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The natriuretic and diuretic response to dopamine is maintained during rat pregnancy

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Abstract

During pregnancy, there is a marked plasma volume expansion due to renal sodium retention. Pregnant rats exhibit a blunted response to natriuretic stimuli that signal via cGMP, and expression and activity of the cGMP phosphodiesterase PDE-5 are upregulated in the inner medullary collecting duct during pregnancy. Here, we tested the hypothesis that the natriuretic response to a cAMP agonist, dopamine, is maintained during pregnancy. Anesthetized pregnant (*day 16*) and age-matched virgin Sprague-Dawley rats were used to determine whether dopamine-cAMP-mediated natriuresis remains intact in pregnant rats. Blood pressure, renal clearances of inulin and *p*-aminohippuric acid, and excretion of sodium were measured during baseline and dopamine infusion periods. Pregnant rats had a lower blood pressure and hematocrit at baseline than their age-matched virgin counterparts. Dopamine infusion decreased blood pressure and increased glomerular filtration rate and renal plasma flow in virgin but not pregnant rats. Dopamine infusion also increased urine volume, sodium excretion, and the fractional excretion of sodium to a similar extent in virgin and pregnant rats. These results indicate that a cAMP-mediated natriuresis and diuresis (stimulated by dopamine) persists in pregnant rats.

Keywords

pregnancy; plasma volume; sodium retention; cAMP

Normal pregnant women undergo a progressive plasma volume expansion which begins in the first trimester, peaks near gestational *week* 32, and remains elevated until term (9, 18). The demands of fetal development require this increased circulating volume, and failure to expand plasma volume is correlated with complications of pregnancy and intrauterine growth retardation (5, 11). This plasma volume expansion of pregnancy also occurs in animal models and is the result of net, continual renal sodium and fluid retention (1). Previous findings in our laboratory and others have indicated that in normal pregnancy the natriuretic response to atrial natriuretic peptide (ANP) is blunted, therefore promoting sodium retention and plasma volume expansion (7, 12, 15, 16).

ANP signals through the second messenger cGMP, and we have reported that PDE-5 activity and protein abundance in the inner medullary collecting duct are selectively increased in normal pregnancy (14). In addition, selective, intrarenal PDE-5 inhibition with sildenafil reverses the blunted response to infused ANP seen in normal pregnancy (10). Increased renal PDE-5 will degrade cGMP and inhibit the normal natriuretic response, contributing to the volume expansion in normal pregnancy. However, it is currently unknown whether the blunted natriuresis of pregnancy is specific to cGMP-dependent pathways.

Therefore, the purpose of this study was to test the hypothesis that a cAMP-mediated natriuresis remains intact in pregnant rats. To test this hypothesis, we compared the natriuretic response to intravenous dopamine, an adenylyl cyclase agonist that promotes natriuresis via production of cAMP, in virgin and 16 day pregnant rats.

METHODS

All experiments were performed using female Sprague-Dawley rats (3–5 mo old; Harlan Laboratories, Indianapolis, IN) in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and approved and monitored by the University of Florida Institutional Animal Care and Use Committee. Rats were housed at the University of Florida Animal Care Services unit at a constant temperature and humidity with 12:12-h light-dark cycles and given free access to tap water and standard laboratory chow. Females chosen to become pregnant were housed with males, daily vaginal smears were taken, and the presence of sperm was taken as *day 1* of pregnancy and confirmed by the presence of fetuses in utero at the time of acute study.

Experiments were conducted on rats at *day 16* of pregnancy ($n = 7$) and age-matched virgins ($n = 8$). Rats were anesthetized with 100 $\mu\text{l}/100\text{ g}$ body wt of 120 mg/ml Inactin (Sigma, St. Louis, MO), placed on a heated table to maintain body temperature at $37 \pm 1^\circ\text{C}$, a tracheotomy was performed, and oxygen was blown across the tracheal tube throughout the experiment. The left femoral vein was cannulated with PE-50 tubing, and an infusion was started at $5\text{ }\mu\text{l} \cdot 100\text{ g body wt}^{-1} \cdot \text{min}^{-1}$ of 0.09% NaCl, 6.4 mg/ml FITC-inulin, and 11.52 mg/ml *p*-aminohippuric acid (PAH; Sigma). The left femoral artery was cannulated with PE-50 tubing to monitor blood pressure (BP) and to collect blood samples. A suprapubic midline abdominal incision was made, and the bladder was exposed and catheterized for the collection of urine with PE-50 tubing. The bladder was placed back under the skin, and the area was covered with parafilm (Pechiney). After a 60-min stabilization period two $\times$ 20-min baseline urine collections and midpoint blood samples (250 μl) were taken. Then, dopamine (Sigma) was added to the left femoral vein infusion at $12.5\text{ }\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. After a 15-min period, two further 20-min urine collections and blood samples were taken. The uterus and pups were checked to confirm viability at the end of the experiment, then the rat was killed with an overdose of anesthetic, and the left kidney was removed and weighed. The blood was centrifuged, and plasma was used to measure inulin, PAH, and sodium concentration.

To calculate glomerular filtration rate (GFR), inulin concentration in urine and plasma was measured in a black, clear bottom 96-well plate on a Tecan Safire optical system (Tecan) reading fluorescence at 485-nm excitation/530-nm emission. PAH concentration was established using a colorimetric assay previously described (2) to calculate renal plasma flow (RPF). Sodium and potassium concentrations were measured on a flame photometer using cesium as the internal standard (1:100 dilution of sample in 1.5 mmol/l CsCl solution, Instrumentation Laboratory).

Results are presented as means $\pm$ SE. Paired and unpaired *t*-tests with a two-tailed *P* value were used to compare between treatments and time points using Prism 4 software (GraphPad Software, Inc., San Diego, CA).

RESULTS

The pregnant rats had a lower mean arterial pressure (MAP) and hematocrit (Hct) at baseline than their age-matched virgin counterparts (Table 1). Dopamine infusion decreased MAP in virgin rats but had no effect on MAP in the pregnant rats, and dopamine infusion had no effect on Hct in either group. At baseline, GFR, RPF, and urine volume were not different between virgin and pregnant rats. Dopamine infusion significantly increased GFR and RPF in the virgin rats, but there was no change in GFR or RPF in the pregnant group. Dopamine infusion produced a diuresis in both virgin and pregnant rats.

As shown in Fig. 1A, there was no significant difference in baseline sodium excretion ($U_{Na}V$) between the virgin and pregnant rats. Dopamine infusion resulted in a significant rise in sodium excretion in both groups, and the magnitude of the natriuresis was similar (Fig. 1B). The fractional excretion of sodium (FE_{Na}) was similar at baseline between virgin and pregnant rats, and the dopamine-induced increase in FE_{Na} was similar (Fig. 2A) as was the percent rise in FE_{Na} with dopamine (Fig. 2B).

DISCUSSION

The main finding in this study is that dopamine has a similar diuretic and natriuretic action in both virgin and pregnant rats. These data suggest that the renal response to natriuretic stimuli that act via the adenylyl cyclase-cAMP pathway is preserved during pregnancy. This is in contrast to the guanylyl cyclase-GMP pathway as the natriuretic response to ANP is blunted during pregnancy (7, 12, 15, 16).

One limitation of this study is that rats were anesthetized during the measurements of renal function. This is of particular concern because barbiturate anesthesia has been shown to influence renal tubular sodium reabsorption (19) and because the pregnant rat is highly sensitive to general anesthesia and acute surgery (3). However, in previous studies using infusion of ANP as the natriuretic stimulus (10, 12), we have seen similar findings in anesthetized and conscious rats; i.e., in both cases, the pregnant rats showed blunted natriuresis compared with virgin rats. Therefore, the anesthetized pregnant rat preparation is valid for the study of renal tubular responsiveness to natriuretic signals.

During pregnancy, women and rats undergo a massive plasma volume expansion that is the result of net, continual renal sodium and fluid retention (1, 9). In the rat, metabolic cage studies have demonstrated a positive sodium balance during pregnancy, predominately during the last trimester (6). It has also been shown that inhibition of volume expansion by restricting sodium intake in the rat compromises pregnancy close to term (17). In women, plasma volume expansion is an accurate predictor of fetal outcome, with suboptimal plasma volume expansion in pregnancy being associated with “small for gestational age” babies (5). Because of the importance of volume expansion to pregnancy and fetal development, we need to understand the mechanisms involved in renal sodium retention. However, volume regulation in pregnancy is complicated with increases in both natriuretic and antinatriuretic stimuli. Sodium-retaining signals such as activation of the renin-angiotensin-aldosterone system, increased ureteral pressure, and a fall in blood pressure all occur. At the same time, there is a large increase in GFR and increased production of progesterone, ANP, and renal nitric oxide (NO), all of which are expected to promote sodium excretion (1, 4, 8). The reasons that sodium retention prevails remain unknown.

One possible explanation for sodium retention in pregnancy is that there is a selective renal resistance to natriuretic agents. Indeed, we and others have shown that the natriuretic response to exogenous and endogenous ANP is significantly blunted in the pregnant rat (7, 12, 14). Also, the pressure-natriuresis response, likely mediated via NO, is markedly attenuated in late pregnancy (13). Because the tubular natriuretic responses to both ANP and NO are mediated by the second messenger cGMP, impaired cGMP action could account for the blunted natriuresis to these signals during pregnancy.

We have previously shown that there is a selective increase in PDE-5, an enzyme that specifically degrades cGMP, in the inner medulla of pregnant rats (14). In inner medullary collecting duct cells isolated from pregnant rats, cGMP accumulation in response to ANP is reduced, and cGMP hydrolysis is increased compared with cells from virgin controls, and this is normalized by inhibition of PDE-5. Furthermore, PDE-5 inhibition in vivo reversed the blunted natriuresis to an infusion of ANP in pregnant rats (10).

The current study extends these previous observations by illustrating the selectivity of the blunted natriuresis in pregnancy. The natriuretic response to dopamine, a cAMP agonist, is maintained during pregnancy, suggesting that the tubular resistance to natriuretic stimuli observed during pregnancy is specific to agents that activate the cGMP signaling pathway.

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Fig. 1.

A: urinary sodium excretion ($U_{Na}V$) in the baseline state and during intravenous infusion of dopamine ($12.5 \mu\text{g} \cdot \text{kg body wt}^{-1} \cdot \text{min}^{-1}$) in virgin and pregnant rats. $*P < 0.05$ vs. respective baseline. *B:* summary of the increase in $U_{Na}V$ during dopamine infusion in virgin and pregnant rats.

Fig. 2.

A: fractional excretion of sodium (FE_{Na}) in the baseline state and during intravenous infusion of dopamine ($12.5 \mu\text{g} \cdot \text{kg body wt}^{-1} \cdot \text{min}^{-1}$) in virgin and pregnant rats. $*P < 0.05$ vs. respective baseline. B: summary of the increase in FE_{Na} during dopamine infusion in virgin and pregnant rats.

Table 1

Effect of dopamine infusion on mean arterial pressure, hematocrit, glomerular filtration rate, renal plasma flow, and urine flow in virgin and 16-day pregnant rats.

	MAP, mmHg	Hct, ml/100 ml	GFR, ml/ min	RPF, ml/min	UV, ml/min
Virgin baseline	116 ± 2	44.8 ± 0.3	2.4 ± 0.2	4.3 ± 0.3	7.7 ± 1.3
Virgin dopamine	108 ± 2 [*]	44.9 ± 0.6	3.0 ± 0.2 [*]	5.1 ± 0.2 [*]	11.4 ± 1.5 [*]
Pregnant baseline	100 ± 2 [†]	39.0 ± 6.1 [†]	2.2 ± 0.4	3.9 ± 0.7	10.6 ± 1.9
Pregnant dopamine	97 ± 1	38.9 ± 1.1	2.5 ± 0.3	4.1 ± 0.6	13.9 ± 2.0 [*]

Values are means ± SE.

MAP, mean arterial pressure; Hct, hematocrit; GFR, glomerular filtration rate; RPF, renal plasma flow; UV, urine flow.

^{*} $P < 0.05$ vs. respective baseline.

[†] $P < 0.05$ vs. age-matched virgin.