Defective renal dopamine function and sodium-sensitive hypertension in adult ovariectomized Wistar rats: role of the cytochrome P-450 pathway

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Di Ciano LA, Azurmendi PJ, Colombero C, Levin G, Oddo EM, Arrizurieta EE, Nowicki S, Ibarra FR. Defective renal dopamine function and sodium-sensitive hypertension in adult ovariectomized Wistar rats: role of the cytochrome P-450 pathway. Am J Physiol Renal Physiol 308: F1358–F1368, 2015. First published April 29, 2015; doi:10.1152/ajprenal.00450.2014.—We have previously shown that ovariectomy in adult Wistar rats under normal sodium (NS) intake results in an overexpression of the total Na⁺-K⁺-ATPase (NKA) α1-subunit (Di Ciano LA, Azurmendi PJ, Toledo JE, Oddo EM, Zotta E, Ochoa F, Arrizurieta EE, Ibarra FR. Clin Exp Hypertens 35: 475–483, 2013). Upon high sodium (HS) intake, ovariectomized (oVx) rats developed defective NKA phosphorylation, a decrease in sodium excretion, and an increment in mean blood pressure (MBP). Since NKA phosphorylation is modulated by dopamine (DA), the aim of this study was to compare the intracellular response of the renal DA system leading to NKA phosphorylation upon sodium challenge in intact female (IF) and oVx rats. In IF rats, HS caused an increase in urinary DA and sodium, in NKA phosphorylation state, in cytochrome P-450A4 (CYP4A) expression, and in 20-HETE production, while MBP kept normal. Blockade of the D1 receptor (D1R) with the D1-like receptor antagonist SCH 23390 in IFHS rats shifted NKA into a more dephosphorylated state, decreased sodium excretion by 50%, and increased MBP. In oVx rats, D1R expression was reduced and D3R expression was increased, and under HS intake sodium excretion was lower and MBP higher than in IFHS rats (both P < 0.05). NKA phosphorylation state varied among the groups. The alteration of the renal DA system produced by ovariectomy could account for the defective NKA phosphorylation, the inefficient excretion of sodium load, and the development of salt-sensitive hypertension.

Sexual hormones play an important role in the regulation of water and sodium balance (34). In previous studies, we have reported that ovariectomy results in a higher renal plasma flow (RPF) and a lower renal vascular resistance together with lower blood pressure compared with control intact female (IF) rats (4). Further results showed that ovariectomy causes an over-expression of the total Na⁺-K⁺-ATPase (t-NKA) α1-subunit in tubular epithelial cells as well as an altered balance between the phosphorylation/dephosphorylation of NKA to a more dephosphorylated (more active) state. As a consequence, when ovariectomized (oVx) rats were shifted from a normal-sodium diet (NS) to a high-sodium diet (HS), they were unable to achieve a normal sodium balance and developed salt-sensitive hypertension (13).

It is well documented that abnormalities in intrarenal dopamine (DA) production or DA receptor signaling can predispose to salt-sensitive hypertension (21, 47). Renal DA is involved in the regulation of sodium excretion under both low (12) and high (1, 6, 30) sodium intake. In this last scenario, it is responsible for almost 50–60% of urinary sodium excretion (11, 20), the decrease in tubular sodium reabsorption being the main mechanism responsible for its natriuretic effect. In addition to its action on other tubular sodium transporters (3), DA inhibits NKA activity (7, 9). This latter effect is, in part, the consequence of NKA phosphorylation by PKC at the Ser 23 residue, which renders a less active enzyme (18, 31, 40).

Based on data from other authors and on our previous findings that suggests an increase in renal DA production and its normal tubular function are necessary to eliminate the sodium overload under HS in intact rats (1); 2) stimulation of DA receptors activates PKC, which phosphorylates NKA, causing the inhibition of its activity (31, 32); and 3) altered NKA phosphorylation at the PKC site was found in oVx rats on HS intake together with the development of salt-sensitive hypertension (13), we hypothesized that the renal DA system could be involved in the altered sodium balance in oVx rats on HS.

Thus the aim of the present study was to explore the role of renal DA production, the known DA-triggered intracellular pathways that might be responsible for the abnormal NKA phosphorylation state in ovariectomized rats under HS intake, and to assess its role on sodium excretion and blood pressure regulation compared with intact female rats.
Fig. 1. Twenty-four-hour urinary dopamine (DA) excretion in intact females (IF; open bars) and ovariectomized rats (oVx; filled bars) under normal salt (NS) or high salt (HS) intake. Values are means ± SE (n = 6 rats/group). *P < 0.05 vs. respective NS rats by 1-way ANOVA.

MATERIALS AND METHODS

Animals. Female Wistar rats aged 150 days, from the Animal Breeding Facility of Instituto de Investigaciones Médicas A. Lanari, University of Buenos Aires, were used in this study. All protocols were performed according to the guidelines recommended by the National Institutes of Health and were reviewed by the Institutional Committee for Animal Welfare of the Faculty of Medicine, University of Buenos Aires (CICUAL). They were housed at 22 ± 2.2°C with a 12:12-h dark-light cycle.

At the age of 60 days, rats were anesthetized with a combination of ketamine (40 mg/kg body wt) and xylazine (5 mg/kg ip). Half of the animals were ovariectomized (oVx) while the others were subjected to an identical surgical procedure but ovaries were left intact (intact female; IF). After surgery, IF and oVx rats had free access to tap water and to a standard NS diet (0.24% NaCl) from Alimentos Cooperación (San Nicolás, Buenos Aires, Argentina). At the age of 145 days, rats from both groups were randomly assigned to a group on NS intake that was fed a standard diet and tap water, or a group on HS intake that was fed a standard diet and received 1% NaCl in the drinking water. On day 150, animals from the four experimental groups (IF-NS, IF-HS, oVx-NS, and oVx-HS) were placed in individual cages for 24-h urine collections. To minimize the effect of stress, all rats were previously acclimatized to individual cages.

Glomerular filtration rate and blood pressure recording. Two days before clearance studies subgroups of HS rats (IF and oVx) were treated with the D1-like receptor antagonist SCH 23390 (1 mg/kg body wt sc twice a day; R (+)-SCH 23390 hydrochloride, Sigma-Aldrich, St. Louis, MO) or with vehicle (normal saline). Animals were anesthetized with Inactin (50 mg/kg body wt ip) and surgically prepared for a renal clearance experiment as described previously (13). The trachea was cannulated to facilitate breathing. Catherets were placed in the carotid artery for blood pressure measurements and blood sampling and the jugular vein for intravenous infusions. Enough insulin (Fresenius Kabi, Graz, Austria) to provide plasmatic concentrations of 0.2 mg/ml was given as a prime, and a sustaining infusion diluted in saline was administered at 0.035 ml/min. The depth of anesthesia was controlled by testing the lack of response to stimulation of posterior limbs and by visual observation of stable and regular breathing. After a 45-min stabilization period, three consecutive 30-min urine samples were collected: 1) basal period; 2) DA or the D1-like receptor agonist fenoldopam (Sigma-Aldrich, St. Louis, MO) infusion (1 μg·kg body wt ·min⁻¹ for both); and 3) recovery (after the infusion was ceased). Volume of blood samples and fluid losses during surgery were replaced by corresponding amounts of normal saline solution.

Analysis of urine and plasma samples. Diuresis and urine sodium concentration were determined by gravimetry and flame photometry, respectively, whereas inulin was determined in plasma and urine samples by conventional methods (46). Urinary sodium excretion was calculated as UNa = V for each period. For DA analysis, 24-h urine samples were collected on 500 μl 6N HCl to prevent DA degradation. DA was extracted from urine samples using alumina, separated by reverse-phase high-pressure liquid chromatography using a 4.6 × 150-mm, 5-μm C18 column (Agilent Life Sciences and Chemical Analysis, Santa Clara, CA), and quantified amperometrically by a triple-electrode system (ESA, Bedford, MA) (14).

Renal tissue samples. Animals were euthanized at the end of the study, and the kidneys were removed, and homogenates from the cortex and outer medulla were prepared in protein homogenization buffer (in mM: 20 Tris, 2 EGTA, 2 EDTA, 1 PMSF, and 10 β-mercaptoethanol, and 100 KIU/ml aprotinin, pH 7.4) and were kept at −70°C. Protein concentration was measured using the Bradford method.

Western blotting. Proteins (15 μg) were separated on a SDS-PAGE gel and transferred to polyvinylidene difluoride membranes (PVDF Transfer Membranes, Pierce Biotechnology, Rockford, IL). Blots were probed with the following primary antibodies: D1R, D2R, D3R, PKCa, and cytochrome P-4504A (CYP4A). To normalize for protein loading, membranes were stripped, washed, and rebotted with mouse anti-β-actin antibody.

Phosphorylation of NKA was detected with a dephosphorylation degree-specific monoclonal antibody McK-1, which binds NKA at Ser-23 when this residue is dephosphorylated and not when it is phosphorylated.
phosphorylated by PKC. Thus the immunosignal from McK1 increases when PKC site Ser 23 becomes more dephosphorylated (8, 17, 23, 29). t-NKA was immunodetected by a regular monoclonal antibody, which recognizes NKA independently of its phospho-state. The phosphorylation state of NKA is expressed in density units from the dephosphorylated NKA/t-NKA ratio (d-NKA/t-NKA). Immunoreactive proteins were visualized using peroxidase-conjugated antibodies and an ECL Western Blotting substrate detection system (Pierce ECL Western Blotting Substrate, Pierce Biotechnology). Densitometric analysis of bands was performed by ImageJ 1.34s National Institutes of Health software.

**Antibodies.** The following antibodies were used: mouse anti-α1-NKA sc-21712 (1:7,000, Santa Cruz Biotechnology, Santa Cruz, CA); mouse McK1 (1:1,000, a kind gift from Dr. K. J. Sweadner); mouse anti-dopamine D1R monoclonal antibody MAB 5290 (1:500, Millipore, Solna, Sweden); goat polyclonal anti-D2R sc-7522 (1:2,500, Santa Cruz Biotechnology); rabbit polyclonal anti-dopamine D3 sc-9114 (1:500, Santa Cruz Biotechnology); rabbit polyclonal antibody to cytochrome P4504A (1:1,500, Abcam, Cambridge, UK); mouse anti-β-actin sc-47778 (1:2,000, Santa Cruz Biotechnology); peroxidase-conjugated anti-goat and peroxidase-conjugated anti-mouse antibodies (Vector Laboratories, Burlingame, CA); and peroxidase-conjugated anti-rabbit antibody (Abcam, Cambridge, MA).

c**AMP assay.** cAMP levels were assessed in renal cortical slices from IF and oVx rats under a NS or HS diet, incubated with or without DA (10⁻⁶ M) in modified Hank’s solution, pH 7.4, in the presence of phosphodiesterase inhibitor IBMX (10⁻³ M). cAMP was determined using the Cyclic AMP EIA kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s protocol. cAMP levels in each sample were assayed in triplicate and normalized by milligrams of tissue.

ω**Hydroxylase activity.** Renal cortical homogenates (0.5 mg protein) were incubated for 60 min at 37°C in 1 ml of 100 mM potassium phosphate buffer (pH 7.4), containing 10 mM MgCl₂ and 500 mM EDTA, 1 mM reduced NADPH, and 40 μM cold arachidonic acid in a shaking bath, with 100% O₂ superfusion. Reactions were terminated by acidification to pH 3.5 with formic acid. Samples were extracted twice with 1 ml of ethyl acetate, and the concentration of 20-HETE was determined by an ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

**Statistical analysis.** The results are reported as means ± SE. For three or more groups, one- or two-way ANOVA followed by a least significant difference post hoc test was used. A two-sided unpaired Student’s t-test was used for two-group comparisons. A P < 0.05 was considered as significant. The statistical analysis was performed using the SPSS statistics 17.0 program.

**RESULTS**

Body weight was 288 ± 5 g in oVx and 267 ± 7 g in IF rats (P < 0.05). Figure 1 shows that under NS daily urinary DA excretion was similar between IF and oVx rats. When challenged...
lenged with HS, both groups increased DA excretion to a similar extent over their respective NS values (P < 0.05 vs. NS for both).

The natriuretic response to DA or D1-like receptor agonist fenoldopam infusion was then tested in IF and oVx rats under NS or HS intake. Baseline sodium excretion was similar between IF and oVx rats. DA elicited a significant increase in sodium excretion only in IF rats either on NS or HS intake. On the contrary, in oVx rats the effect of exogenous DA on natriuresis was negligible (Fig. 2). A similar pattern of response was observed when fenoldopam was infused (n /H11005 4/group). In IF rats under NS or HS, fenoldopam significantly increased UNa /H11001 V (mol·30 min /H11002 1·100 g body wt /H11002 1) from 8.4 /H11006 3.8 to 29.7 /H11006 6.8 and from 15.0 /H11006 2.2 to 29.0 /H11006 6.4, respectively, both P < 0.05. Instead, in oVx rats, fenoldopam did not change UNa /H11001 V under NS or HS intake, from 7.5 /H11006 4.1 to 10.3 /H11006 5.0 and from 14.0 /H11006 5.4 to 10.4 /H11006 4.4, respectively, both P = NS.

We next examined whether ovariectomy affects renal expression of DA receptors. Analysis of the expression of D1R is shown in Fig. 3. Two bands were visualized at 75 and 55 kDa in both the renal cortex and renal medulla. Under NS, the expression of the two bands in the renal cortex was significantly lower in oVx compared with IF rats (Fig. 3A), and at the 75-kDa band the two-way ANOVA showed a significant interaction (oVx × sodium intake, P = 0.047). The same difference was observed in the renal medulla for the band at 55 kDa (Fig. 3B) with a significant interaction (oVx × sodium intake, P = 0.044). HS intake induced a reduction in the expression of the 75-kDa band only in the renal cortex of IF rats. No changes were observed after HS for the other analyzed bands.

The expression of D2R in the renal cortex and medulla was similar in IF and oVx rats under either NS or HS (Fig. 4), while D3R abundance in the renal cortex and medulla was increased in oVxNS rats compared with IFNS rats (Fig. 5). HS intake did not modify D3R expression in IF or oVx rats.

Table 1 depicts the functional response brought about by D1R blockade with the D1-like receptor antagonist SCH 23390. Treatment with SCH 23390 did not affect diuresis, sodium excretion, MBP, or glomerular filtration rate in IF or oVx rats under NS intake. The challenge with HS intake resulted in a lower sodium excretion together with increased levels of MBP in oVxHS compared with IFHS rats. Moreover, while in IFHS rats D1R blockade caused a marked decrease in diuresis and natriuresis together with a significant increment in MBP, in oVxHS rats it did not change any of these parameters. Glomerular filtration rate was similar between IFHS and oVxHS rats whether treated with D1-like receptor antagonist SCH 23390 or not.

Basal cAMP content was similar in outer cortical slices isolated from IF and oVx rats on NS and incubated with normal saline (0.69 ± 0.16 and 0.52 ± 0.11 pmol/mg, respectively, n = 5). Incubation with DA resulted in an equivalent increase

![Fig. 4. D2 receptor (D2R) expression in IF (open bars) and oVx rats (filled bars) under NS or HS intake. A: renal cortex. B: renal medulla. Top: representative blot for D2R (55-kDa band) and the corresponding β-actin from 6 independent experiments. Bottom: D1R/β-actin ratio in density units. Values are means ± SE. No differences were found among groups.](http://ajprenal.physiology.org/Downloadedfrom)
Renal function and systemic hemodynamic parameters of rats on high and normal sodium intake

<table>
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<tr>
<th>Parameter</th>
<th>Groups</th>
<th>IF NS</th>
<th>IF HS</th>
<th>oVx NS</th>
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<tr>
<td>Diuresis</td>
<td>ml-day^{-1}·100 g body wt^{-1}</td>
<td>11.51 ± 0.60</td>
<td>3.14 ± 0.03</td>
<td>112 ± 2</td>
<td>0.67 ± 0.06</td>
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<tr>
<td>U_{Na+}·V</td>
<td>mmol·day^{-1}·100 g body wt^{-1}</td>
<td>6.13 ± 0.19</td>
<td>1.65 ± 0.09</td>
<td>140 ± 2</td>
<td>0.71 ± 0.11</td>
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<td>MBP</td>
<td>mmHg</td>
<td>8.67 ± 1.28</td>
<td>2.08 ± 0.03</td>
<td>135 ± 4</td>
<td>0.64 ± 0.06</td>
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<tr>
<td>GFR</td>
<td>ml·min^{-1}·100 g body wt^{-1}</td>
<td>9.31 ± 2.26</td>
<td>2.41 ± 0.56</td>
<td>138 ± 4</td>
<td>0.69 ± 0.08</td>
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High sodium
- IFHS: 11.51 ± 0.60
- IFHS + SCH: 6.13 ± 0.19
- oVxHS: 8.67 ± 1.28
- oVxHS + SCH: 9.31 ± 2.26

Normal sodium
- IFNS: 3.94 ± 0.24
- IFNS + SCH: 3.48 ± 0.39
- oVxNS: 4.32 ± 0.16
- oVxNS + SCH: 4.00 ± 0.28

Values are means ± SE; n = 20 rats/group [diuresis, sodium excretion (U_{Na+}·V), and mean blood pressure (MBP)] and 5 rats/group (glomerular filtration rate; GFR). Intact female (IF) and ovariectomized (oVx) rats under high sodium (HS) or normal sodium (NS) intake were treated with the D1-like receptor antagonist SCH 23390 (SCH) or not. *P < 0.05, **P < 0.01 vs. IFHS group by 1-way ANOVA. In NS groups, values of diuresis, U_{Na+}·V, MBP, and GFR for rats treated with SCH 23390 or not did not show significant differences among them. The oVxNS group had the lowest MBP. Diuresis and natriuresis in NS groups were, however, always significantly lower than in HS groups (P < 0.01 for both).

Table 1. Renal function and systemic hemodynamic parameters of rats on high and normal sodium intake

Fig. 5. D3 receptor (D3R) expression in IF (open bars) and oVx rats (filled bars) under NS or HS intake. A: renal cortex. B: renal medulla. Top: representative blot for D3R and corresponding β-actin from 4–5 independent experiments. Bottom: D3R/β-actin ratio in density units. Values are means ± SE. A significant interaction (oVx × Na intake) was found in renal cortex (P < 0.012) and medulla, (P < 0.049). *P < 0.03 oVxNS vs. IFNS in renal cortex and P < 0.02 oVxNS vs. IFNS in renal medulla by 2-way ANOVA.

In cAMP in cortical slices from IF and oVx rats (1.09 ± 0.10 and 0.91 ± 0.17 pmol/mg, respectively, n = 5, P < 0.05 vs. their respective basal values for both).

The expression of the t-NKA α-1 subunit and its phosphorylation state were next explored. The NKA α-1 subunit is phosphorylated at Ser 23 residue by PKC. It has been previously shown that NKA is less active when phosphorylated and more active when the dephosphorylated state (d-NKA) predominates (8, 29, 31, 40). Figure 6 analyzes the role of D1R in the regulation of t-NKA α1-subunit expression and phosphorylation in IF and in oVx rats on HS intake. There was no difference in the expression of t-NKA in the renal cortex and medulla in IF or oVx rats, and it was not modified by the administration of the D1-like receptor antagonist SCH 23390 to either IF or oVx rats. On the contrary, the d-NKA signal in renal cortex and medulla was significantly lower in IF rats than in oVx rats. Therefore, the NKA α1-subunit is significantly more phosphorylated in IF than in oVx rats. Since D1R plays an important role in DA-induced phosphorylation of the NKA α1-subunit at Ser 23 residue, we tested whether the blockade of D1R may alter NKA phosphorylation state. Treatment with SCH 23390 caused a marked and significant increase in the d-NKA immunosignal in IFHS rat renal cortex and medulla (P < 0.05). This indicates that NKA turned more dephosphorylated as a consequence of D1R blockade in IFHS rats. In
oVxHS rats, on the other hand, NKA Ser 23 phosphorylation state was not altered by treatment with SCH 23390 in either the renal cortex or medulla.

Since the NKA α1-subunit is phosphorylated at the Ser 23 residue by PKCα, we next explored the expression of PKCα in renal homogenates from IF and oVx rats. Figure 7 shows that the expression of PKCα in the renal cortex and renal medulla was similar between rats on NS or HS irrespective of whether they were IF or oVx.

The catalytic activity of PKCα is regulated by the CYP4A-20-HETE pathway. Therefore, we analyzed the expression of CYP4A in renal cortex and medulla isolated from IF and oVx rats on NS or HS intake (Fig. 8). CYP4A expression in both the cortex and medulla was significantly increased under HS intake only in IF rats whereas it was not increased by sodium intake in oVx rats. Moreover, a significant decrease in CYP4A expression was observed in renal medullas from oVx rats. The renal cortical formation of 20-HETE, the product of CYP4A α-hydroxylase activity, was similar between IFNS and oVxNS rats. In line with changes in CYP4A expression, HS intake resulted in a significant increase in 20-HETE production only in IF but not in oVx rats (Fig. 9).

**DISCUSSION**

The results of this investigation point to an important role of the renal DA system in the development of salt-sensitive hypertension in oVx rats on HS intake. This conclusion is based on the abnormal DA signaling pathway in oVx rats that leads to the defective NKA phosphorylation state, which in its turn results in the inability to handle HS intake.

In the epithelial cells of proximal tubules of normal rats, DA is formed by the enzymatic decarboxylation of L-dopa ultrafiltered from plasma. DA release, mainly into tubular lumen, is triggered among other stimuli by sodium load (6, 42). Indeed, the natriuretic effect of DA is dependent on the state of Na⁺ balance. Under Na⁺ depletion, D1R-mediated natriuresis does not occur, whereas under Na⁺ repletion the stimulation of D1R induces a robust natriuretic response (36). Recent studies confirmed the importance of an intact dopaminergic system in preventing the increase in blood pressure in mice fed on a HS diet (47).

In our hands, the increase in sodium intake was associated with a similar increase in renal DA excretion in both intact and oVx rats, suggesting an intact tubular capacity for DA synthet-
sis in oVx rats. However, despite the similar DA excretion, sodium output after HS intake was lower in oVx than in control rats, and this was followed by the increase in blood pressure in oVx animals. The lack of changes in glomerular filtration rate among groups suggests that the lower sodium excretion in oVx rats may be mainly due to defective tubular transport in these animals. Interestingly, treatment of IFNS with the D1-like receptor antagonist SCH 23390 had no effect on sodium excretion and blood pressure whereas it significantly reduced sodium excretion and increased blood pressure in IFHS rats. On the other hand, none of these parameters was changed by administration of SCH 23390 to oVx rats even under HS intake. This prompted us to speculate that ovariectomy results in an alteration in D1R function. In addition, the infusion of DA or the D1-like receptor agonist fenoldopam resulted in increased sodium excretion in IF rats under either NS or HS intake but not in oVx rats, strengthening the hypothesis that D1R function is deficient in oVx rats.

While the expression of D2R was similar among all groups, the expression of D1R (75- and 55-kDa bands) was lower in renal cortex from oVxNS compared with IFNS rats. A reduction in D1R protein abundance at the 75-kDa band was observed under HS intake only in renal cortex from IF rats. The 75-kDa band has been reported to be the most important one since it is placed in the lipid rafts at the plasma membrane (45). Our results are in line with previous observations from other authors who reported lower expression of D1R in hypertensive patients (5, 10) or animal models of experimental hypertension (41). Regarding sodium intake, while some reports describe that HS intake does not modify D1R abundance (41), a decrease in D1R expression during HS intake has been reported (5, 10).

In our hands, under NS intake, D3R expression was higher in oVx than in IF rats. However, this difference was no longer kept under HS intake. Other authors have reported that D3R may have a compensatory role in pathological conditions where D1R expression is decreased (10, 41). Thus in oVxNS rats the increment in D3R abundance might help to maintain sodium excretion and blood pressure within normal levels under NS intake, being that this compensatory mechanism is lost after sodium challenge.

Further experiments performed in rats on HS intake showed a different phosphorylation state of NKA when IF were compared with oVx rats. It is well known that upon PKC phosphorylation NKA becomes less active, resulting in lower tubular sodium reabsorption (2, 8). In IF rats on HS intake, a tonic PKC phosphorylation of NKA was evidenced by the low d-NKA/t-NKA ratio in the renal cortex and medulla. Administration of the D1-like receptor antagonist SCH 23390 to IF rats on HS intake increased the d-NKA/t-NKA ratio compared with nontreated IFHS animals, thus confirming that stimulation of D1R is necessary to maintain the phosphorylated state of NKA under HS intake. On the contrary, in oVx rats on HS intake phosphorylation of NKA was significantly lower than in IFHS rats (higher d-NKA/t-NKA ratio) and was not further modified by SCH 23390.

It is well known that stimulation of D1R in tubule cells is linked to both activation of adenylyl cyclase and PLC. The activation of adenylyl cyclase leads to an increase in cAMP levels and the subsequent activation of PKA, whereas activation of PLC leads to the generation of inositol triphosphate and diacylglycerol (DAG), a physiological PKC activator (18). Additionally, DA induces receptor-mediated stimulation of PLA2, which releases arachidonic acid from membrane phos-
pholipids (32). We have previously shown that the main product of arachidonic acid metabolism by CYP4A-20-HETE synergizes with DAG for the activation of PKC, which in turn phosphorylates NKA (26, 31). 20-HETE inhibits sodium transport in the proximal tubule (35) and in the thick ascending limb of Henle (TALH) (15). Moreover, previous results from our laboratory have demonstrated in vivo the contribution of 20-HETE to the inhibition of sodium transport at the proximal tubule and TALH levels (16). As shown by our present results, neither the content of cAMP nor the expression of PKC was affected by hormonal status. Therefore, we explored the CYP4A pathway, which has an important role in the modulation of PKC activity through the generation of 20-HETE. In IF rats, CYP4A expression and activity were increased by sodium intake. This result is in line with previous observations by other authors using Sprague-Dawley (38) or Dahl salt-resistant rats (39). In oVx rats instead, neither CYP4A expression nor its activity in renal cortex were changed by HS intake. Thus we may speculate that the molecular mechanisms responsible for the sodium-related CYP4A overexpression are hormone dependent. The expression of CYP4A is under the transcriptional control of the peroxisome proliferator activated-α receptors (PPARα) that are ligand-activated transcription factors of the nuclear hormone receptor superfamily. PPARα modulates transcription via binding to a specific DNA sequence element called a peroxisome proliferator response element (PPRE) (25). Interestingly, it has been shown that estrogen deficiency induced by ovariectomy reduces the expression of PPARα in rat liver and skeletal muscle (33, 24). Therefore, if a similar regulatory mechanism to the one previously reported in other tissues was also present in the kidney, the lower expression of PPARα in oVx rats might serve as an explanation for the inability of oVx rats to overexpress CYP4A in response to HS intake, as shown by our present results.

In line, Yanes and colleagues (44) have reported lower renal microsomal CYP4A protein expression and activity together with an increase in blood pressure in postmenopausal spontaneously hypertensive rats compared with young matches. The authors speculate that a decrease in 20-HETE synthesis in renal tubules would be prohypertensive due to its inhibitory effect on sodium reabsorption (44). Accordingly, the inability to upregulate CYP4A expression or to increase 20-HETE production in response to a HS diet derived in the development of salt-sensitive hypertension in rats (22, 39) and humans (27). Thus we may hypothesize that a lack of increment in CYP4A expression in response to HS intake in oVx rats may have contributed to sodium retention and development of hypertension in our experimental model.

Taken together, these findings suggest that either a reduction in D1R or a defective response of CYP4A to sodium load, or a combination of both may account for altered PKC phosphorylation of NKA, leading to the oVx rats’ inability to increase sodium excretion.

To our knowledge, this is the first report of studies performed in vivo showing evidence that connect D1R stimulation to changes in NKA phosphorylation state and its implication in sodium excretion during HS intake. Previous studies have emphasized the role of nitric oxide and angiotensin II in gender- and age-related alterations in renal function (34). This work shows that the renal DA system can also be compromised.

Fig. 8. Cytochrome P450A4 (CYP4A) expression in IF (open bars) and oVx rats (filled bars) rats under NS or HS intake. A: renal cortex. B: renal medulla. Top: representative blot for CYP4A and β-actin from 4–5 independent experiments. Bottom: CYP4A/β-actin ratio in density units. Values are means ± SE. *P < 0.05, **P < 0.025 by unpaired Student’s t-test.
in the salt-sensitivity pattern described in menopausal and postmenopausal states. The presence or alteration of female hormones seems to be relevant to this pathophysiological condition. Indeed, the decrease in estrogen levels in aging female spontaneously hypertensive rats (SHR) has been shown to be associated with a decrease in endothelial nitric oxide synthase expression and nitric oxide production, resulting in poor vasodilation and higher blood pressure levels compared with adult rats (43). Besides, other researchers have shown that in old uninephrectomized-ovariectomized SHR, estrogen supplementation ameliorates the renal damage observed in sham-treated rats (19). The results of the present work are in line with these reports, highlighting the importance of estrogens, or in opposition, of their lack, in keeping an efficient tubular DA pathway to ensure normal renal function and blood pressure levels.

It is worth noting that oVx rats, even on NS intake, already had a reduced abundance of D1R together with an increase in D3R expression, and a lack of response to infused DA. In line, other authors have described that ovariectomy induces a decrease in the density of striatal D1R that was corrected by chronic treatment with 17β-estradiol (28). This observation was confirmed by other authors who demonstrated that estrogens induce the upregulation of D1 gene transcription and provide a mechanism for modulation of central dopaminergic functions (37). If a similar modulatory mechanism was operative in the kidney, we may hypothesize that the lack of estrogens may account at least in part for the decreased D1R expression in our experimental model. Thus our findings may indicate the presence of a prehypertensive condition in oVx rats that was uncovered by sodium load.

The schematic diagram shown in Fig. 10 illustrates the similarities and differences in the response of the DA system to HS intake in IF and oVx rats.

In summary, our results show that with the exception of renal DA generation, all the other components of the renal dopaminergic system analyzed in this study are completely...
asymmetric when IF rats are compared with oVx rats. The renal DA system is greatly distorted in ovary hormone-deprived rats and, as a consequence, they are unable to handle HS intake. Then, a normotensive rat strain develops salt-sensitive hypertension. Data support the crucial role of gonadal steroids in regulating renal function and systemic hemodynamics as well as renal sodium handling, particularly under HS intake. A better understanding of the renal DA system in postmenopausal women as related to renal disease and blood pressure control could provide insights into new preventive and therapeutic strategies.

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AUTHOR CONTRIBUTIONS

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