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Time course of renal sodium transport in the pregnant rat

Crystal A. West [a,*], Steven D. Beck [a], Shyama M.E. Masilamani [b]

[a] Department of Biology, Appalachian State University, North Carolina Research Campus, Kannapolis, NC, USA
[b] Department of Internal Medicine, Division of Nephrology, Virginia Commonwealth University Medical Center, Richmond, VA, USA

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ABSTRACT

Progressive sodium retention and cumulative plasma volume expansion occur to support the developing fetus during pregnancy. Sodium retention is regulated by individual tubular transporters and channels. An increase or decrease in any single transporter could cause a change in sodium balance. Understanding the time-course for changes in each sodium transporter during pregnancy will enable us to understand progressive sodium retention seen in pregnancy. Here, we examined the activity of the major apical sodium transporters found in the nephron using natriuretic response tests in virgin, early pregnant, mid-pregnant, and late pregnant rats. We also measured renal and serum aldosterone levels. We found that furosemide sensitive sodium transport (NKCC2) is only increased during late pregnancy, thiazide sensitive sodium transport (NDCBE/pendrin) is increased in all stages of pregnancy, and that benzamil sensitive sodium transport (ENaC) is increased beginning in mid-pregnancy. We also found that serum aldosterone levels progressively increased throughout gestation and kidney tissue aldosterone levels increased only during late pregnancy. Here we have shown progressive turning on of specific sodium transport mechanisms to help support progressive sodium retention through the course of gestation. These mechanisms contribute to the renal sodium retention and plasma volume expansion required for an optimal pregnancy.

1. Introduction

Progressive sodium retention is required to support a healthy pregnancy (Alexander et al., 1980). During pregnancy, renal sodium retention drives the plasma volume expansion which supports nutrient delivery to the growing fetus (West et al., 2016). When nutrient delivery to the fetus is compromised by volume contraction, fetal growth restriction occurs. Fetal growth restriction increases the baby's risk for metabolic syndrome (Barker et al., 1989) and renal disease (Brenner et al., 1988) later in life.

The reabsorption of sodium is determined by the regulation of the individual tubular transporters and channels such that an increase in any single transporter could cause a change in sodium balance (Knepper et al., 2003). We and others have characterized adaptations of the sodium transporters along the renal tubule during pregnancy (de Souza and West, 2018). The primary finding from this work established an evolutionarily conserved increase in the epithelial sodium channel (ENaC) during pregnancy (West et al., 2010; Nielsen et al., 2017; Fu et al., 2019; Walter et al., 2020). Furthermore, it has been established that this increase in ENaC activity is critical for sodium balance, plasma volume expansion, and fetal growth (West et al., 2014; Fu et al., 2019). Another conserved feature in both rats and mice is an upregulation of pendrin and down regulation of the sodium chloride co-transporter (NCC) in pregnancy (West et al., 2015a, 2015b; Walter et al., 2020). We found in pregnant rats that despite the down regulated NCC (West et al., 2015a), thiazide mediated sodium transport was increased (West et al., 2015b) and was sensitive to regulation by the proteinase activated receptor type 2 (PAR2) (West et al., 2021). Thiazide sensitive sodium transport, independent of NCC, has previously been described in the cortical collecting duct of male rats (Nielsen et al., 2002; Morla et al., 2013) and non-pregnant mice (Leviel et al., 2010). This transport mechanism is mediated by the sodium driven chloride bicarbonate exchanger (NDCBE) and pendrin (Morla et al., 2013; Leviel et al., 2010) and sensitive to regulation by PAR2 (Morla et al., 2013). Since, we previously demonstrated a decrease in NCC but an increase in pendrin and thiazide sensitive sodium transport in the pregnant rat that was sensitive to regulation by PAR2, this is likely mediated by the NDCBE/pendrin mediated mechanism (West et al., 2015a, 2015b,

* Corresponding author. Appalachian State University Department of Biology, North Carolina Research Campus, 150 N. Research Campus Dr. Kannapolis, NC, 28081, USA.
E-mail address: westca1@appstate.edu (C.A. West).

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2021).

The majority of the work to date has focused on the late pregnant period. The purpose of this study is to determine the time-course for changes in sodium transport during pregnancy. Specifically, the *in vivo* activity of the furosemide-sensitive cotransporter (NKCC2), the thiazide sensitive sodium transporter (although classically thought to be NCC, the current hypothesis in pregnancy supports the NDCBE/pendrin mechanism [West et al., 2015a; West et al., 2015b]), and the benzamil sensitive sodium channel (ENaC) in early, mid, and late pregnant rats.

2. Methods

Animals were housed in the Virginia Commonwealth University animal facility in agreement with institutional guidelines. All animal protocols were approved by the Institutional Animal Care and Use Committee (VCU IACUC, Protocol #AM10087) and in accord with the NIH Guide for animal use. Virgin, Day 6–8 early pregnant (EP), Day 12–14 mid pregnant (MP), and Day 18–20 late pregnant (LP) female Sprague Dawley (SD) rats from Harlan (Indianapolis, Indiana) were used for these experiments. Rats destined to become pregnant were placed with a fertile male and day 1 of pregnancy was designated as the day that sperm was present in vaginal smears. Rat gestation is ~21 days. Time of day was not recorded for animal experiments or tissue harvest in this study.

2.1. Natriuretic-response tests

A total of 28 rats were used for the natriuretic response tests. Each rat was used at multiple time-points, but only received one drug per pregnant time point. Rats were randomized into treatment groups and each rat had a minimum washout period of 3 days for virgins and 5 days for pregnant animals between drug administration.

As a measure of NKCC2 *in vivo* activity, sodium excreted in response to single subcutaneous (sc) injection of furosemide (18 mg/kg body wt) was determined in virgin (n = 6), EP (n = 5), MP (n = 5), and LP (n = 6) rats respectively. Urine was collected in metabolic cages for 0–3 h following furosemide. Urine volume was determined gravimetrically and sodium concentration by flame photometry. Before this test, a baseline natriuretic response test to vehicle (water) sc was performed on each rat. Net sodium excretion in response to furosemide, above that particular rat's response to vehicle was determined and considered as an index of *in vivo* activity of NKCC2.

As a measure of NDCBE/pendrin *in vivo* activity, sodium excreted in response to single subcutaneous (sc) injection of hydrochlorothiazide (5.625 mg/kg body wt) was determined in virgin (n = 5), EP (n = 4), MP (n = 5), and LP (n = 6) rats respectively. Urine was collected in metabolic cages for 0–6 h following hydrochlorothiazide. Urine volume was determined gravimetrically and sodium concentration by flame photometry. Before this test, a baseline natriuretic response test to vehicle (water) sc was performed on each rat. Net sodium excretion in response to hydrochlorothiazide, above that particular rat's response to vehicle was determined and considered as an index of *in vivo* activity of NDCBE/pendrin.

As a measure of ENaC *in vivo* activity, sodium excreted in response to single subcutaneous (sc) injection of benzamil (BZ, 1.05 mg/kg body wt) was determined in virgin (n = 6), EP (n = 5), MP (n = 6), and LP (n = 7) rats respectively. Urine was collected in metabolic cages for 0–3 h following benzamil. Urine volume was determined gravimetrically and sodium concentration by flame photometry. Before this test, a baseline natriuretic response test to vehicle (water) sc was performed on each rat. Net sodium excretion in response to benzamil, above that particular rat's response to vehicle was determined and considered as an index of *in vivo* activity of ENaC.

2.2. Determination of aldosterone concentration

A second group of rats (n = 30) were used for determination of serum and kidney aldosterone in virgin (n = 8), EP (n = 7), MP (n = 7), and LP (n = 5–8) rats. Each rat was used for a single time-point for kidney and serum levels of aldosterone. Rats were euthanized by exsanguination under 1–4% isoflourane (Baxter Healthcare Corp., Deerfield, IL) anesthesia, blood was collected from the vena cava, and the kidneys were rapidly removed. The kidneys were processed as a whole kidney homogenates (WKH) for radioimmunoassay (RIA) analysis (Diagnostic Products Corp, Los Angeles, CA). Kidneys were homogenized using a tissue homogenizer (Pro Scientific, Oxford, CT) in a chilled isolation solution of 10 mM triethanolamine, 250 mM sucrose (Mallinckrodt Baker Inc., Phillipsburg, NJ), and protease inhibitor cocktail.

2.3. Statistical analysis

Data are given as mean ± standard error (SE). A one-way analysis of variance (ANOVA) with Tukey post hoc was performed. The null hypothesis was rejected at $p < 0.05$.

3. Results

3.1. In vivo NKCC2 activity

To determine if there is a functional increase in NKCC2 activity in pregnant rats we examined the natriuretic response to NKCC2 blockade with acute administration of furosemide (18 mg/kg, sc), an index for *in vivo* NKCC2 activity. Most of this natriuretic response occurred within 3 h of furosemide administration. As shown in Fig. 1, the net natriuretic response to NKCC2 blockade was markedly increased only in late pregnant rats. Early pregnant and mid pregnant rats had similar NKCC2 activity as virgin rats.

3.2. In vivo NDCBE/pendrin activity

To determine if there is a functional increase in the NDCBE/pendrin mediated sodium transport throughout gestation in the pregnant rat, we examined the natriuretic response to blockade with acute administration of hydrochlorothiazide (5.625 mg/kg, sc), an index for *in vivo* NDCBE/pendrin activity in pregnancy. Most of this natriuretic response occurred within 6 h of hydrochlorothiazide administration. As shown in Fig. 2, the net natriuretic response to hydrochlorothiazide was markedly increased in all stages of pregnancy compared to virgin rats at 0–6 h post hydrochlorothiazide.

3.3. In vivo ENaC activity

To determine if there is a functional increase in ENaC activity throughout gestation in the pregnant rat, we examined the natriuretic response to ENaC blockade with acute administration of benzamil (0.7 mg/kg, sc), an index for *in vivo* ENaC activity. Most of this natriuretic response occurred within 3 h of benzamil administration. As shown in Fig. 3, the net natriuretic response to ENaC blockade was markedly increased only in mid and late pregnant compared to virgin rats at 0–3 h post benzamil.

3.4. Serum and renal aldosterone

To determine renal and serum aldosterone levels we used RIA. We found that serum aldosterone levels progressively increased through gestation (Fig. 4A). Although circulating aldosterone levels are increased through the course of pregnancy, local renal aldosterone levels are only increased in late pregnancy (Fig. 4B).

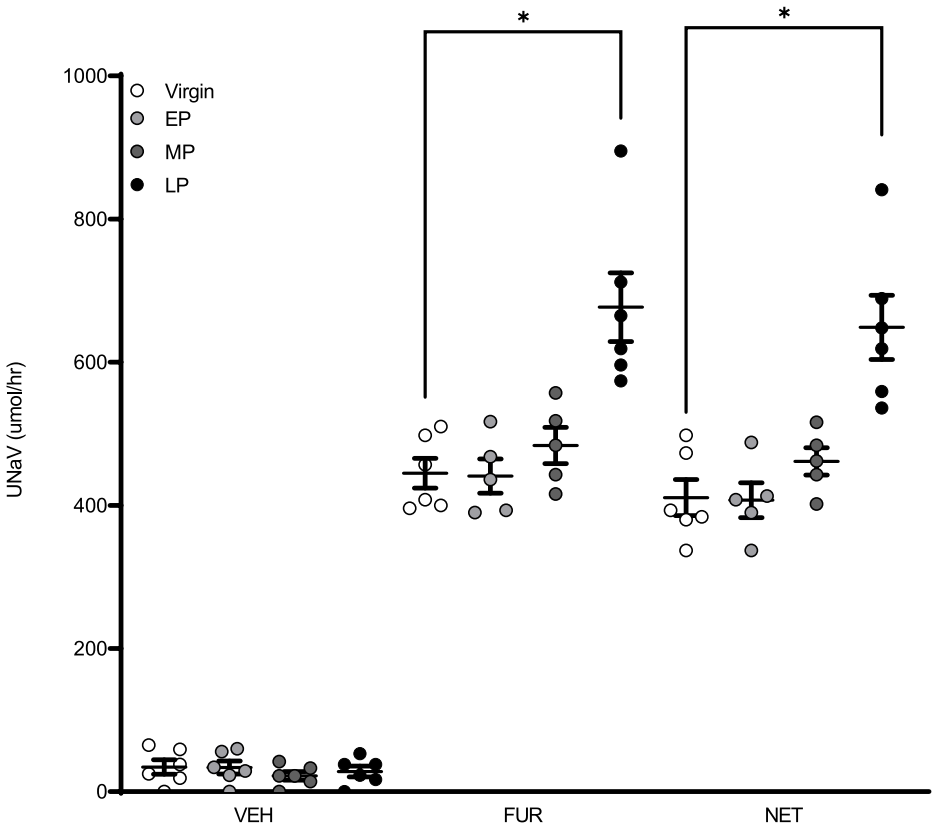

Fig. 1. In vivo furosemide-sensitive cotransporter (NKCC2) activity. Renal sodium excretion (UNaV) in response to furosemide (FUR; 18 mg/kg, sc), in virgins ($n = 6$), early pregnant (EP; $n = 5$), mid pregnant (MP; $n = 5$), and late pregnant (LP; $n = 6$) rats. Sodium excretion was measured for 3 h following vehicle (VEH) and furosemide (FUR). A 1-Way ANOVA with Tukey pot-hoc was performed. Mean ± standard error, *p < 0.05 vs. Virgin.

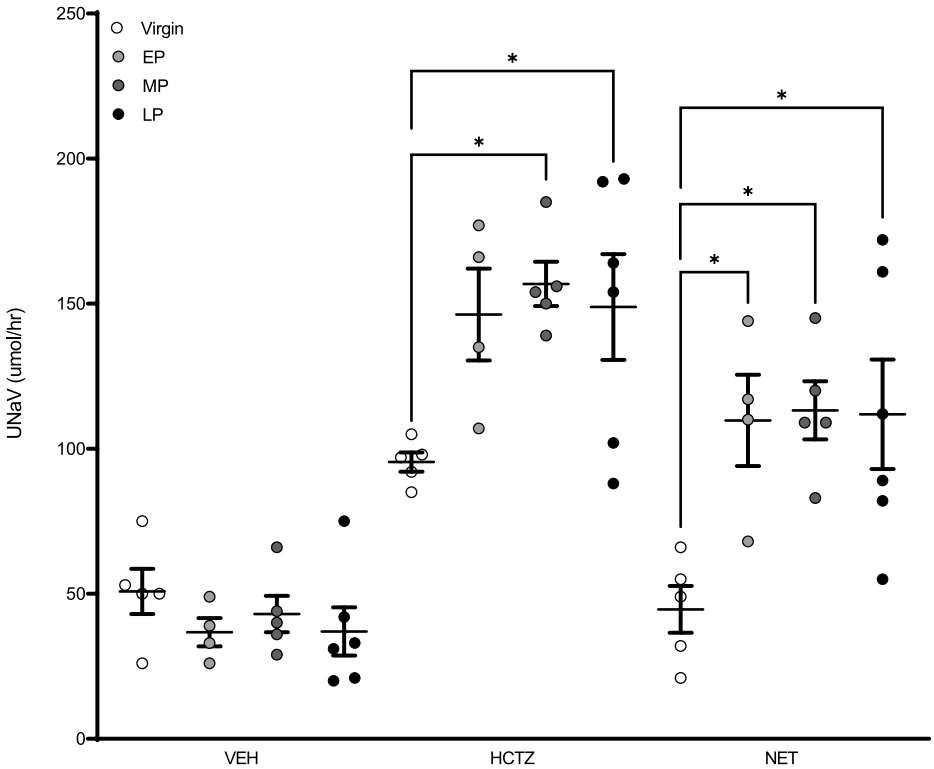

Fig. 2. *In vivo* thiazide sensitive sodium transporter (NDCBE/pendrin) activity. Renal sodium excretion (UNaV) in response to hydrochlorothiazide (HCTZ; 5.625 mg/kg, sc), in virgins ($n = 5$), early pregnant (EP; $n = 4$), mid pregnant (MP; $n = 5$), and late pregnant (LP; $n = 6$) rats. Sodium excretion was measured for 6 h following vehicle (VEH) and hydrochlorothiazide (HCTZ). A 1-Way ANOVA with Tukey pot-hoc was performed. Mean ± standard error, *p < 0.05 vs. Virgin.

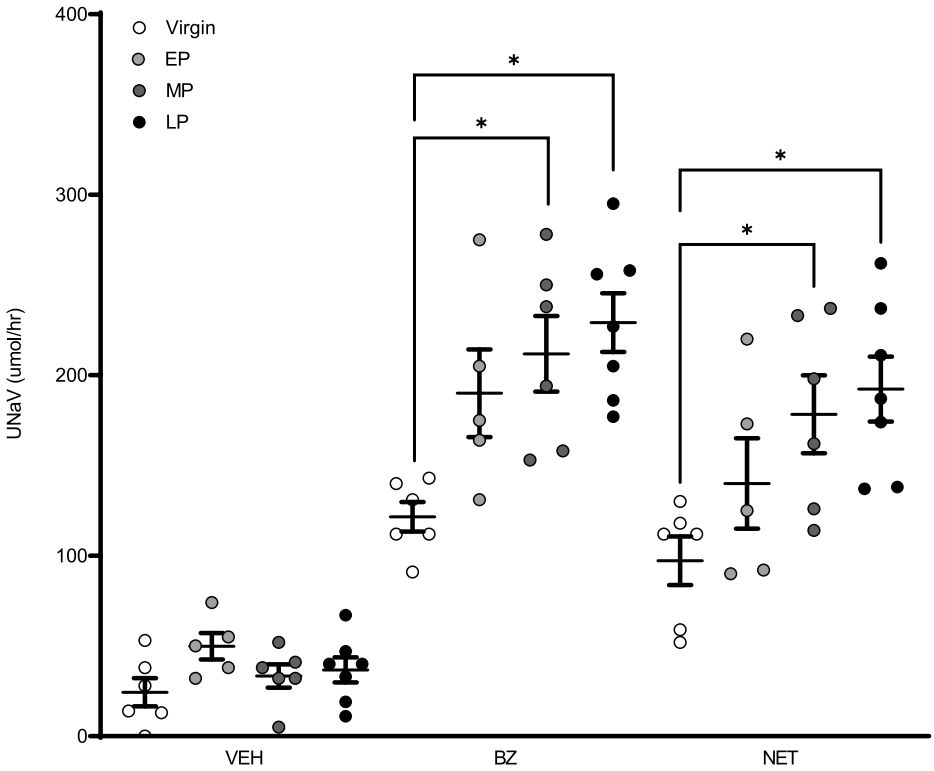

Fig. 3. *In vivo* benzamil sensitive sodium channel activity (ENaC) activity. Renal sodium excretion (UNaV) in response to Benzamil (BZ; 1.05 mg/kg, sc), in virgins ($n = 6$), early pregnant (EP; $n = 5$), mid pregnant (MP; $n = 6$), and late pregnant (LP; $n = 7$) rats. Sodium excretion was measured for 3 h following vehicle (VEH) and Benzamil (BZ). A 1-Way ANOVA with Tukey pot-hoc was performed. Mean ± standard error, *p < 0.05 vs. Virgin.

4. Discussion

The novel findings of this study are 1) that NKCC2 activity is only increased during late pregnancy, 2) that thiazide sensitive sodium transport, likely mediated by NDCBE/pendrin, is increased in all stages of pregnancy, and 3) that ENaC activity is increased beginning in mid-pregnancy.

NKCC2 is best known to be regulated by vasopressin and glomerular filtration rate (GFR) (Hebert et al., 1981; Ares et al., 2011). Vasopressin increases cAMP in the thick ascending limb, which activates protein kinase A, stimulating the reabsorption of sodium through NKCC2. Late pregnancy corresponds to both the time vasopressin is circulating at its highest levels and activation of sodium transport through NKCC2 (Schrier, 2010). Another factor known to increase NKCC2 activity is increased GFR. Increased GFR increases the filtered load of sodium which activates NKCC2. GFR is known to be increased in mid to late pregnant rats (Baylis, 1980) and may be a contributing factor to the increased NKCC2 activity seen in late pregnancy. This late adaptation could be necessary to further increase sodium retention in late pregnancy, where there are mounting natriuretic factors. Alternatively, increased NKCC2 activity coupled with decreased ROMK may be a mechanism to support the increased potassium retention of late pregnancy (West et al., 2018). Further work will be necessary to elucidate the physiological role of increased NKCC2 activity in late pregnancy.

The early increase in the NDCBE/pendrin activity could be due to increased circulating levels of aldosterone. Aldosterone is increased in pregnancy and is critical for sodium retention and plasma volume expansion. Pregnant rats that have undergone adrenalectomy and aldosynthase knockout mice have reduced plasma volume expansion, decreased blood pressure and restricted fetal growth (Barron et al., 1993; Todkar et al., 2012). Aldosterone regulates pendrin expression in non-pregnant rats by increasing total protein abundance and apical plasma membrane protein abundance through subcellular redistribution (Verlander et al., 2003). We have previously shown an increase in pendrin protein and subcellular redistribution in mid and late pregnant rats (West et al., 2015b). Coupling of pendrin to NDCBE permits sodium chloride reabsorption (Morla et al., 2013; Leviel et al., 2010) which is regulated by PAR2 (Morla et al., 2013). Research in male rodents has shown that in addition to stimulating renal sodium chloride reabsorption, activation of PAR2 can produce peripheral vasodilatation and inhibit renal potassium secretion, all of which are features of normal pregnancy. We recently found that normal pregnant rats are more sensitive to the sodium retaining and blood pressure lowering effects of PAR2 activation compared to virgin rats. Further that the PAR2 mediated sodium retention of pregnancy is through a thiazide mediated pathway (likely NDCBE/pendrin) (West et al., 2021). The data from the present study suggests activation of NDCBE/pendrin may be the first adaptation to support the increased sodium balance of pregnancy.

The increase in ENaC activity in mid and late pregnancy is likely due to elevated aldosterone. Aldosterone increases ENaC activity through increased protein abundance, increased trafficking to the plasma membrane, and increased open probability of the channel through phosphorylation. We have previously demonstrated that the increased ENaC activity in pregnancy is mediated through the mineralocorticoid receptor (West et al., 2010). Furthermore, inhibition of ENaC during pregnancy, creates the same phenotype (reduced plasma volume expansion, decreased blood pressure and restricted fetal growth) as pregnant rats that have undergone adrenalectomy and aldosynthase knockout mice (Barron et al., 1993; West et al., 2010, 2014; Todkar et al., 2012).

In conclusion, pregnancy requires continued sodium retention and here we have shown progressive turning on of specific sodium transport mechanisms through the course of gestation to help support this sodium requirement. Understanding these mechanisms is necessary to treat the pathologies of pregnancy associated with volume depletion.

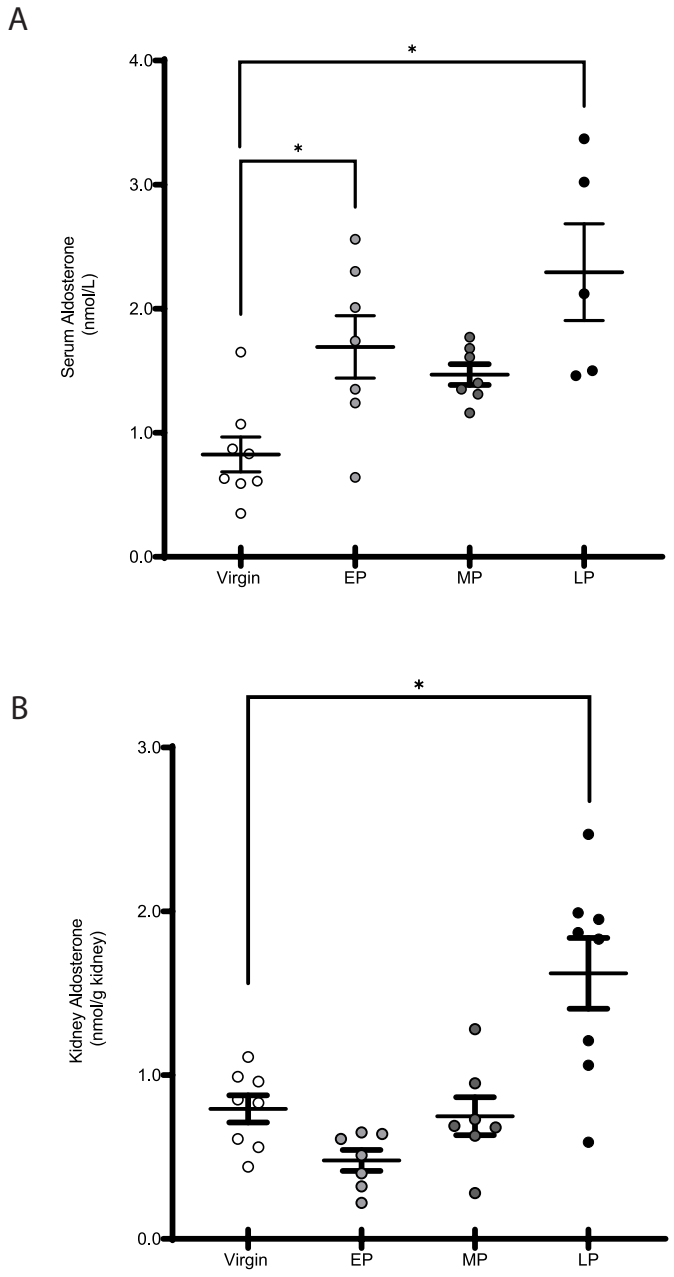

Fig. 4. A) Serum aldosterone as determined by radioimmunoassay in virgin (n = 8), early pregnant (EP; n = 7), mid pregnant (MP; n = 7), and late pregnant (LP; n = 5) rats. B) Kidney aldosterone in V ($n = 8$), EP ($n = 7$), MP ($n = 7$), LP ($n = 8$). A 1-Way ANOVA with Tukey pot-hoc was performed. Mean ± standard error, *p < 0.05 vs. Virgin.

Author contributions

Experiments were performed in the laboratories of S. Masilamani. C. West and S. Masilamani were involved in the conception and design of the experiments. C. West was involved in the data collection. C. West and S. Beck were involved in data entry, analysis, and interpretation. C. West, S. Beck, and S. Masilamani were involved in drafting the article and revising it critically for important intellectual content. All authors approved the final version of this manuscript. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Data availability statement

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

CRediT authorship contribution statement

Crystal A. West: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Steven D. Beck:** Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Shyama M.E. Masilamani:** Conceptualization, Resources, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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