the contributions of NO and EDHF to endothelium-dependent relaxation differ between males and females. Thus, Hayashi et al. (1995) and Sanchez et al. (1996) both described a predominant role for NO in acetylcholine-induced relaxation in the aorta of female rabbits and rats compared with male controls, whereas others reported a dominant role for EDHF in acetylcholine-induced endothelium-dependent vasorelaxation in female animals (McCulloch and Randall, 1998; White et al., 2000). At first sight, these data may appear to conflict with the present results. However, numerous studies have demonstrated the tissue- and species-variability of endothelium-derived relaxing factors (Campbell and Gauthier, 2002; Furchgott and Vanhoutte, 1989; Garland et al., 1995; Hecker, 2000; Vanhoutte, 1993).

In conclusion, the present results indicate that the attenuated renal pressor responsiveness to vasoconstrictors in female preparations is related to an increased activity of endothelium-derived relaxing factors, NO and EDHF. No differences were found in the responsiveness to endothelium-dependent or -independent vasodilators between male and female rat kidneys. Finally, the relative contribution of NO and EDHF to acetylcholine-induced endothelium-dependent vasodilation in the renal vascular bed was similar between male and female rats.

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References

Adeagbo, A.S., Triggle, C.R., 1993. Varying extracellular [K^+]: a functional approach to separating EDHF- and EDNO-related mechanisms in perfused rat mesenteric arterial bed. J. Cardiovasc. Pharmacol. 21, 423–429.

Altura, B.M., 1972. Sex as a factor influencing the responsiveness of arterioles to catecholamines. Eur. J. Pharmacol. 20, 261–265.

Bhargava, K.P., Dhawan, K.N., Saxena, R.C., 1967. Enhancement of noradrenaline pressor responses in testosterone-treated cats. Br. J. Pharmacol. 31, 26–31.

Brayden, J.E., Quayle, J.M., Standen, N.B., Nelson, M.T., 1991. Role of potassium channels in the vascular response to endogenous and pharmacological vasodilators. Blood Vessels 28, 147–153.

Campbell, W.B., Gauthier, K.M., 2002. What is new in endothelium-derived hyperpolarizing factors? Curr. Opin. Nephrol. Hypertens. 11, 177–183.

Chen, Y.F., 1996. Sexual dimorphism of hypertension. Curr. Opin. Nephrol. Hypertens. 6, 181–185.

Collis, M.G., Vanhoutte, P.M., 1977. Vascular reactivity in isolated perfused kidneys from male and female spontaneously hypertensive rats. Circ. Res. 41, 759–767.

Ercan, Z.S., Soydan, A.S., Turker, R.K., 1990. Possible involvement of endothelium in the responses of various vasoactive agents in rabbit isolated perfused kidney. Gen. Pharmacol. 21, 205–209.

Furchgott, R.F., Vanhoutte, P.M., 1989. Endothelium-derived relaxing and contracting factors. FASEB J. 3, 2007–2018.

Garland, C.J., Plane, F., Kemp, B.K., Cocks, T.M., 1995. Endothelium-dependent hyperpolarization: a role in the control of vascular tone. Trends Pharmacol. Sci. 16, 23–30.

Golding, E.M., Kepler, T.E., 2001. Role of estrogen in modulating EDHF-mediated dilations in the female rat middle cerebral artery. Am. J. Physiol, Heart Circ. Physiol. 280, H2417–H2423.

Hayashi, T., Yamada, K., Esaki, T., Kuzuya, M., Satake, S., Ishikawa, T., Hidaka, H., Iguchi, A., 1995. Estrogen increases endothelial nitric oxide by a receptor-mediated system. Biochem. Biophys. Res. Commun. 214, 847–855.

Hecker, M., 2000. Endothelium-derived hyperpolarizing factor—fact or fiction? News Physiol. Sci. 15, 1–5.

Huang, A., Sun, D., Koller, A., Kaley, G., 1997. Gender difference in myogenic tone at rat arterioles is due to estrogen-induced, enhanced release of NO. Am. J. Physiol. 272, H1804–H1809.

Ignarro, L.J., Lippton, H., Edwards, J.C., Baricos, W.H., Hyman, A.L., Kadowitz, P.J., Gruetter, C.A., 1981. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside, and nitric oxide: evidence for the involvement of *S*-nitrosothiols as active intermediates. J. Pharmacol. Exp. Ther. 218, 739–749.

Ishii, K., Chang, B., Kerwin Jr., J.F., Huang, Z.J., Murad, F., 1990. N^{ω}-nitro-L-arginine. A potent inhibitor of endothelium-derived relaxing factor formation. Eur. J. Pharmacol. 176, 219–223.

Kauser, K., Rubanyi, G.M., 1994. Gender difference in bioassayable endothelium-derived nitric oxide from isolated rat aortae. Am. J. Physiol. 267, H2311–H2317.

Li, Z., Duckles, S.P., 1994. Influence of gender on vascular reactivity in the rat. J. Pharmacol. Exp. Ther. 268, 1426–1431.

Liu, M.Y., Hattori, Y., Fukao, M., Sato, A., Sakuma, I., Kanno, M., 2001. Alterations in EDHF-mediated hyperpolarization and relaxation in mesenteric arteries of female rats in long term deficiency of oestrogen and during oestrus cycle. Br. J. Pharmacol. 132, 1035–1046.

McCulloch, A.I., Randall, M.D., 1998. Sex differences in the relative contribution of nitric oxide and EDHF to agonist-stimulated endothelium-dependent relaxations in the rat isolated mesenteric arterial bed. Br. J. Pharmacol. 123, 1700–1706.

Millatt, J.L., Abdel-Rahman, E.M., Siragy, H.M., 1999. Angiotensin II and nitric oxide: a question of balance. Regul. Pept. 81, 1–10.

Moreno, J.M., Wangensteen, R., Sainz, J., Rodríguez-Gómez, Y., Chamorro, V., Osuna, A., Vargas, F., 2003. Role of endothelium-derived relaxing factors in the renal response to vasoactive agents in hypothyroid rats. Am. J. Physiol: Endocrinol. Metab. 285, E182–E188.

Munger, K., Baylis, C., 1988. Sex differences in renal hemodynamics in rats. Am. J. Physiol. 254, F223–F231.

Reckelhoff, J.F., 2001. Gender differences in the regulation of blood pressure. Hypertension 37, 1199–1208.

Rubanyi, G.M., Freay, A.D., Kauser, K., Johns, A., Harder, D.R., 1990. Mechanoreception by the endothelium: mediators and mechanisms of pressure- and flow-induced vascular responses. Blood Vessels 27, 246–257.

Sainz, J., Osuna, A., Wangensteen, R., Duarte, J., Vargas, F., 2000. Influence of gender on the development of nitric oxide inhibition-induced hypertension. Hypertension 36, 660 (Abstract).

Sanchez, A., Gomez, M.J., Dorantes, A.L., Rosales, J.L., Pastelin, G., Diaz, V., Posadas, F., Escalante, B., 1996. The effect of ovariectomy on depressed contractions to phenylephrine and KCl and increased relaxation to acetylcholine in isolated aortic rings of female compared to male rabbits. Br. J. Pharmacol. 118, 2017–2022.

Savineau, J.P., Marthan, R., 1993. Effect of cromakalim on KCl-, noradrenaline- and angiotensin II-induced contractions in the rat pulmonary artery. Pulm. Pharmacol. 6, 41–48.

Stallone, J.N., Crofton, J.T., Share, L., 1991. Sexual dimorphism in vasopressin-induced contraction of rat aorta. Am. J. Physiol. 260, H453–H458.

Vanhoutte, P.M., 1993. Other endothelium-derived vasoactive factors. Circulation 87 (suppl. V), V9–V17.

Vargas, F., Osuna, A., 1996. Modulatory role of endothelium-derived relaxing factors on the response to vasoconstrictors and flow–pressure curve in the isolated perfused rat kidney. J. Vasc. Res. 33, 119–123.

Vargas, F., Sabio, J.M., Luna, J.D., 1994. Contribution of endothelium-derived relaxing factors to acetylcholine-induced vasodilatation in the rat kidney. Cardiovasc. Res. 28, 1373–1377.

Vargas, F., Fernandez-Rivas, A., Osuna, A., 1996. Vascular reactivity and flow–pressure curve in isolated perfused kidneys from L-NAME hypertensive rats. J. Hypertens. 14, 373–379.

Weiner, C.P., Lizasoain, I., Baylis, S.A., Knowless, R.G., Charles, I.G., Moncada, S., 1994. Induction of calcium-dependent nitric oxide synthase by sex hormones. Proc. Natl. Acad. Sci. U. S. A. 91, 5212–5216.

White, R.M., Rivera, C.O., Davison, C.A., 2000. Nitric oxide-dependent and -independent mechanisms account for gender differences in vasodilation to acetylcholine. J. Pharmacol. Exp. Ther. 292, 375–380.

Zhang, Y., Davidge, S.T., 1999. Effect of estrogen replacement on vasoconstrictor responses in rat mesenteric arteries. Hypertension 34, 1117–1122.