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ORIGINAL RESEARCH

Evidence for G-Protein–Coupled Estrogen Receptor as a Pronatriuretic Factor

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BACKGROUND: The novel estrogen receptor, G-protein–coupled estrogen receptor (GPER), is responsible for rapid estrogen signaling. GPER activation elicits cardiovascular and nephroprotective effects against salt-induced complications, yet there is no direct evidence for GPER control of renal Na^+ handling. We hypothesized that GPER activation in the renal medulla facilitates Na^+ excretion.

METHODS AND RESULTS: Herein, we show that infusion of the GPER agonist, G1, to the renal medulla increased Na^+ excretion in female Sprague Dawley rats, but not male rats. We found that GPER mRNA expression and protein abundance were markedly higher in outer medullary tissues from females relative to males. Blockade of GPER in the renal medulla attenuated Na^+ excretion in females. Given that medullary endothelin 1 is a well-established natriuretic factor that is regulated by sex and sex steroids, we hypothesized that GPER activation promotes natriuresis via an endothelin 1–dependent pathway. To test this mechanism, we determined the effect of medullary infusion of G1 after blockade of endothelin receptors. Dual endothelin receptor subtype A and endothelin receptor subtype B antagonism attenuated G1-induced natriuresis in females. Unlike males, female mice with genetic deletion of GPER had reduced endothelin 1, endothelin receptor subtype A, and endothelin receptor subtype B mRNA expression compared with wild-type controls. More important, we found that systemic GPER activation ameliorates the increase in mean arterial pressure induced by ovariectomy.

CONCLUSIONS: Our data uncover a novel role for renal medullary GPER in promoting Na^+ excretion via an endothelin 1–dependent pathway in female rats, but not in males. These results highlight GPER as a potential therapeutic target for salt-sensitive hypertension in postmenopausal women.

Key Words: endothelin 1 ■ estrogen ■ GPER ■ hypertension ■ kidney ■ sodium excretion

Premenopausal women have a lower risk of cardiovascular and renal diseases compared with age-matched men. This female advantage is lost when women reach menopause, pointing to a fundamental role for ovarian hormones, particularly estradiol, in protecting the cardiovascular and renal health in premenopausal women. In addition to the well-established classic genomic effects of this sex steroid, rapid nongenomic responses to estradiol have also been reported. The G-protein–coupled estrogen receptor (GPER) is a membrane-associated receptor linked to short-term estrogenic signaling pathways.[1–6] More important, GPER is widely expressed throughout the body, including the kidney.[7–9] However, the physiological and pathophysiological role of renal GPER is poorly understood.

Similar to classic steroid receptors, estrogen receptors α and β, GPER is implicated as a protective pathway in cardiovascular and kidney disease.[8,10–12] Activation of GPER acutely lowers blood pressure in normotensive rats[13] and ameliorates renal and salt-induced injury.[8,10,12] GPER activation reduces oxidative

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CLINICAL PERSPECTIVE

What Is New?

- This study identifies a novel mechanism by which females efficiently excrete sodium and maintain blood pressure.
- We also report that the G-protein–coupled estrogen receptor facilitates sodium excretion, at least in part, through activation of the renal medullary endothelin system.

What Are the Clinical Implications?

- This study highlights the importance of the G-protein–coupled estrogen receptor in regulating blood pressure and sodium excretion in females.
- G-protein–coupled estrogen receptor activation is a potential therapeutic target for salt-sensitive hypertension in postmenopausal women.

Nonstandard Abbreviations and Acronyms

ER	estrogen receptors
ET-1	endothelin 1
ET_A	endothelin receptor subtype A
ET_B	endothelin receptor subtype B
GPER	G protein-coupled estrogen receptor
MAP	mean arterial pressure
U_KV	urinary K^+ excretion
$U_{Na}V$	Urinary Na^+ excretion
UV	urine flow

stress and proteinuria as well as attenuates aortic remodeling in the salt-sensitive female mRen2.Lewis rats.[8,10] Activation of GPER blunts diastolic dysfunction induced by a high salt diet in intact female mRen2 rats.[14] In addition, the GPER locus in the human genome has been associated with hypertension.[15]

A series of recent studies have reported sex-dependent discrepancies in mechanisms that regulate Na^+ handling within the renal medulla.[16–21] Given the critical role of the renal medulla in fine-tuning of Na^+ excretion and blood pressure, we designed experiments to identify the effect of GPER on Na^+ handling within the renal medulla. We hypothesized that GPER within the renal medulla promotes natriuresis. We tested our hypothesis by determining the excretory response to intramedullary infusion of the GPER agonist, G1, in age-matched male and female Sprague Dawley rats. To provide further mechanistic insight, we explored a possible relationship between GPER and the renal endothelin 1 (ET-1) system because of its importance in Na^+ handling in the renal medulla. To elucidate the role of the endogenous ligand, estradiol, on GPER pronatriuretic effects, we determined the effect of ovariectomy on GPER activation within the renal medulla. Additional experiments determined whether GPER has long-term effects on blood pressure in ovariectomized rats.

METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request.

Animals

Male and female (16–20 weeks of age) Sprague Dawley rats from Envigo (Indianapolis, IN) were used. Rats used in intramedullary infusion experiments were maintained on our standard 7917 Irradiated NIH-31 Mouse/Rat diet (0.8% NaCl; Envigo). Rats used in telemetry experiments were maintained on TD 96208 (0.49% NaCl [Envigo], matched control diet for our high salt diet, TD 92034, 4% NaCl [Envigo]). Male and female wild-type and GPER knockout mice (18–25 weeks of age) were generated and genotyped as previously described.[22,23] Mice were maintained on 5053 Picolab Rodent diet 20 (0.82% NaCl; LabDiet, St. Louis, MO). Animals were conventionally housed in a temperature (18°C–23°C) controlled room with a 12:12-hour light-dark cycle with free access to food and water. All protocols were in accordance with the *Guide for the Care and Use of Laboratory Animals* and were approved in advance by the University of Alabama at Birmingham or Tulane University Institutional Animal Care and Use Committee.

Short-Term Intramedullary Infusion

Rats were anesthetized using thiobutabarbitone (Inactin, hydrate, 100 mg/kg, IP, T133; Sigma-Aldrich Co, St. Louis, MO) at 8 to 11 AM and surgically prepared, as detailed in our previous studies.[17] Animals were then allowed to equilibrate for 60 to 80 minutes, before a 20-minute baseline urine collection, during which the vehicle (0.02% dimethyl sulfoxide in saline) was infused into the renal medulla. This was followed by intramedullary infusion of the GPER agonist, G1 (5 pmol/kg per minute, 41004001, purity ≥98%; Sandia Biotec Inc, Albuquerque, NM; n=8 female rats, n=9 male rats, n=8 ovariectomized rats) and/or GPER antagonist, G15 (5 pmol/kg per minutes, 41004003, purity ≥99%; Sandia Biotec Inc; n=4 and 9 female rats, respectively) for an additional 20-minute period (Figure 1A). In an additional group of female rats (n=6), we continued to infuse the vehicle for the second 20-minute urine collection period to serve as a time control. At the end

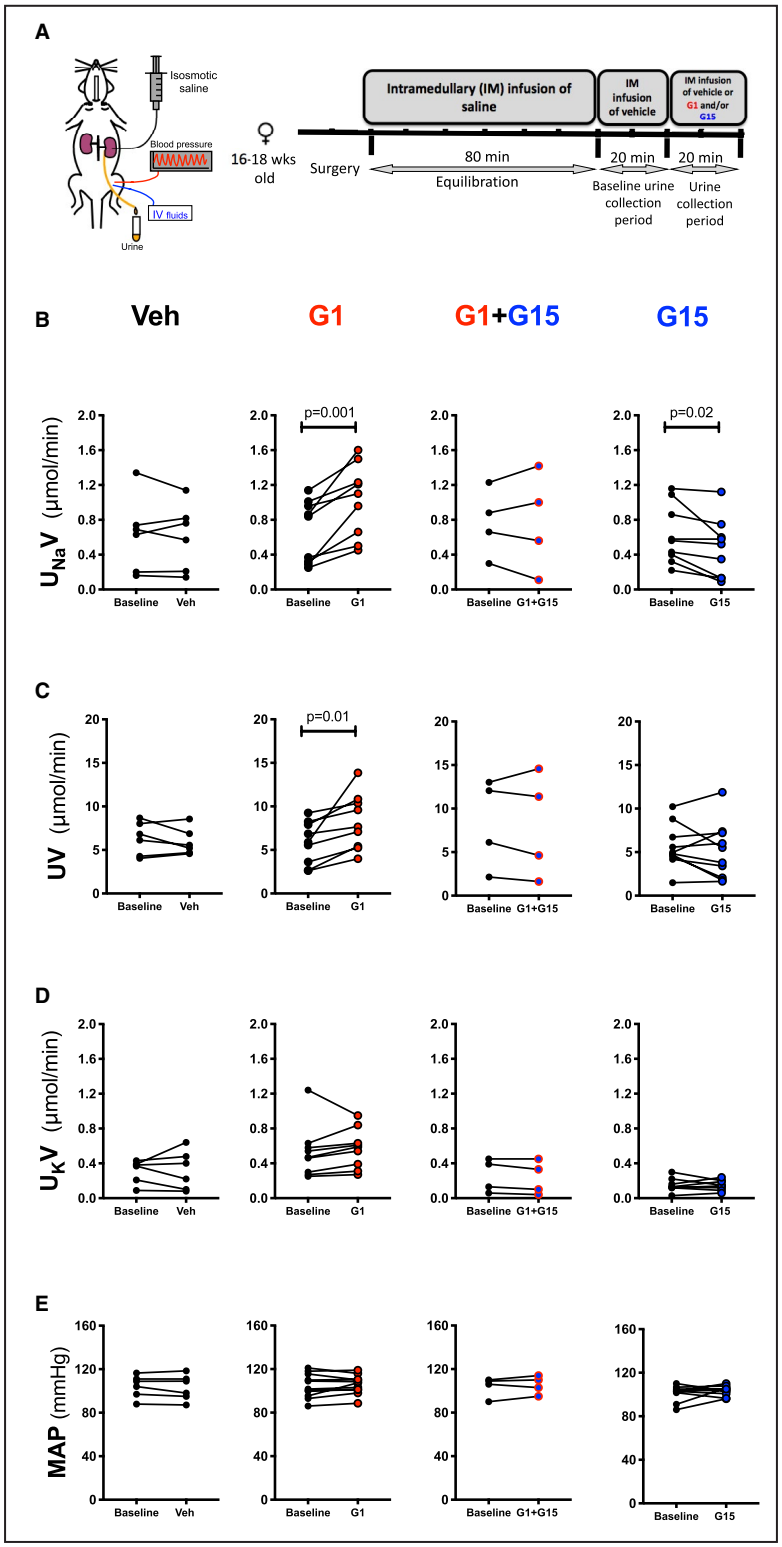

Figure 1. Activation of G-protein–coupled estrogen receptor (GPER) in the renal medulla facilitates urinary Na^+ excretion in female rats.
Schematic presentation of the surgical procedure and experimental time line employed (**A**). Urinary Na^+ excretion ($U_{Na}V$) (**B**), urine flow (UV) (**C**), urinary K^+ excretion (U_KV) (**D**), and mean arterial pressure (MAP) (**E**) in anesthetized intact female Sprague Dawley rats receiving renal medullary interstitial infusions of vehicle (Veh), G1 (GPER agonist, 5 pmol/kg per minute), G1+G15 (GPER antagonist, 5 pmol/kg per minute), or G15 alone, respectively. n=4 to 9 in each group. Statistical comparisons performed by paired Student *t* test.

of each experiment, the kidney was dissected to confirm correct positioning of the catheter. Urinary Na^+ and K^+ concentrations were measured using an atomic absorption spectrometer (iCE 3000 series paired with a CETAC ASX-520 AutoSampler; Thermo Fisher Scientific, Waltham, MA).

A separate group of female rats (n=5) was used to determine the role of endothelin receptor subtype A (ET_A) and endothelin receptor subtype B (ET_B) in response to medullary GPER activation. Rats received an IV bolus injection (0.5 mL/kg via femoral vein catheter) of a combination of the ABT-627 (selective ET_A receptor antagonist, 5 mg/kg; AbbVie Inc, Abbott Park, IL) simultaneously with the selective ET_B receptor antagonist A-192621 (10 mg/kg; PepTech Corp, Bedford, MA) 30 minutes before the end of the equilibration period. These doses are known to maintain efficient blockade of ET_A and ET_B receptors, respectively, in male rats for the duration of our experimental protocol, as previously demonstrated.[17] After the equilibration period and the 20-minute baseline urine collection during which vehicle was infused, G1 was infused for an additional 20-minute urine collection period.

RNA Extraction and Gene Expression Assessment by Reverse Transcription–Polymerase Chain Reaction

Tissue RNA was isolated using Purelink Mini RNA extraction kit (12183018A; Thermo Fischer Scientific), according to manufacturer's instructions. The isolated RNA was reverse transcribed using QuantiTect Reverse Transcription kit (Qiagen, Hilden, Germany). The resulting cDNA was used to quantify mRNA by reverse transcription–polymerase chain reaction (CFX96 Real-Time System; BIORAD) using TaqMan primer gene expression assays with rat GPER (Rn00592091_s1), mouse ET-1 (Mm00438656_m1), mouse ET_A receptor (Mm01243722_m1), mouse ET_B receptor (Mm00432989_m1), mouse β-actin (Mm02619580_g1), and rat β-actin (Rn00667869_m1) primers. Gene expression was quantified relative to β-actin using $2^{-\Delta\Delta Ct}$ method.

Western Blotting

Samples were processed as previously described.[24] Briefly, 20 µg of renal outer and inner medulla was separated, transferred, and incubated in goat GPER primary antibody (AF5534; R&D, Minneapolis, MN) at 1:500 dilution or Na^+/K^+ ATPase α-subunit primary antibody (Developmental Studies Hybridoma Bank at the University of Iowa) at 1:1000 dilution. Donkey anti-goat (H+L) cross-adsorbed secondary antibody, Alexa Fluor 680 (A-21109; Invitrogen, Waltham, MA) at 1:500 dilution, and goat anti-mouse IgG (H+L) secondary antibody DyLight 680 (35518; Thermo Fisher Scientific) at 1:1000 were used for GPER and Na^+/K^+ ATPase α-subunit, respectively. Coomassie Blue staining was performed to ensure equal loading.

Na^+/K^+ ATPase Activity

Renal outer medullary Na^+/K^+ ATPase activity was measured using Na^+/K^+ ATPase Microplate Assay Kit (MBS8243226; MyBioSource, San Diego, CA), according to manufacturer's instructions.

Telemetry

Blood pressure measurements were made using HD-S10 transmitters (Data Sciences International, Duluth, MN), as previously described.[25] Rats were allowed to recover for 7 to 10 days postsurgically before baseline blood pressure and heart rate recording.

Ovariectomy

Female rats were subjected to bilateral ovariectomy, as described previously.[19] Three weeks after ovariectomy, rats were used in short-term intramedullary infusion experiments.

Osmotic Mini Pump Implantation

At the same time of ovariectomy, rats underwent subcutaneous implantation of an Alzet osmotic pump (model 2ML4; Cupertino, CA) that infused G1, a GPER agonist (400 µg/kg per day, 41004003; Sandia Biotec Inc; n=6) or vehicle (75% dimethyl sulfoxide in saline; n=6). This dose of G1 has been shown in previous studies to elicit cardiovascular protection in rats.[26,27] More important, G1 is a potent and selective GPER agonist. Even at high concentrations, G1 (1–10 µmol/L) displays no activity toward classic estrogen receptors.[28,29] G1 showed no activity toward a panel of 25 other important G-protein–coupled receptors,[30] and elicited no activity in GPER knockout mice.[13,23,31]

Metabolic Cages

Rats were placed into metabolic cages, allowing daily quantitative urine collections and measurements of intake on day 14 after performing ovariectomy and pump implantation surgeries. The 24-hour urine samples were collected at 8 AM.

Statistical Analysis

Data are presented as mean±SEM. Statistical tests used for each data set are specified in each figure legend. This includes analysis by paired and unpaired Student *t* test. Two-way ANOVA followed by Sidak's post hoc tests were also used for data analysis, where indicated. A probability of $P<0.05$ was considered statistically significant. Statistical analysis was performed using GraphPad Prism version 8.

RESULTS

GPER-Induced Natriuresis in Female Rats

Medullary infusion of G1 significantly increased Na^+ excretion in female Sprague Dawley rats by 70±22% relative to baseline (Figure 1B). Urine flow also significantly increased at commencing intramedullary infusion of G1 (Figure 1C). In time control experiments where vehicle was infused, urine flow and electrolyte excretion were not significantly changed (Figure 1B and 1C). Medullary infusion of the GPER antagonist, G15, simultaneously with G1 prevented G1-evoked natriuretic and diuretic effects (Figure 1B and 1C). Urinary K^+ excretion (Figure 1D) and mean arterial pressure (MAP) (Figure 1E) were not significantly changed during intramedullary infusions of vehicle, G1 alone, or when combined with G15. Of note, many females elicited a small increase in urinary K^+ in response to G1 infusion; however, this effect did not reach statistical significance (Figure 1D). G1 infusion slightly decreased medullary blood flow, 7±2%, relative to baseline (n=5). This was not significantly different compared with the change observed during vehicle infusion, 14±5% (n=5).

To determine the contribution of GPER activation by its endogenous ligand, estradiol, in facilitating basal Na^+ excretion in females, we performed experiments with intramedullary infusion of G15. G15 significantly decreased urinary Na^+ excretion by 30±9% relative to baseline (Figure 1B). Urine flow did not significantly change during medullary blockade of GPER (Figure 1C). Urinary K^+ excretion and MAP were not significantly altered by G15 (Figure 1D and 1E).

In contrast to data from female rats, medullary activation of GPER did not significantly increase urinary Na^+ excretion or urine flow in male rats (Figure 2A and 2B). Urinary K^+ excretion and MAP also remained unchanged during G1 infusion in males (Figure 2C and 2D). GPER mRNA expression was 5-fold higher in outer medulla from female rats, compared with males (Figure 2E). Similarly, outer medullary GPER protein abundance was higher in females relative to males (Figure 2E). We did not observe a significant sex-related difference in inner medullary GPER mRNA expression or protein abundance; however,

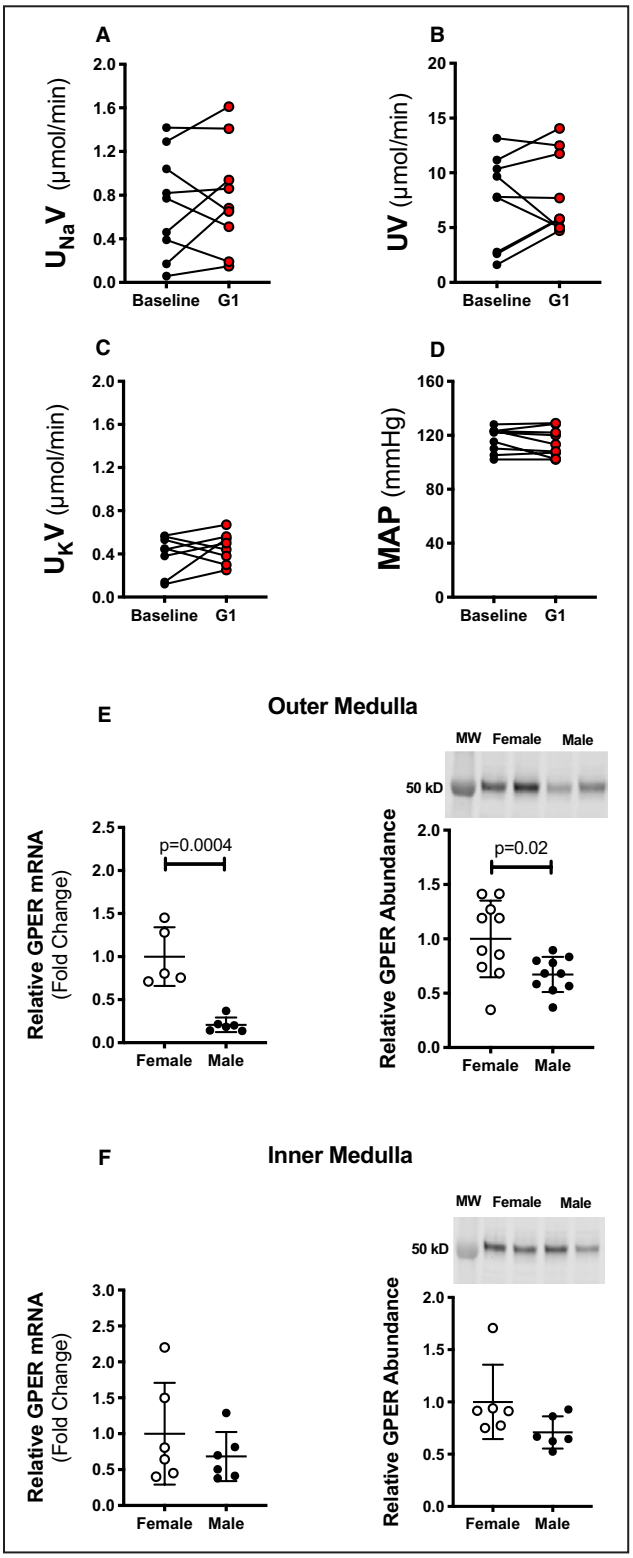

Figure 2. Activation of G-protein–coupled estrogen receptor (GPER) in the renal medulla did not change urinary Na^+ excretion in male rats.

Effect of renal medullary interstitial infusions of the GPER agonist, G1 (5 pmol/kg per minute) on urinary Na^+ excretion ($U_{Na}V$) (**A**), urine flow (UV) (**B**), urinary K^+ excretion (U_KV) (**C**), and mean arterial pressure (MAP) (**D**) in anesthetized male Sprague Dawley rats. Relative mRNA expression and protein abundance of GPER in outer medulla (**E**) and inner medulla (**F**) from male and female rats (representative Western blots are presented). Gene expression values represent fold change from corresponding female levels. Protein abundance is presented relative to female levels. n=5 to 10 rats in each group. Statistical comparisons performed by paired Student *t* test (**A** through **D**) and unpaired Student *t* test (**E** and **F**).

there was a trend toward lower abundance in males that did not reach statistical significance (Figure 2F).

GPER and ET-1 Pathway Interactions

Dual blockade of ET_A and ET_B receptors completely abolished the natriuretic and diuretic response to medullary GPER activation (Figure 3A and 3B). Urinary K^+ excretion and MAP remained unchanged during these experiments (Figure 3C and 3D). We also determined the effect of genetic ablation of GPER on mRNA expression of renal ET-1, ET_A, and ET_B. GPER knockout resulted in significant reductions in the expression of ET-1, ET_A, and ET_B receptors only in female mice, but not in males (Figure 3E). ET_B receptor expression in wild-type mice was significantly lower in males relative to females (Figure 3E).

Na^+/K^+ ATPase

Given that ET receptor activation mediates GPER pronatriuretic effects and ET-1 has been previously shown to inhibit Na^+/K^+ ATPase activity,[32] we tested whether GPER activation reduces Na^+/K^+ ATPase activity and protein abundance. Specifically, we determined the effect of medullary GPER activation on the activity of Na^+/K^+ ATPase and the protein abundance of its α-subunit in the outer medulla, where we have observed a significant sex difference in GPER expression and protein abundance. We found that infusion of G1 to the renal medulla in females significantly reduced Na^+/K^+ ATPase activity in the outer medulla, in comparison to the contralateral kidneys (0.45±0.02 versus 0.53±0.02 U/100 mg; n=5 each; Figure S1). However, the protein abundance was not different between G1-infused and contralateral kidneys (Figure S1).

Medullary GPER in Ovariectomized Rats

To test whether the loss of estradiol, endogenous GPER ligand, exaggerates the natriuretic response

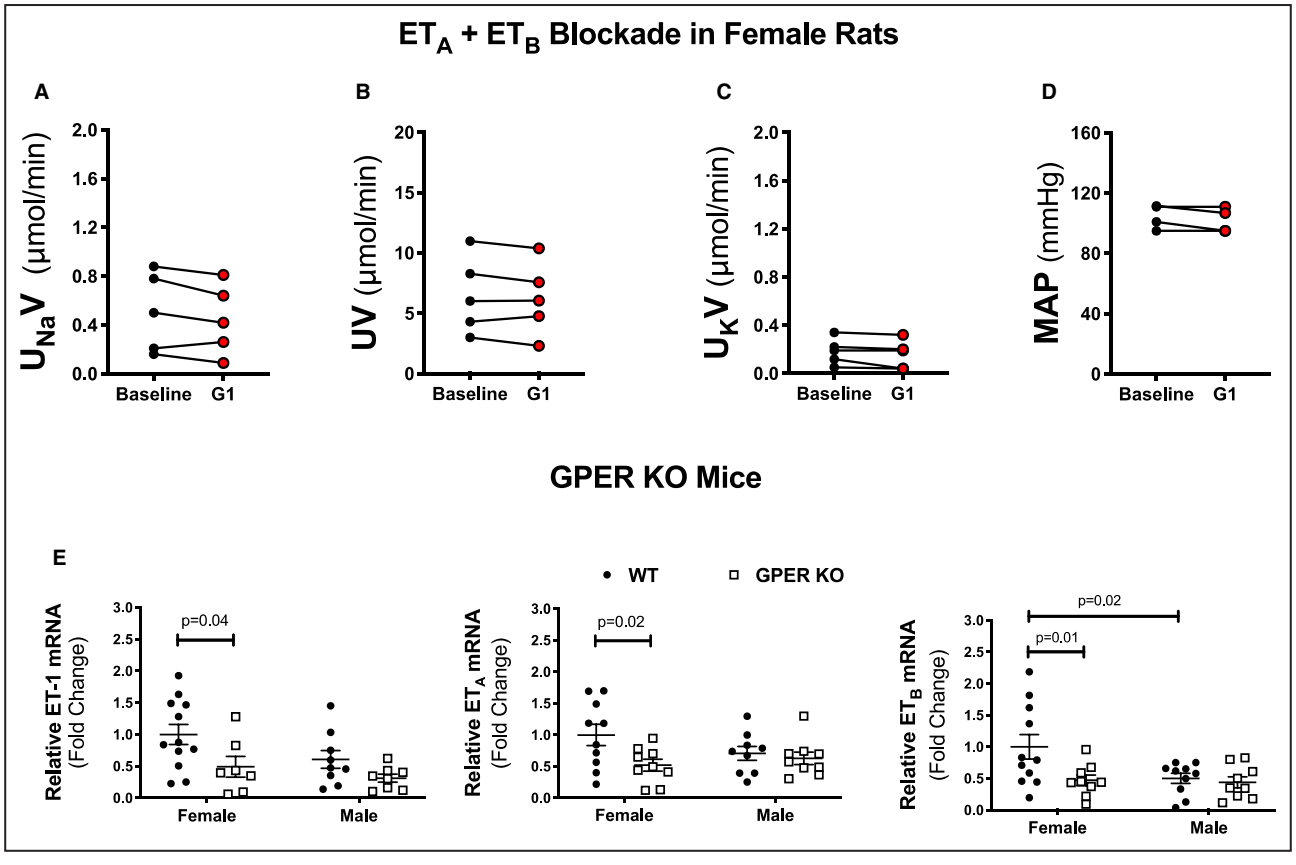

Figure 3. Pharmacologic and genetic evidence for the interaction between G-protein–coupled estrogen receptor (GPER) and renal endothelin 1 (ET-1) system in females.

Effect of renal medullary interstitial infusions of the GPER agonist, G1 (5 pmol/kg per minute), after a bolus injection of the selective endothelin receptor subtype A (ET_A) receptor blocker (ABT-627) and the selective endothelin receptor subtype B (ET_B) receptor blocker (A-192621) on urinary Na^+ excretion ($U_{Na}V$) (**A**), urine flow (UV) (**B**), urinary K^+ excretion (U_KV) (**C**), and mean arterial pressure (MAP) (**D**) in anesthetized intact female Sprague Dawley rats. mRNA expression of renal ET-1, ET_A, and ET_B receptors (**E**) in male and female wild-type (WT) and GPER knockout (KO) mice. n=5 to 12 animals in each group. Statistical comparisons performed by paired Student *t* test (**A** through **D**) and 2-way ANOVA with Sidak's post hoc test for multiple comparisons (**E**).

to exogenous GPER activation, ovariectomized rats were subjected to the same experimental protocol depicted in Figure 1A. In ovariectomized rats, medullary activation of GPER significantly increased urinary Na^+ excretion by 64±16% relative to baseline (Figure 4A). No significant changes in urinary K^+ or MAP were observed (Figure 4C and 4D). No significant differences in G1-induced natriuresis or diuresis between intact female rats and ovariectomized rats were observed (Figure 4A and 4B). GPER mRNA expression in the outer medulla from ovariectomized rats was increased relative to intact females (Figure 4E). In contrast, ovariectomy resulted in a decrease in GPER mRNA expression within the inner medulla (Figure 4F). However, ovriectomy did not have significant impact on GPER protein abundance in either the outer medulla or the inner medulla (Figure 4E and 4F).

Long-Term Systemic Activation of GPER

To determine whether GPER activation contributes to long-term blood pressure regulation, we determined the effect of systemic activation of GPER on blood pressure elevation in ovariectomized rats (Figure 5A). Ovariectomy significantly increased MAP in vehicle-treated rats, compared with their baseline before ovariectomy (Figure 5B). This increase in MAP was prevented by G1 (Figure 5B). Similar hypertensive responses to ovariectomy were observed in both systolic and diastolic blood pressure in vehicle-treated rats (Figure 5C and 5D). G1 abolished ovariectomy-induced increase in systolic blood pressure, but not diastolic blood pressure (Figure 5C and 5D). Heart rate was increased in both vehicle- and G1-treated rats in response to ovariectomy (Figure 5E). After day 11 postovariectomy, the increase in heart rate was normalized in vehicle-treated rats only; however, heart rate remained increased in G1-treated animals relative to baseline (Figure 5E). G1 supplementation to ovariectomized rats did not have a significant impact on body weight or food or water intake (Figure 6A through 6C). Urine volume and excretion of Na^+ and K^+ remained unchanged in G1-treated rats, relative to vehicle-treated ovariectomized rats (Figure 6D through 6F).

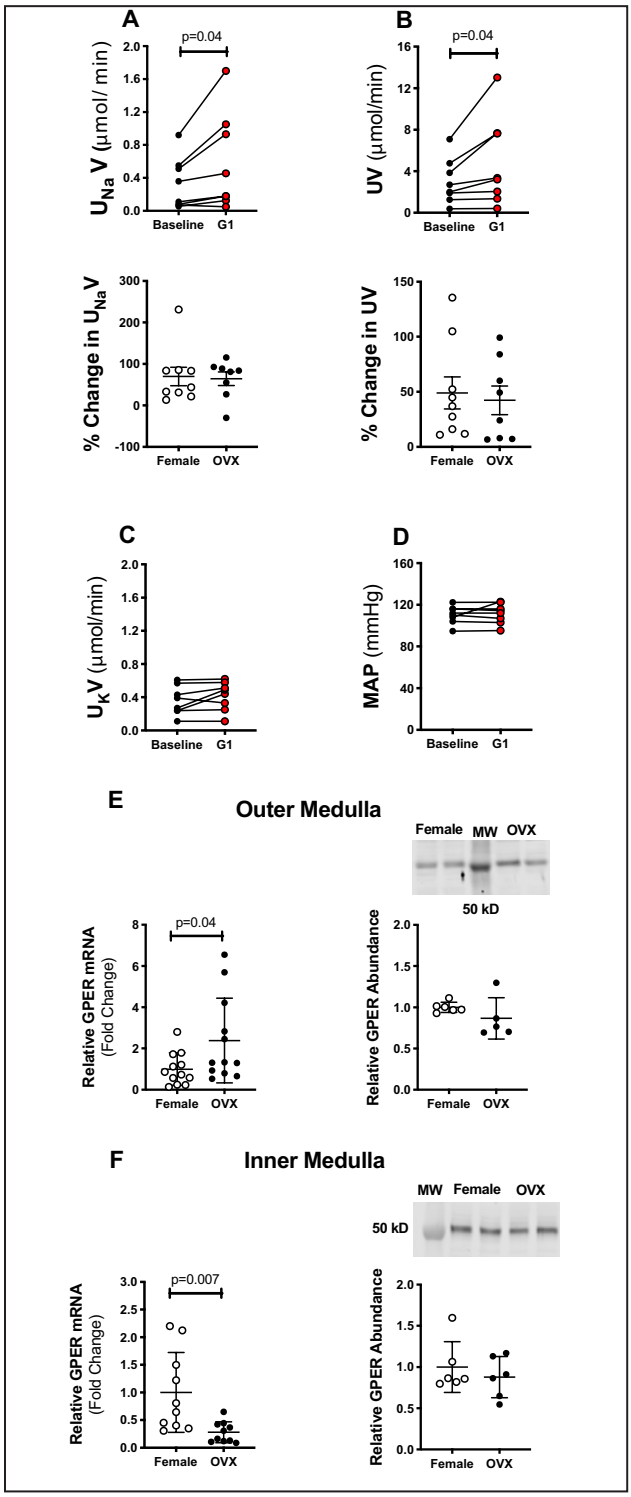

Figure 4. Ovariectomy (OVX) does not impact the natriuretic response to medullary G-protein–coupled estrogen receptor (GPER) activation.
Urinary Na^+ excretion ($U_{Na}V$) and percentage change in $U_{Na}V$ (**A**), urine flow (UV) and percentage change in UV (**B**), urinary K^+ excretion (U_KV) (**C**), and mean arterial pressure (MAP) (**D**) in anesthetized OVX Sprague Dawley rats receiving renal medullary interstitial infusions of G1 (GPER agonist, 5 pmol/kg per minute). Percentage change in $U_{Na}V$ and UV in response to intramedullary infusion of G1 in intact females, and OVX is represented relative to baseline values. Relative mRNA expression and protein abundance of GPER in outer medulla (**E**) and inner medulla (**F**) from intact female and OVX rats (representative Western blots are presented). Gene expression values represent fold change from corresponding female levels. Protein abundance is presented relative to female levels. n=6 to 12 rats in each group. Statistical comparisons performed by Student *t* test (**A** through **F**).

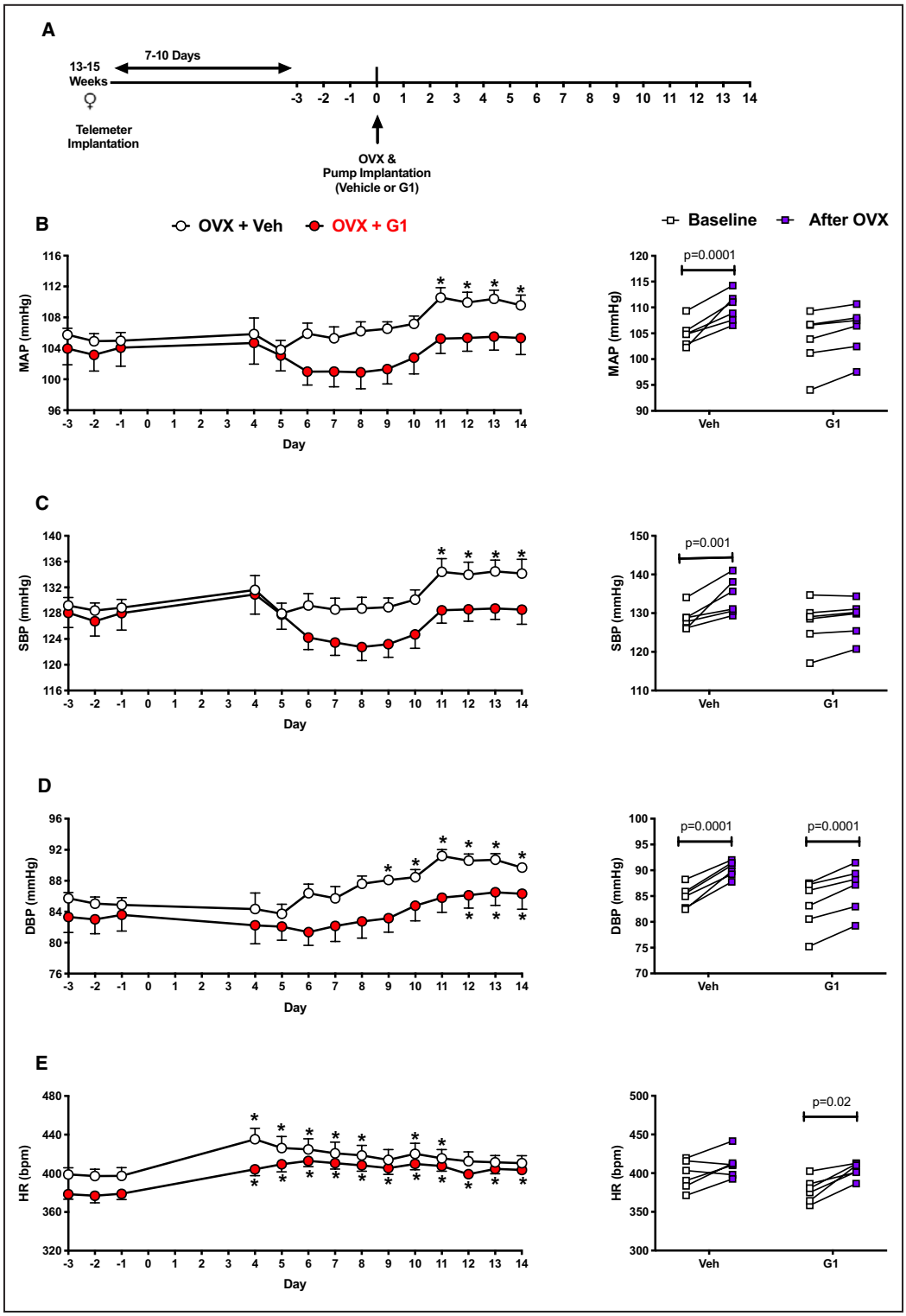

Figure 5. Systemic activation of G-protein–coupled estrogen receptor prevents the ovariectomy (OVX)–induced increase in blood pressure.

Experimental time line (**A**). The 24-hour mean arterial pressure (MAP) (**B**), systolic blood pressure (SBP) (**C**), diastolic blood pressure (DBP) (**D**), and heart rate (HR) (**E**) along the experimental time line in conscious female rats subjected to OVX and vehicle (Veh) or G1 (400 μg/kg per day, osmotic minipump). Panels on the right-hand side show MAP, SBP, DBP, and HR at baseline (before OVX, average of blood pressure at days -1 and -2) and 2 weeks after OVX (average of blood pressure at days 13 and 14). Data are means±SE of 6 rats in each group. Statistical comparisons performed by repeated measures 2-way ANOVA with Sidak's post hoc test for multiple comparisons. *P<0.05 vs corresponding baseline values.

DISCUSSION

The current study shows that activation of GPER within the renal medulla provoked a natriuretic response via the ET-1 pathway in female but not male rats. In addition, we observed that systemic GPER activation ameliorated the ovariectomy-induced increase in mean and systolic blood pressure but not diastolic blood pressure. Together, these findings indicate an interaction between renal GPER and ET-1 systems in females to efficiently excrete Na^+ and maintain blood pressure. Furthermore, these findings provide in vivo evidence for estradiol-dependent GPER activation as a novel female-specific natriuretic pathway accounting for more efficient Na^+ excretion in females.[33,34]

Estradiol plays an integral role in the maintenance of cardiovascular and renal health.[35–38] However, the impact of estradiol on renal Na^+ handling, which plays a central role in blood pressure regulation, is not totally clear. Sexual dimorphism in the abundance of Na^+ transporters along the nephron has been recently described.[33] Renal Na^+ excretion was higher in women compared with men during short-term hypertonic saline infusion,[34] presumably attributable to estradiol actions along the nephron, but the evidence is limited.

Previous evidence suggests a potential contribution of GPER to the maintenance of Na^+ homeostasis. Our study demonstrated that exogenous GPER activation by G1 provoked natriuresis that was abolished by simultaneous infusion of the GPER antagonist, G15. Infusion of G15 alone reduced basal Na^+ excretion, indicating that endogenous activation of medullary GPER exerts an inhibitory tone on tubular reabsorption of Na^+. The effects of estradiol on renal Na^+ handling may not be mediated solely through GPER, especially under long-term conditions. Future studies are needed to determine the role of estrogen receptors α and β in regulating Na^+ excretion.

The renal medullary ET-1 system, which plays a central role in the regulation of blood pressure and Na^+ excretion, contributes to sex-related differences in blood pressure control.[39–41] Previous evidence

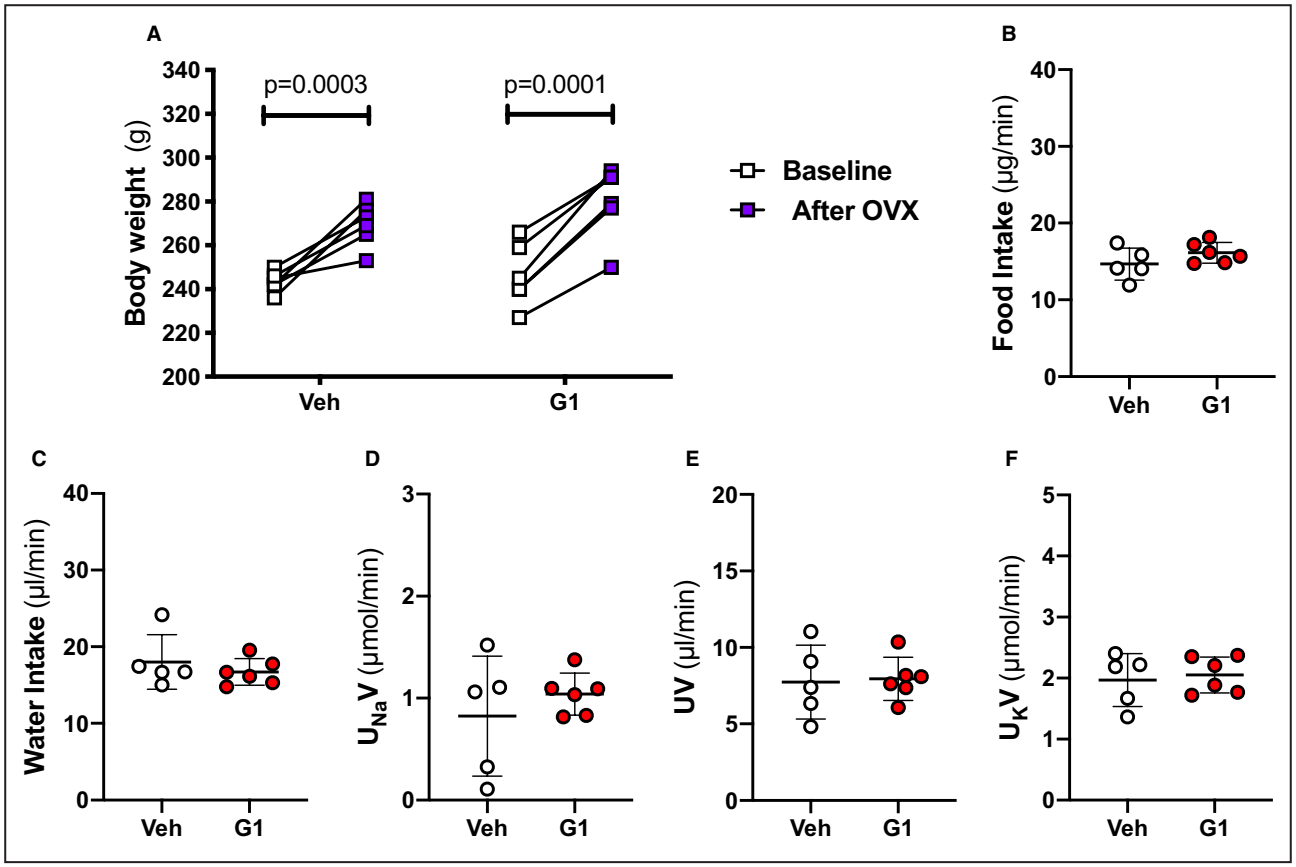

Figure 6. Effect of systemic activation of G protein–coupled estrogen receptor on body weight and basal metabolic cage parameters.

Body weight at baseline and 2 weeks after ovariectomy (OVX) (**A**), food intake (**B**), water intake (**C**), urinary Na^+ excretion ($U_{Na}V$) (**D**), urine flow (UV) (**E**), and urinary K^+ excretion (U_KV) (**F**) in OVX Sprague Dawley rats supplemented with vehicle (Veh) or G1 (400 g/kg per day, osmotic minipump) for 2 weeks. Data are mean±SE of 5 to 6 rats in each group. Statistical comparisons performed by repeated measures 2-way ANOVA with Sidak's post hoc test for multiple comparisons (**A**) and unpaired Student *t* test (**B** through **F**).

indicates that GPER regulates ET-1–dependent effects on the cardiovascular system. GPER agonists inhibit ET-1–dependent vasoconstriction[5,42,43] and ET-1–dependent vascular tone in carotid arteries.[44] In addition, GPER facilitates the age-dependent increase in myocardial ET_B expression[45] and antagonizes ET-1–induced hypertrophy in cultured neonatal cardiomyocytes.[46] Taken together, a link between GPER and ET-1 signaling has been demonstrated in the cardiovascular system; however, the renal interaction between these 2 systems has not been established.

In the current study, a sex-specific regulatory role of GPER on ET-1 signaling was supported by use of both pharmacological and genetic approaches. Specifically, dual blockade of ET_A and ET_B abolished the natriuretic and diuretic response to G1 infusion in female rats. More important, previously published studies from our laboratory showed no time-dependent effect of ET_A and ET_B blockers on the response to intramedullary infusion of isosmotic NaCl.[47] In addition, genetic deletion of GPER diminishes ET-1, ET_A, and ET_B mRNA expression in female but not male mice. The influence of GPER in provoking ET-1–induced natriuresis introduces a novel pathway by which estradiol maintains Na^+ homeostasis in females. Previous studies showed the existence of GPER mRNA and protein in the kidney.[7–9] Early studies demonstrated smooth muscle cells in renal pelvis and to a lesser extent the renal medulla.[9] However, multiple studies reported that GPER expression is mainly in tubular epithelial cells.[8,48,49] Cheng and colleagues demonstrated that GPER is within or in close proximity to the basolateral membrane of epithelia within proximal and distal convoluted tubules and the loop of Henle and in the renal medullary, cortical, and pelvic areas.[48,49] However, Lindsey et al[8] observed that GPER is localized to the apical aspect of the renal epithelium in the hypertensive mRen2.Lewis females. Whether the differences in cellular localization of GPER within renal tubular epithelia are based on sex or physiological versus pathophysiological conditions remain to be determined.

Interestingly, it has been demonstrated that GPER expression in the kidney and brain is modulated during the estrus cycle.[48,50] Experiments involving intact cycling female rats in the current study were conducted in a periodic manner by performing experiments with cohoused rats over 4 consecutive days to maximize use across the estrus cycle. Modulation of GPER expression by estrus cycle may contribute to the observed variability in the mRNA of ET-1, ET_A, and ET_B receptors in wild-type animals. Future studies are needed to identify the impact of estrus cycle on the renal GPER function and its interaction with ET-1.

The nature of the interaction between GPER and ET-1 signaling at the cellular level is not clear yet. It is possible that GPER activation in a short-term setting directly triggers ET-1 release to activate ET_A and ET_B receptors. As G-protein–coupled receptors, GPER, ET_A, and ET_B are capable of forming dimers or even higher-order oligomers that might contribute to functional synergy. Despite the fact that GPER deletion resulted in changes in ET_A and ET_B receptor mRNA expression, we do not anticipate that the short-term response to G1 involves changes in ET_A and ET_B receptors at the mRNA level. We cannot exclude the possibility of changes in protein expression or localization, so future experiments are required to identify the nature of the interaction between GPER, ET_A, and ET_B receptors. Experiments directed toward assessing potential posttranslational modifications in response to short-term GPER activation will advance our understanding of rapid estrogenic signaling in the kidney.

Na^+/K^+ ATPase plays a key role in Na^+ reabsorption. Enhanced activity of Na^+/K^+ ATPase results in Na^+ retention.[51,52] Early studies have shown that ET-1 reduces Na^+/K^+ ATPase activity in inner medullary collecting duct.[32] Of note, our data demonstrate that intramedullary infusion of G1 resulted in decreased Na^+/K^+ ATPase activity in the renal outer medulla, which may contribute to G1-induced natriuretic effect. Ovariectomy decreased the expression and activity of Na^+/K^+ ATPase in the renal cortex, which was normalized by supplementation of estradiol to ovariectomized rats.[53] Similarly, estradiol induces the expression and activity of Na^+/K^+ ATPase in rat arterioles.[54] Contrasting effects of estradiol may relate to stimulating different subtypes of ER. Future studies directed toward identifying the effect of specific activation of ER on Na^+ channels and transporters are needed.

Multiple studies have established a vasodilatory role for GPER.[44,55–60] Thus, we tested whether the natriuretic response to G1 resulted from an increase in medullary blood flow. Our finding that GPER activation did not increase medullary blood flow excludes a role for GPER-induced renal medullary vasodilatation in mediating G1 natriuretic effects.

The current study did not show a difference in renal medullary GPER abundance or short-term G1-pronatriuretic effects in response to ovariectomy. It was also reported that mesenteric and aortic relaxation responses to G1 were not different in vessels from ovariectomized versus intact female rats,[61,62] suggesting that ovariectomy does not alter renal medullary or vascular responsiveness to GPER activation. However, ovariectomy appears to elicit differential effects on brain GPER expression based on the region of interest.[50,63]

More important, our data also showed that systemic GPER activation ameliorated the increase in blood pressure induced by ovariectomy, suggesting

that GPER contributes to the antihypertensive effect of ovarian hormones. Short-term intrarenal GPER activation was used in the current study to specifically identify the role of renal medullary GPER on Na^+ excretion, whereas systemic infusion established an overall effect of GPER on blood pressure control. It is known that GPER triggers vasodilation,[57,62,64,65] a potential underlying mechanism that may have contributed to the antihypertensive actions of GPER demonstrated in the current study. Despite the relatively high abundance of GPER in the brain,[66] the contribution of central GPER to blood pressure regulation is not defined yet. Thus, it is possible that the antihypertensive effect of GPER in the current study may involve multiple systems, including peripheral vascular and central mechanisms, and not solely a kidney-specific mechanism. Additional studies are needed to identify the contribution of intrarenal versus extrarenal GPER in the control of Na^+ homeostasis and blood pressure.

ARTICLE INFORMATION

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Disclosures

Dr Gohar is affiliated with Department of Pharmacology and Toxicology, Faculty of Pharmacy, Alexandria University, Egypt. The remaining authors have no disclosures to report.

Supplementary Material

Figure S1

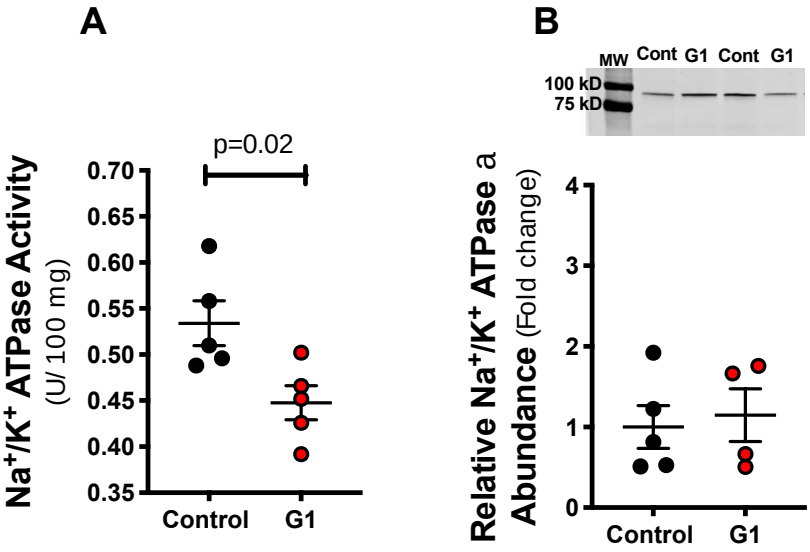
REFERENCES

1. Molina L, Figueroa CD, Bhoola KD, Ehrenfeld P. GPER-1/GPR30 a novel estrogen receptor sited in the cell membrane: therapeutic coupling to breast cancer. *Expert Opin Ther Targets*. 2017;21:755–766.
2. Filardo EJ, Thomas P. Minireview: G protein-coupled estrogen receptor-1, GPER-1: its mechanism of action and role in female reproductive cancer, renal and vascular physiology. *Endocrinology*. 2012;153:2953–2962.
3. Thomas P, Pang Y, Filardo EJ, Dong J. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology*. 2005;146:624–632.
4. Carmeci C, Thompson DA, Ring HZ, Francke U, Weigel RJ. Identification of a gene (GPR30) with homology to the G-protein-coupled receptor superfamily associated with estrogen receptor expression in breast cancer. *Genomics*. 1997;45:607–617.
5. Prossnitz ER, Barton M. The G-protein-coupled estrogen receptor GPER in health and disease. *Nat Rev Endocrinol*. 2011;7:715–726.
6. Deschamps AM, Murphy E. Activation of a novel estrogen receptor, GPER, is cardioprotective in male and female rats. *Am J Physiol Heart Circ Physiol*. 2009;297:H1806–H1813.
7. Cheema MU, Irsik DL, Wang Y, Miller-Little W, Hyndman KA, Marks ES, Frokiaer J, Boesen EI, Norregaard R. Estradiol regulates AQP2 expression in the collecting duct: a novel inhibitory role for estrogen receptor alpha. *Am J Physiol Renal Physiol*. 2015;309:F305–F317.
8. Lindsey SH, Yamaleyeva LM, Brosnihan KB, Gallagher PE, Chappell MC. Estrogen receptor GPR30 reduces oxidative stress and proteinuria in the salt-sensitive female mRen2.Lewis rat. *Hypertension*. 2011;58:665–671.
9. Hazell GG, Yao ST, Roper JA, Prossnitz ER, O'Carroll AM, Lolait SJ. Localisation of GPR30, a novel g protein-coupled oestrogen receptor, suggests multiple functions in rodent brain and peripheral tissues. *J Endocrinol*. 2009;202:223–236.
10. Liu L, Kashyap S, Murphy B, Hutson DD, Budish RA, Trimmer EH, Zimmerman MA, Trask AJ, Miller KS, Chappell MC, et al. GPER activation ameliorates aortic remodeling induced by salt-sensitive hypertension. *Am J Physiol Heart Circ Physiol*. 2016;310:H953–H961.
11. Ikeda M, Swide T, Vayl A, Lahm T, Anderson S, Hutchens MP. Estrogen administered after cardiac arrest and cardiopulmonary resuscitation ameliorates acute kidney injury in a sex- and age-specific manner. *Crit Care*. 2015;19:332.
12. Kurt AH, Bozkus F, Uremis N, Uremis MM. The protective role of G protein-coupled estrogen receptor 1 (GPER-1) on methotrexate-induced nephrotoxicity in human renal epithelium cells. *Ren Fail*. 2016;38:686–692.
13. Haas E, Bhattacharya I, Brailoiu E, Damjanovic M, Brailoiu GC, Gao X, Mueller-Guerre L, Marjon NA, Gut A, Minotti R, et al. Regulatory role of G protein-coupled estrogen receptor for vascular function and obesity. *Circ Res*. 2009;104:288–291.
14. Jessup JA, Lindsey SH, Wang H, Chappell MC, Groban L. Attenuation of salt-induced cardiac remodeling and diastolic dysfunction by the GPER agonist G-1 in female mRen2.Lewis rats. *PLoS One*. 2010;5:e15433.
15. Lafferty AR, Torpy DJ, Stowasser M, Taymans SE, Lin JP, Huggard P, Gordon RD, Stratakis CA. A novel genetic locus for low renin hypertension: familial hyperaldosteronism type II maps to chromosome 7 (7p22). *J Med Genet*. 2000;37:831–835.
16. Gohar EY, Kasztan M, Becker BK, Speed JS, Pollock DM. Ovariectomy uncovers purinergic receptor activation of endothelin-dependent natriuresis. *Am J Physiol Renal Physiol*. 2017;313:F361–F369.
17. Gohar EY, Speed JS, Kasztan M, Jin C, Pollock DM. Activation of purinergic receptors (P2) in the renal medulla promotes endothelin-dependent natriuresis in male rats. *Am J Physiol Renal Physiol*. 2016;311:F260–F267.
18. Kittikulsuth W, Pollock JS, Pollock DM. Sex differences in renal medullary endothelin receptor function in angiotensin II hypertensive rats. *Hypertension*. 2011;58:212–218.
19. Gohar EY, Yusuf C, Pollock DM. Ovarian hormones modulate endothelin A and B receptor expression. *Life Sci*. 2016;159:148–152.
20. Sullivan JC, Sasser JM, Pollock DM, Pollock JS. Sexual dimorphism in renal production of prostanoids in spontaneously hypertensive rats. *Hypertension*. 2005;45:406–411.
21. Taylor TA, Gariepy CE, Pollock DM, Pollock JS. Gender differences in ET and NOS systems in ET_B receptor-deficient rats: effect of a high salt diet. *Hypertension*. 2003;41:657–662.
22. Wang C, Dehghani B, Magrisso IJ, Rick EA, Bonhomme E, Cody DB, Elenich LA, Subramanian S, Murphy SJ, Kelly MJ, et al. GPR30 contributes to estrogen-induced thymic atrophy. *Mol Endocrinol*. 2008;22:636–648.
23. Liu S, Le May C, Wong WP, Ward RD, Clegg DJ, Marcelli M, Korach KS, Mauvais-Jarvis F. Importance of extranuclear estrogen receptor-alpha and membrane G protein-coupled estrogen receptor in pancreatic islet survival. *Diabetes*. 2009;58:2292–2302.
24. Hyndman KA, Mironova EV, Giani JF, Dugas C, Collins J, McDonough AA, Stockand JD, Pollock JS. Collecting duct nitric oxide synthase 1ss activation maintains sodium homeostasis during high sodium intake through suppression of aldosterone and renal angiotensin

ll pathways. *J Am Heart Assoc*. 2017;6:e006896. DOI: 10.1161/JAHA.117.006896.
25. Jin C, Jeon Y, Kleven DT, Pollock JS, White JJ, Pollock DM. Combined endothelin A blockade and chlorthalidone treatment in a rat model of metabolic syndrome. *J Pharmacol Exp Ther*. 2014;351:467–473.
26. Alencar AK, Montes GC, Montagnoli T, Silva AM, Martinez ST, Fraga AG, Wang H, Groban L, Sudo RT, Zapata-Sudo G. Activation of GPER ameliorates experimental pulmonary hypertension in male rats. *Eur J Pharm Sci*. 2017;97:208–217.
27. Alencar AKN, Montes GC, Costa DG, Mendes LVP, Silva AMS, Martinez ST, Trachez MM, Cunha V, Montagnoli TL, Fraga AGM, et al. Cardioprotection induced by activation of GPER in ovariectomized rats with pulmonary hypertension. *J Gerontol A Biol Sci Med Sci*. 2018;73:1158–1166.
28. Albanito L, Madeo A, Lappano R, Vivacqua A, Rago V, Carpino A, Oprea TI, Prossnitz ER, Musti AM, Ando S, et al. G protein-coupled receptor 30 (GPR30) mediates gene expression changes and growth response to 17beta-estradiol and selective GPR30 ligand G-1 in ovarian cancer cells. *Cancer Res*. 2007;67:1859–1866.
29. Bologa CG, Revankar CM, Young SM, Edwards BS, Arterburn JB, Kiselyov AS, Parker MA, Tkachenko SE, Savchuck NP, Sklar LA, et al. Virtual and biomolecular screening converge on a selective agonist for GPR30. *Nat Chem Biol*. 2006;2:207–212.
30. Blasko E, Haskell CA, Leung S, Gualtieri G, Halks-Miller M, Mahmoudi M, Dennis MK, Prossnitz ER, Karpus WJ, Horuk R. Beneficial role of the GPR30 agonist G-1 in an animal model of multiple sclerosis. *J Neuroimmunol*. 2009;214:67–77.
31. Wang C, Dehghani B, Li Y, Kaler LJ, Proctor T, Vandenbark AA, Offner H. Membrane estrogen receptor regulates experimental autoimmune encephalomyelitis through up-regulation of programmed death 1. *J Immunol*. 2009;182:3294–3303.
32. Zeidel ML, Brady HR, Kone BC, Gullans SR, Brenner BM. Endothelin, a peptide inhibitor of Na^+-K^+-ATPase in intact renal tubular epithelial cells. *Am J Physiol*. 1989;257:C1101–C1107.
33. Veiras LC, Girardi ACC, Curry J, Pei L, Ralph DL, Tran A, Castelo-Branco RC, Pastor-Soler N, Arranz CT, Yu ASL, McDonough AA. Sexual dimorphic pattern of renal transporters and electrolyte homeostasis. *J Am Soc Nephrol*. 2017;28:3504–3517.
34. Stachenfeld NS, Splenser AE, Calzone WL, Taylor MP, Keefe DL. Sex differences in osmotic regulation of AVP and renal sodium handling. *J Appl Physiol*. 2001;91:1893–1901.
35. Gouva L, Tsatsoulis A. The role of estrogens in cardiovascular disease in the aftermath of clinical trials. *Hormones*. 2004;3:171–183.
36. Teede HJ, Vincent A. Hormone therapy—where are we now? *Aust Fam Physician*. 2011;40:280–285.
37. Suzuki H, Kondo K. Chronic kidney disease in postmenopausal women. *Hypertens Res*. 2012;35:142–147.
38. Inada A, Inada O, Fujii NL, Nagafuchi S, Katsuta H, Yasunami Y, Matsubara T, Arai H, Fukatsu A, Nabeshima YI. Adjusting the 17beta-estradiol-to-androgen ratio ameliorates diabetic nephropathy. *J Am Soc Nephrol*. 2016;27:3035–3050.
39. Tostes RC, Fortes ZB, Callera GE, Montezano AC, Touyz RM, Webb RC, Carvalho MH. Endothelin, sex and hypertension. *Clin Sci (London)*. 2008;114:85–97.
40. Kittikulsuth W, Sullivan JC, Pollock DM. ET-1 actions in the kidney: evidence for sex differences. *Br J Pharmacol*. 2013;168:318–326.
41. Nakano D, Pollock DM. Contribution of endothelin a receptors in endothelin 1-dependent natriuresis in female rats. *Hypertension*. 2009;53:324–330.
42. Lee MY, Leung SW, Vanhoutte PM, Man RY. Genistein reduces agonist-induced contractions of porcine coronary arterial smooth muscle in a cyclic AMP-dependent manner. *Eur J Pharmacol*. 2004;503:165–172.
43. Tsang SY, Yao X, Essin K, Wong CM, Chan FL, Gollasch M, Huang Y. Raloxifene relaxes rat cerebral arteries in vitro and inhibits l-type voltage-sensitive Ca^{2+} channels. *Stroke*. 2004;35:1709–1714.
44. Meyer MR, Field AS, Kanagy NL, Barton M, Prossnitz ER. GPER regulates endothelin-dependent vascular tone and intracellular calcium. *Life Sci*. 2012;91:623–627.
45. Meyer MR, Fredette NC, Sharma G, Barton M, Prossnitz ER. GPER is required for the age-dependent upregulation of the myocardial endothelin system. *Life Sci*. 2016;159:61–65.
46. Goncalves GK, Scalzo S, Alves AP, Agero U, Guatimosim S, Reis AM. Neonatal cardiomyocyte hypertrophy induced by endothelin-1 is blocked by estradiol acting on GPER. *Am J Physiol Cell Physiol*. 2018;314:C310–C322.
47. Boesen EI, Pollock DM. Cooperative role of ET_A and ET_B receptors in mediating the diuretic response to intramedullary hyperosmotic NaCl infusion. *Am J Physiol Renal Physiol*. 2010;299:F1424–F1432.
48. Cheng SB, Dong J, Pang Y, LaRocca J, Hixon M, Thomas P, Filardo EJ. Anatomical location and redistribution of G protein-coupled estrogen receptor-1 during the estrus cycle in mouse kidney and specific binding to estrogens but not aldosterone. *Mol Cell Endocrinol*. 2014;382:950–959.
49. Cheng SB, Graeber CT, Quinn JA, Filardo EJ. Retrograde transport of the transmembrane estrogen receptor, G-protein-coupled-receptor-30 (GPR30/GPER) from the plasma membrane towards the nucleus. *Steroids*. 2011;76:892–896.
50. Spary EJ, Chapman SE, Sinfield JK, Maqbool A, Kaye J, Batten TF. Novel G protein-coupled oestrogen receptor GPR30 shows changes in mRNA expression in the rat brain over the oestrous cycle. *Neurosignals*. 2013;21:14–27.
51. Deschenes G, Doucet A. Collecting duct (Na^+/K^+)-ATPase activity is correlated with urinary sodium excretion in rat nephrotic syndromes. *J Am Soc Nephrol*. 2000;11:604–615.
52. Katz AI. Renal Na-K-ATPase: its role in tubular sodium and potassium transport. *Am J Physiol*. 1982;242:F207–F219.
53. Gracelli JB, Souza-Menezes J, Barbosa CM, Ornellas FS, Takiya CM, Alves LM, Wengert M, Feltran Gda S, Caruso-Neves C, Moyses MR, et al. Role of estrogen and progesterone in the modulation of CNG-A1 and Na^+/K^+-ATPase expression in the renal cortex. *Cell Physiol Biochem*. 2012;30:160–172.
54. Palacios J, Marusic ET, Lopez NC, Gonzalez M, Michea L. Estradiol-induced expression of Na^+-K^+-ATPase catalytic isoforms in rat arteries: gender differences in activity mediated by nitric oxide donors. *Am J Physiol Heart Circ Physiol*. 2004;286:H1793–H1800.
55. Arefin S, Simoncini T, Wieland R, Hammarqvist F, Spina S, Goglia L, Kublickiene K. Vasodilatory effects of the selective GPER agonist G-1 is maximal in arteries of postmenopausal women. *Maturitas*. 2014;78:123–130.
56. Meyer MR, Prossnitz ER, Barton M. The G protein-coupled estrogen receptor GPER/GPR30 as a regulator of cardiovascular function. *Vascul Pharmacol*. 2011;55:17–25.
57. Lindsey SH, Liu L, Chappell MC. Vasodilation by gper in mesenteric arteries involves both endothelial nitric oxide and smooth muscle camp signaling. *Steroids*. 2014;81:99–102.
58. Zimmerman MA, Budish RA, Kashyap S, Lindsey SH. GPER-novel membrane oestrogen receptor. *Clin Sci*. 2016;130:1005–1016.
59. Meyer MR, Fredette NC, Barton M, Prossnitz ER. G protein-coupled estrogen receptor inhibits vascular prostanoid production and activity. *J Endocrinol*. 2015;227:61–69.
60. Meyer MR, Amann K, Field AS, Hu C, Hathaway HJ, Kanagy NL, Walker MK, Barton M, Prossnitz ER. Deletion of G protein-coupled estrogen receptor increases endothelial vasoconstriction. *Hypertension*. 2012;59:507–512.
61. Lindsey SH, Cohen JA, Brosnihan KB, Gallagher PE, Chappell MC. Chronic treatment with the G protein-coupled receptor 30 agonist G-1 decreases blood pressure in ovariectomized mRen2.Lewis rats. *Endocrinology*. 2009;150:3753–3758.
62. Lindsey SH, Carver KA, Prossnitz ER, Chappell MC. Vasodilation in response to the GPR30 agonist G-1 is not different from estradiol in the mRen2.Lewis female rat. *J Cardiovasc Pharmacol*. 2011;57:598–603.
63. Broughton BR, Brait VH, Guida E, Lee S, Arumugam TV, Gardiner-Mann CV, Miller AA, Tang SC, Drummond GR, Sobey CG. Stroke increases G protein-coupled estrogen receptor expression in the brain of male but not female mice. *Neurosignals*. 2013;21:229–239.
64. Fredette NC, Meyer MR, Prossnitz ER. Role of GPER in estrogen-dependent nitric oxide formation and vasodilation. *J Steroid Biochem Mol Biol*. 2018;176:65–72.
65. Tropea T, De Francesco EM, Rigiracciolo D, Maggiolini M, Wareing M, Osol G, Mandala M. Pregnancy augments g protein estrogen receptor (GPER) induced vasodilation in rat uterine arteries via the nitric oxide—cGMP signaling pathway. *PLoS One*. 2015;10:e0141997.
66. Hutson DD, Gurrala R, Ogola BO, Zimmerman MA, Mostany R, Satou R, Lindsey SH. Estrogen receptor profiles across tissues from male and female rattus norvegicus. *Biol Sex Differ*. 2019;10:4.

SUPPLEMENTAL MATERIAL

Figure S1. Activation of GPER in the renal medulla reduces outermedullary Na^+/K^+ ATPase activity in female rats.



Renal outer medullary Na^+/K^+ ATPase activity (A) and protein abundance (B) in kidneys obtained from anesthetized intact female SD rats receiving renal medullary interstitial infusions of G1 (GPER agonist, 5 pmol/kg/min) or contralateral un-infused kidneys. Protein abundance is presented relative to control (contralateral kidney) levels. n=4-5 in each group. Statistical comparisons performed by Student *t*-test.