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Roles of estrogen and progesterone in modulating renal nerve function in the rat kidney

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Abstract

The maintenance of extracellular Na^+ and Cl^- concentrations in mammals depends, at least in part, on renal function. It has been shown that neural and endocrine mechanisms regulate extracellular fluid volume and transport of electrolytes along nephrons. Studies of sex hormones and renal nerves suggested that sex hormones modulate renal function, although this relationship is not well understood in the kidney. To better understand the role of these hormones on the effects that renal nerves have on Na^+ and Cl^- reabsorption, we studied the effects of renal denervation and oophorectomy in female rats. Oophorectomized (OVX) rats received 17β-estradiol benzoate (OVE, 2.0 $mg \cdot kg^{-1} \cdot day^{-1}$, *sc*) and progesterone (OVP, 1.7 $mg \cdot kg^{-1} \cdot day^{-1}$, *sc*). We assessed Na^+ and Cl^- fractional excretion (FE_{Na+} and FE_{Cl-}, respectively) and renal and plasma catecholamine release concentrations. FE_{Na+}, FE_{Cl-}, water intake, urinary flow, and renal and plasma catecholamine release levels increased in OVX *vs* control rats. These effects were reversed by 17β-estradiol benzoate but not by progesterone. Renal denervation did not alter FE_{Na+}, FE_{Cl-}, water intake, or urinary flow values *vs* controls. However, the renal catecholamine release level was decreased in the OVP (236.6 ± 36.1 ng/g) and denervated rat groups (D: 102.1 ± 15.7; ODE: 108.7 ± 23.2; ODP: 101.1 ± 22.1 ng/g). Furthermore, combining OVX + D (OD: 111.9 ± 25.4) decreased renal catecholamine release levels compared to either treatment alone. OVE normalized and OVP reduced renal catecholamine release levels, and the effects on plasma catecholamine release levels were reversed by ODE and ODP replacement in OD. These data suggest that progesterone may influence catecholamine release levels by renal innervation and that there are complex interactions among renal nerves, estrogen, and progesterone in the modulation of renal function.

Key words: Estrogen; Na^+ and Cl^- reabsorption; Progesterone; Renal nerve

Introduction

Estrogen (E_2) and progesterone (P_4) are steroid hormones implicated principally in the control of female reproductive functions by genomic and non-genomic mechanisms (1,2). Among their other actions, E_2 and P_4 can also modulate Na^+ and Cl^- reabsorption along the mammalian nephron and alter the physiological hydroelectrolyte balance (3). It is known that extracellular volume increases in women during the pre-ovulatory phase of the menstrual cycle when estrogen levels are rising (4). Furthermore, salt and water retention occurs during pregnancy (5) and in postmenopausal women who receive E_2 (6). These findings could be associated with the actions of E_2 and P_4 on renal function, leading to hydrosaline retention (5). E_2 stimulates epithelial sodium channel mRNA expression in the rat kidney (7), primarily in the proximal and distal renal tubules (1,8). P_4 is known to compete with aldosterone for mineralocorticoid receptors (expressed mainly in distal tubules), which can lead to receptor activation and increased Na^+ reabsorption in this nephron segment (9).

Biochemical studies suggest that there are at least three distinct estrogen receptors (ER) and five progesterone receptors (PR) expressed in the kidney. Two ER (ER-α and ER-β) and two PR (PR-A and PR-B) belong to the ligand-activated transcription factors. The third ER, G-protein coupled ER (GPR30) (10), and the membrane progestin receptors α and γ (11) have been studied and belong to the G-protein coupled receptor superfamily.

There are several mechanisms through which the kidney is able to adjust the hydroelectrolyte balance in the body. Among them is the autonomic nervous system, specifically the renal sympathetic nerves (RSN), and circulating endocrine factors (12,13), such as P_4 and E_2 (1-3,5-9). The RSN innervate renal tubules (the proximal tubule, except for the S_1 segments, followed by the thick ascending limb of Henle's loop, the distal convoluted

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tubule, and the collecting duct). Additionally, adrenergic innervation of the renal vasculature is distributed throughout the renal cortex and the outer stripe of the renal medulla, with the greatest density in the juxtamedullary region of the inner cortex. This distribution allows for the modulation of multiple renal functions (at least in part by the release of catecholamines), such as renal blood flow (RBF), the glomerular filtration rate (GFR), renin release, and Na^+ reabsorption (12,13). Although the involvement of P_4 and E_2 in renal ion reabsorption is clear, the relationship with the RSN that are involved is not well known.

Catecholamines are phenylalanine-derived amines synthesized and produced within the chromaffin cells of the adrenal medulla and postganglionic fibers of the sympathetic nervous system (PSNS). Catecholamines act as hormones and neurotransmitters (13). The principal source of circulating catecholamines is the adrenal medulla, which responds to sympathetic stimulation. The major source of plasma norepinephrine is spillover from PSNS (14). In the kidney, norepinephrine is released in response to RSN fiber activation and binds to specific post-junctional receptors (15), which induces changes in renal function, such as increased Na^+ and Cl^- reabsorption (13).

Investigations suggest that sex hormones can modulate catecholamine metabolism in various tissues (13,14,16). P_4 dose-dependently inhibited catecholamine secretion from bovine chromaffin cells (16). In rat oophorectomies, intravenous injection of E_2 into the nucleus tractus solitarius (17) and central nucleus of the amygdala (18) decreased RSN activity. Although the participation of P_4 and E_2 in modulation of the sympathetic nervous system is clear (17), the role of these hormones in the control of catecholamines involved in the renal tissue is not well understood (16).

Based on the knowledge that female hormones and renal nerves can modulate corporal body Na^+ and Cl^- balance by modulating renal function and catecholamine release levels, we proposed a study of the possible involvement of P_4 and E_2 in the modulation of catecholamine levels (renal and plasma) in renal nerves.

Material and Methods

All procedures were conducted in accordance with the Biomedical Research Guidelines for the Care and Use of Laboratory Animals, as stated by the Brazilian College of Animal Experimentation (COBEA). The experimental protocol was approved by the Ethics Committee in Animal Experimentation of Universidade Federal do Espírito Santo (CEUA) under No. 012/2008 (for oophorectomy) and No. 014/2008 (for renal denervation).

Experimental animals

Female Wistar rats (~250 g, 3 months old) were maintained in temperature- and humidity-controlled cages with 12:12-h light-dark cycles and free access to water and food. All animals showed regular 4- to 5-day estrous cycles that were monitored by vaginal smears collected each morning for at least two consecutive weeks before starting the experiments, as previously described (19). All experimental protocols were performed by a single researcher.

The rats were divided into 2 groups: control (C, n = 8) and oophorectomized rats (OVX, n = 24). Afterwards, we evaluated E_2 and P_4 plasma concentrations and uterine weight. The OVX rats were kept for 7 days after surgery and then further subdivided into 3 groups: an OVX group that received daily subcutaneous (*sc*) injections of corn oil as a vehicle (OVX, *sc*, n = 8); an OVX group that received 17β-estradiol benzoate (OVE, 2.0 $\mu g \cdot kg^{-1} \cdot day^{-1}$ 17β-estradiol benzoate, *sc*, n = 8), and an OVX progesterone-treated group (OVP, 1.7 $mg \cdot kg^{-1} \cdot day^{-1}$ progesterone, *sc*, n = 8). The 17β-estradiol benzoate and progesterone were purchased from Sigma Chemical Co. (USA).

In addition, 5 other groups were included: sham-operated, mimicking bilateral renal denervation (CD, n = 8); bilateral sympathetic renal denervated (D, n = 8); OVX and bilateral sympathetic renal denervated treated only with the same vehicle (OD, n = 8); and OVX and bilateral sympathetic renal denervated, treated with the same doses of 17β-estradiol benzoate (ODE, n = 8) and progesterone (ODP, n = 8), as described earlier. These groups recovered for 7 days after the surgical procedures and 7 days of daily hormonal treatment. During the 7 days of treatment, the rats were placed individually in metabolic cages for 24-h urine collection and renal function assessment. At the end of the experiment, the animals were killed by decapitation, and blood samples were collected to determine renal parameters and plasma hormone levels of E_2 (pg/mL) and P_4 (ng/mL). The kidneys were perfused with saline, then removed and frozen for later catecholamine measurements. This procedure was performed for all groups.

Gonadectomy

Bilateral gonadectomy was performed in female rats under ketamine [30 mg/kg, intramuscular injection, (*im*)] and xylazine (3 mg/kg, *im*) anesthesia. The females were subjected to a muscular incision to open the peritoneal cavity, identify the posterior connection of the uterine tubules, and remove the ovaries (19). The peritoneal cavity was closed with 4.0 silk sutures, and the animals were allowed to recover. The female sham group only underwent an incision that was also closed with 4.0 silk sutures. After that, the animals were allowed to recover.

Bilateral renal denervation

Bilateral renal denervation was performed to eliminate the influence of renal nerves on renal excretory function. The rats underwent chronic bilateral renal denervation together with bilateral gonadectomy. Under ketamine (30 mg/kg, *im*) and xylazine (3 mg/kg, *im*) anesthesia, the left kidney was exposed via a flank incision. The

adventitia surrounding the renal artery and vein were stripped, and all visible renal nerves were cut under a microscope (Model 902/18140, DFV, Brazil). The vessels were then treated with 95% alcohol containing 10% phenol. After renal denervation, the flank incision was sutured closed, and the procedure was repeated on the opposite side to denervate the right kidney. This renal denervation procedure prevents the renal vasoconstrictor response to suprarenal lumbar sympathetic nerve stimulation, prevents the anti-natriuretic response to environmental stress, and reduces renal tissue norepinephrine concentrations within 15 days postdenervation (20). After the acute experiments, the kidneys were kept frozen until catecholamine concentrations were measured.

Renal function analysis

Methods for renal function analysis were described previously (18). In brief, the electrolyte balance and GFR were measured at the end of the experiment. For electrolyte measurements, serum and 24-h urine samples were analyzed for Na^+, Cl^-, and creatinine (Cr) concentrations according to standard laboratory procedures using an automatic serum analyzer. Then, fractional excretion (FE) and GFR were calculated in each experimental group. Urine was collected to determine urinary flow, and plasma was collected after the animals were killed. The electrolyte FE and GFR were calculated using the following formulas: $FE(\%) = [(U_x \times V)/(GFR \times P_x)] \times 100$ and $GFR = (U_{cr} \times V)/P_{cr})$, respectively; where U_x = electrolyte urinary concentration (mg/mL), P_x = electrolyte plasma concentration (mg/mL), U_{cr} = Cr urinary concentration (mg/mL), P_{cr} = Cr plasma concentration (mg/mL), and V = urinary flow (mL/min).

Renal and plasma catecholamine measurements

Renal and plasma catecholamine release (RECA and PLCA, respectively) levels were measured, as previously described (19,21). The kidneys were perfused with saline, removed, and frozen for later catecholamine analysis. The same procedure was performed for all groups. The kidneys were weighed and adipose tissue was removed. Each kidney was homogenized in 10 volumes of cold 4 mM ethylenediamine tetraacetic acid solution (buffered with Tris-HCl, pH 7.4). The homogenate was centrifuged at 10,000 *g* for 10 min at 4°C. The resulting supernatant was used for renal catecholamine content measurements. The blood plasma and renal supernatants were subjected to fluorescence spectrophotometry (Hitachi, Model F-2000, Japan) and direct reading (λ Ex: at 285 nm and λ En: at 325 nm). Noradrenaline (L-norepinephrine hydrochloride) was used as a standard.

Statistical analysis

All results are reported as means ± SE. Comparisons between groups were carried out by analysis of variance and the Tukey test. The significance level was set at $P<0.05$.

Results

After oophorectomy, renal denervation, or both, we evaluated changes in the hormone plasma levels and the uterine, body, and kidney weights (Table 1). The OVX group showed significantly decreased E_2 and P_4 serum levels ($P<0.05$) compared to the control group. Oophorectomy also reduced uterine weight in the OVX group ($P<0.01$). Uterine atrophy was prevented by E_2 in the OVE group, but not in the OVP group ($P<0.01$). Uterine weights in the OVE rats were not significantly different compared to the control rats. The E_2 and P_4 concentrations were decreased but were reverted to the original values by 17β-estradiol in the OVE rats and by progesterone in the OVP rats. In addition, the D rats did not show differences in female sex hormone levels or

Table 1. Changes in E_2 and P_4 plasma levels, and uterine, kidney, and body weight in female rats.

Groups (n = 8)	E_2 (pg/mL)	P_4 (ng/mL)	Uterine weight (mg)	Kidney weight/body weight ratio (mg/g)	Body weight (g)
C	98.4 ± 11.2	17.8 ± 2.8	453.8 ± 25.3	3.0 ± 0.1	237.7 ± 7.9
OVX	10.7 ± 1.8**	4.1 ± 0.9**	98.1 ± 3.9**	3.1 ± 0.2	241.4 ± 7.1
OVE	110.1 ± 9.8	6.2 ± 2.8**	407.3 ± 34.8	2.9 ± 0.2	223.5 ± 8.1
OVP	10.2 ± 3.3**	16.9 ± 1.7	159.2 ± 10.9**	2.9 ± 0.2	216.1 ± 9.3
CD	98.4 ± 11.2	17.8 ± 2.8	453.8 ± 25.3	3.0 ± 0.1	237.7 ± 7.9
D	103.1 ± 10.0	17.9 ± 2.7	434.7 ± 42.2	3.3 ± 0.2	238.5 ± 8.3
OD	13.1 ± 1.4^{++}	4.5 ± 1.1^{++}	109.2 ± 14.4^{++}	3.1 ± 0.3	243.3 ± 9.4
ODE	113.0 ± 10.7	8.9 ± 1.6^{++}	397.6 ± 28.6	3.2 ± 0.2	233.6 ± 8.5
ODP	12.4 ± 2.5^{++}	18.3 ± 3.1	164.5 ± 13.9^{+}	3.2 ± 0.2	229.9 ± 9.2

Data are reported as means ± SE (n = 8 per group). C: control; OVX: ovariectomized; OVE: OVX with 17β-estradiol benzoate (E_2, 2.0 mg·kg^{-1}·day^{-1}, *sc*); OVP: OVX with progesterone replacement (P_4, 1.7 mg·kg^{-1}·day^{-1}, *sc*), both for 7 days; CD: sham-denervated; D: denervated; OD: D ovariectomized; ODE: OD with 17β-estradiol benzoate; ODP: OD with progesterone, both for 7 days. **$P<0.01$ compared to C, $^{+}P<0.05$ and $^{++}P<0.01$ compared to CD (ANOVA and Tukey test).

uterine, body, and kidney weights (Table 1). Additionally, the E_2 and P_4 plasma concentrations and uterine weights were lower in the OD group ($P<0.01$) compared to the CD group. The ODE rats had normal E_2 levels but low P_4 levels ($P<0.01$). Conversely, the ODP rats had normal plasma P_4 levels but low E_2 levels and uterine atrophy ($P<0.05$). The administration of sex hormones and surgical procedures did not produce significant changes in kidney or body weight (Table 1).

The RECA and PLCA concentrations were different in renal tissue (Figure 1) and blood plasma (Figure 2), respectively. We observed that OVX rats had significantly increased RECA levels (1514.0 ± 183.3 ng/g) compared to C rats (996.2 ± 150.8 ng/g; $P<0.01$, Figure 1A); OVE rats showed similar RECA values compared to C rats (792.7 ± 84.5 ng/g); and OVP rats showed significantly decreased values (236.6 ± 36.1 ng/g; $P<0.01$, Figure 1A) compared to C rats. As expected, group D rats showed significantly decreased RECA values (D = 102.1 ± 15.7; OD = 111.9 ± 25.4; ODE = 108.7 ± 23.2; ODP = 101.1 ± 22.1 ng/g, $P<0.01$), independent of oophorectomy and/or treatment with estrogen (ODE) or progesterone (ODP), compared to CD rats (1006.7 ± 148.6 ng/g, Figure 1B). We did not observe any differences in RECA values between the C and CD groups. Similar to the RECA results, PLCA levels increased in the OVX group (386.2 ± 10.4 pmol/mL, $P<0.01$, Figure 2A) compared to the C group (222.2 ± 8.5 pmol/mL). The OVE and OVP group values were similar to the C group (OVE = 197.1 ± 26.2; OVP = 202.0 ± 34.6 pmol/mL, respectively, Figure 2A). PLCA levels increased in the OD group (352.2 ± 36.0 pmol/mL, $P<0.01$) compared to the CD group (223.1 ± 8.7 pmol/mL). The D, ODE and ODP group values (199.8 ± 13.2; 179.3 ± 10.5; 165.5 ± 15.7 pmol/mL, respectively) were similar to that of the CD group (Figure 2B). We did not observe any differences in PLCA levels between the CD and C groups.

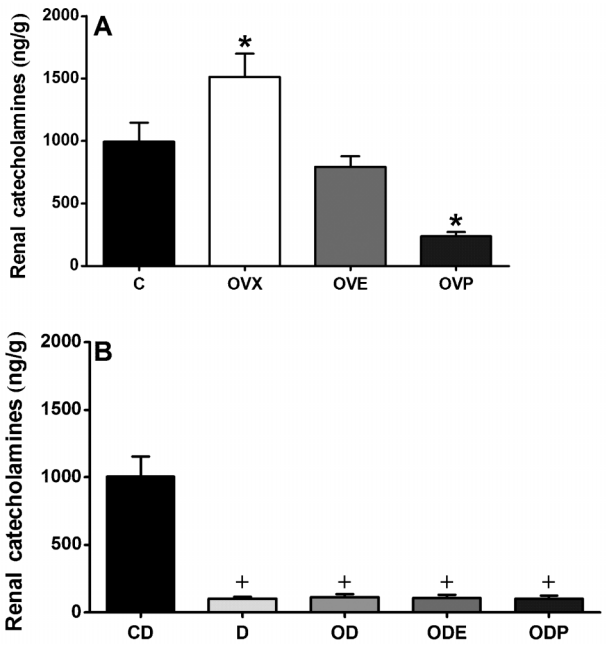

Figure 1. Renal catecholamine content in female rats. *A*, Control (C), ovariectomized (OVX), OVX with 17β-estradiol benzoate (OVE, 2.0 mg·kg^{-1}·day^{-1}, *sc*), and OVX with progesterone (OVP, 1.7 mg·kg^{-1}·day^{-1}, *sc*), both for 7 days. *B*, Sham-denervated (CD), denervated (D), D ovariectomized (OD), OD with 17β-estradiol benzoate (ODE), and OD with progesterone (ODP), both for 7 days. Data are reported as means ± SE (n = 8). *$P<0.01$ and $^{+}P<0.01$ compared to C and CD, respectively (ANOVA and Tukey test).

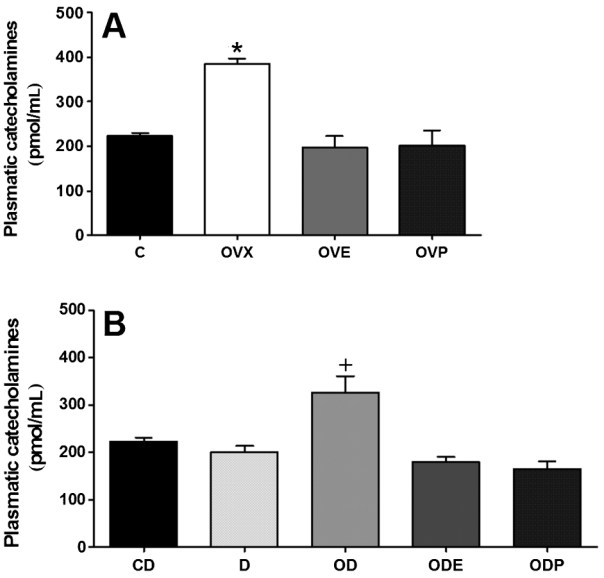

Figure 2. Plasma catecholamine levels in female rats. *A*, Control (C), ovariectomized (OVX), OVX with 17β-estradiol benzoate (OVE, 2.0 mg·kg^{-1}·day^{-1}, *sc*), and OVX with progesterone (OVP, 1.7 mg·kg^{-1}·day^{-1}, *sc*), both for 7 days. *B*, Sham-denervated (CD), denervated (D), D ovariectomized (OD), OD with 17β-estradiol benzoate (ODE), and OD with progesterone (ODP), both for 7 days. Data are reported as means ± SE (n = 8). *$P<0.01$ and $^{+}P<0.01$ compared to C and CD, respectively (ANOVA and Tukey test).

We also analyzed renal function parameters (Table 2). FE_{Na+} and FE_{Cl-} were significantly increased in the OVX and OVP groups compared to the C group (in both, $P<0.01$ and $P<0.05$, respectively). As expected, both urinary flow and water intake increased in the OVX and OVP groups compared to the C group ($P<0.01$, $P<0.05$, $P<0.05$, and $P<0.05$, respectively). The renal parameters in the OVE group did not change significantly compared to the C group. Renal denervation did not change the FE_{Na+}, FE_{Cl-}, urinary flow, and water intake compared to the CD group. However, these parameters increased in the OD group ($P<0.01$, $P<0.05$, $P<0.05$, and $P<0.05$, respectively) and the ODP group ($P<0.01$, $P<0.05$, $P<0.05$, and $P<0.05$, respectively). The ODE group was similar to

Table 2. Renal function in female rats.

Group (n = 8)	FE_{Na+} (%)	FE_{Cl-} (%)	Urinary flow (mL/day)	GFR (mL/min)	Water intake (mL/day)
C	0.12 ± 0.01	0.39 ± 0.01	10.4 ± 0.8	1.7 ± 0.4	14.6 ± 1.2
OVX	0.21 ± 0.02**	0.62 ± 0.01*	13.7 ± 0.1**	1.5 ± 0.2	17.9 ± 1.1*
OVE	0.12 ± 0.01	0.35 ± 0.08	12.2 ± 0.7	1.7 ± 0.4	14.2 ± 1.3
OVP	0.18 ± 0.05**	0.53 ± 0.02*	14.4 ± 0.8*	2.1 ± 0.8	18.2 ± 1.4*
CD	0.12 ± 0.01	0.39 ± 0.01	10.4 ± 0.8	1.7 ± 0.4	14.6 ± 1.2
D	0.11 ± 0.02	0.41 ± 0.02	11.2 ± 0.9	1.8 ± 0.9	15.3 ± 1.8
OD	0.20 ± 0.01^{++}	0.67 ± 0.02^{+}	13.9 ± 0.8^{+}	1.7 ± 0.4	18.1 ± 1.2^{+}
ODE	0.13 ± 0.02	0.42 ± 0.05	12.2 ± 0.9	1.6 ± 0.5	13.3 ± 1.7
ODP	0.19 ± 0.02^{++}	0.57 ± 0.03^{+}	14.2 ± 0.7^{+}	2.0 ± 0.7	17.7 ± 1.3^{+}

Data are reported as means ± SE (n = 8 per group). See Table 1 legend for explanation of groups. *$P<0.05$ and **$P<0.01$ compared to C; $^{+}P<0.05$ and $^{++}P<0.01$ compared to CD (ANOVA and Tukey test).

the CD group. No changes in the GFR were observed in any of the groups studied (Table 2).

Discussion

In the present study, we showed a possible relationship between renal nerve catecholamine release levels and the roles of E_2 and P_4 in renal Na^+ and Cl^- reabsorption in the rat kidney. Our data support the view that oophorectomy increases RECA levels, PLCA levels, FE_{Na+}, and FE_{Cl-}. In similar animal models, our group showed changes in catecholamine release levels (renal and plasma) and renal function at the different estrous cycle phases (19). As expected, E_2 treatment reversed the effect of OVX on RECA levels, PLCA levels, FE_{Na+}, and FE_{Cl-}.

Renal function is modulated (at least in part) by the autonomic nervous system through the PSNS. Catecholamine release levels (α_1 predominantly on adrenergic receptors) from renal tubules and vasculature influence the levels of RBF, GFR, renin release, and Na^+ reabsorption (13,22). Our data support studies that have shown that female hormones attenuate sympathetic activation in the central nervous system (17,23). After an acute infusion of intravenous E_2 in female rats (17) in the nucleus tractus solitarius, rostral ventrolateral medulla, parabrachial nucleus, central nucleus of the amygdala, and intrathecal space, RSN activity decreased (18). Additionally, there is evidence suggesting that E_2 can modulate adrenal and neurogenic catecholamine secretion and inhibit catecholamine release pre-synaptically (24,25). E_2 may also upregulate the catalytic activity of catechol-*O*-methyltransferase, thereby reducing the availability of catecholamines (26).

On the other hand, we showed that P_4 decreased RECA levels to an even lower concentration than control levels and values similar to those in the renal denervated rats. Predictably, renal denervation decreased RECA levels, which were unchanged by other treatments. However, it did not change PLCA levels. Our results are consistent with other studies that showed that P_4 inhibited catecholamine secretion in bovine chromaffin cells of the adrenal medulla via a dose- and time-dependent mechanism and inhibited acetylcholine nicotinic receptors and increased inactivation of Ca^{2+}-voltage-gated channels (16). Additionally, P_4 has a potentially inhibitory effect on gamma-aminobutyric acid in the rostral ventrolateral medulla, which decreases central sympathetic activity (27).

Previous studies have shown that renal denervation induces diuresis and natriuresis (28,29). However, our results showed similar values of renal function between renal denervated and normal animals. Similarly, DiBona and Sawin (30) showed that renal denervation had no effect on the dynamic autoregulation of RBF in control rats and that renal denervation did not produce changes in Na^+ excretion (31) or urinary volume in rats fed a normal Na^+ diet (20).

Several investigations have shown that E_2 and P_4 modify body fluids and electrolytes (5,6,10,19). Female sex hormones have been shown to regulate the expression of different transporters in different tissues in rats, such as renal tissue (7,8). E_2 replacement in the OVX rats increases renal epithelial sodium channel mRNA levels compared to males, and P_4 treatment inhibits the stimulation by E_2 alone (7). E_2 increases ClC-2 mRNA expression in the rat proximal convoluted and proximal straight renal tubules (32). In accordance with previously published data, P_4 acts as a competitive antagonist of the mineralocorticoid receptor, which attenuates the effect of aldosterone on Na^+ reabsorption (5,9). Because oophorectomy reduced Na^+ and Cl^- transporter expression and hormonal replacement normalized or modulated the expression, it was hypothesized that this downregulation could reduce Na^+ and Cl^- reabsorption by the nephron and that hormonal replacement could normalize this possible reduction in Na^+ and Cl^- reabsorption in this animal model. Increases in FE_{Na+} and FE_{Cl-} were observed, and estrogen replacement normalized these increases. Also,

FE_{Na+} and FE_{Cl-} were increased in the OVX rats and not normalized by progesterone replacement.

In addition, E_2 and P_4 act in renal tissues by modulating cyclic nucleotide-gated-A1 and Na^+-K^+-ATPase (mainly in the renal cortex) by genomic mechanisms, and these effects could be important for Na^+, Cl^-, and water balance (33). E_2, *in vitro*, increases the expression of the NaCl cotransporter and Na^+-K^+-ATPase in renal distal tubule cells in culture by non-genomic mechanisms (8). Investigations showed that the kidneys control the expression of nuclear ERs (ER-α and ER-β) and the transmembrane GPR30 (10,11). This effect in the proximal tubules could be mediated directly via its receptors, or it could also act indirectly though the renin-angiotensin-aldosterone system (32).

GFR is considered to be the best and most common parameter for assessing renal function (10,19,33). We did not observe significant changes in the GFR or kidney and body weight following these experimental protocols. Similar to our results, women treated with a GnRH antagonist (to suppress reproductive function for the duration of the study) showed similar urinary volume, osmolality, and GFR after P_4 and $P_4 + E_2$ treatment (5) before, during, and after isotonic saline infusion.

Renal functions are controlled mainly by humoral factors and the sympathetic nervous system (such as catecholamine release), which differ widely in their time of activation (34). The complex interactions of different hormones, such as sex steroid hormones, atrial natriuretic factor, angiotensin II, aldosterone, and vasopressin, may affect tubular reabsorption and water intake (5,35,36). Furthermore, renal cells after oophorectomy and hormonal replacement adapt and alter the expression of several genes that code for transporter proteins (10,33,34).

The present results show that there is a physiological interaction of E_2 and P_4 with renal nerves (catecholamine release levels) that affects Na^+/Cl^- reabsorption in rats. Female sex hormones play an important role in maintaining Na^+/Cl^- reabsorption in rats on a normal Na^+/Cl^- diet. The variations in RECA and PLCA levels related to female sex hormones may influence renal function. On the other hand, changes in the FE of Na^+ and Cl^- may be directly due to the effects of female sex hormones, independent of the main role of the renal nerves while on a normal Na^+/Cl^- diet. Further investigations are required to better elucidate the mechanisms of E_2 and P_4 on the plasma and renal sympathetic system and on renal function.

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