Role of Renal Nerves in Renal Responses to Acute Volume Expansion During Pregnancy in Rats (43585)

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Abstract. **To examine the role of the renal nerves in renal responses to acute volume expansion (VE) at Days 17–19 of pregnancy in rats, the diuretic and natriuretic responses to acute VE were measured from intact and denervated kidneys. One group of pregnant rats (Pregnant 1) was treated with the same amount of VE (1 ml/min for 15 min) as age- and sex-matched virgin control rats, and a second group of pregnant rats (Pregnant 2) was treated with a VE corrected for the higher body weight (presumably expanded blood volume) normally observed in late pregnancy (1.38 ml/min for 15 min). Urine flow and sodium excretion were measured before and after VE from innervated and denervated kidneys in anesthetized (Inactin) rats. Mean arterial pressure was not significantly different among the groups. During VE, the increments in urinary flow (UV) rate and sodium excretion (UNaV) from the innervated kidneys of Pregnant 1 rats were significantly smaller (26.5% for UV and 17.0% for UNaV) than those from the innervated kidneys of virgin rats. Although the UV and UNaV were greater in the Pregnant 2 group than in the Pregnant 1 group, these differences were not statistically significant. However, the values were still significantly smaller than those observed in the control group (39.1% for UV and 52.8% for UNaV). Urine flow and sodium excretion from the denervated kidneys of pregnant rats (both groups) were not significantly different from those of denervated kidneys of control rats. These results demonstrate that the reduced diuresis and natriuresis observed during acute volume expansion in pregnant rats may be due to the contribution of tonic renal nerve activity during the third week of pregnancy.** [P.S.E.B.M. 1993, Vol 203]

Pregnancy in both humans and animals is accompanied by a striking retention of salt and water. During normal gestation in a rat, salt and water are retained, particularly during the final week of a 3-week gestation period (1, 2). This retention of fluid volume results in an increase in extracellular fluid volume and sometimes edema (1, 3). Previous studies have demonstrated that an acute volume load at this stage of pregnancy results in a poor diuresis and natriuresis compared with the control rats (4, 5). Such alterations in renal responses to acute volume expansion have also been observed in various other edema-forming states, such as congestive heart failure, hepatic cirrhosis with ascites, and the nephrotic syndrome (6). Experiments in these forms of edema states demonstrate that renal nerves contribute to the altered renal responses to acute volume expansion, and possibly to the fluid retention observed in these forms of edema. However, the mechanisms responsible for the retention of sodium in the last 2 weeks of pregnancy in rats remain unclear. Among the various neurohumoral systems that may be altered during pregnancy, the activity of the sympathetic nervous system (particularly the renal nerves) affecting sodium and water excretion may also be altered during pregnancy.

This study was designed to determine the contribution of renal nerves to the altered diuresis and natriuresis after acute volume expansion during the third week of pregnancy in rats. To control for the influence of changes in blood pressure and hormonal factors, we denervated one kidney in each rat and examined the hemodynamics and electrolyte excretion in both the

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innervated and denervated kidneys. This preparation is useful in detecting an effect of renal nerves, since each kidney is exposed to the same arterial pressure and circulating hormones. Differences between innervated and denervated kidneys, with respect to renal excretion, in this preparation can be attributed to a direct or indirect effect of the renal nerves.

Methods

Female Sprague-Dawley pregnant and virgin rats were obtained from Sasco River Breeding Laboratories (Omaha, NE). The pregnant and virgin rats were matched for weight and age at the time of breeding. The animal facilities had 12:12-hr light:dark cycles with ambient temperatures maintained at 22°C and humidity at 30–40%. Laboratory chow (Purina) and tap water were available *ad libitum* during these studies. The rats arrived 13 days pregnant. The experiments were performed on each of the rats 4–6 days after their arrival.

General Protocol. On the day of the experiment, rats were anesthetized with Inactin (20 mg/rat, ip). Body temperature was maintained at 37°C via external warming by a heated stage. After tracheal intubation, the animals were allowed to breathe spontaneously. The left femoral artery was cannulated with PE-50 polyethylene tubing and connected to a pressure transducer (Gould P23 ID) for the continuous recording of arterial pressure. The left femoral vein was cannulated with PE-50 tubing and a constant infusion (40 μl/min) of a mixture of inulin and *para*-aminohippuric acid (PAH; 2% inulin and 0.1% PAH) in isotonic saline was started after a 0.7-ml bolus priming dose of a more concentrated mixture (4% inulin and 2% PAH). The clearance of inulin and PAH were used to determine glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), respectively.

Renal Denervation and Ureteral Cannulation. The kidneys were exposed through an abdominal incision and left renal denervation was performed by stripping the sheath and adventitia from the exposed left renal artery and vein. To destroy any remaining nerve fibers, the renal vessels were painted with 95% ethanol. Previously, this technique has been shown to decrease renal norepinephrine concentration to less than 5% of basal endogenous levels (7). In addition, the urine output from the denervated kidney was consistently greater than the contralateral intact kidney. It is recognized that such a preparation will have increased renal nerve activity to the intact kidney because of both Inactin anesthesia (8) and the renorenal reflex (9). However, this effect is not expected to alter the renal sympathoinhibition that should occur in response to volume expansion.

Subsequently, both ureters were cannulated with PE-10 tubing. Surgery was completed within 60 min and an additional 30-min stabilization period was allowed before the start of the first urine collection. Care was taken to ensure that adequate blood flow was maintained to the uterus and that there was no uterine ischemia. The coloration of uterine tissue was stable throughout the study.

Renal Responses to Acute Volume Expansion. The diuretic and natriuretic responses to acute volume expansion (VE) induced by the intravenous infusion of isotonic saline (1.0 ml/min for 15 min, which represents 6.85% of body weight of virgin rats; $n = 8$) were measured in the virgin rats and one subgroup of pregnant rats (Pregnant 1, $n = 8$). Since the late pregnant rats would be massively volume expanded compared with the virgin rats, the percentage of change in volume (change in volume sensed by the volume receptors) produced by the fixed infusion of isotonic saline is predicted to be much less in pregnant than in virgin rats. Therefore, in a second subgroup of pregnant rats (Pregnant 2, $n = 7$), VE was performed by the infusion of 1.38 ml/min of isotonic saline for 15 min. This represents a proportionally equivalent VE (with respect to the pre-existing volume) in the pregnant rats that have an increased blood volume of approximately 40%. Such a correction for an increase in blood volume has been used previously (10). Urine was collected in preweighed tubes from each of the kidneys separately and urine volume was measured gravimetrically. After two 15-min control collection periods, urine was collected during a period of acute volume expansion (15 min) followed by two consecutive 15-min recovery periods. Subsequently, the sodium concentration of each of the urine samples was analyzed using an ion-selective electrode (Beckman Ion analyzer).

The concentrations of PAH and inulin in both plasma and urine were measured by the method of Smith (11) and the anthrone method (12), respectively. Plasma PAH and inulin concentrations were determined for each of the clearance periods before and during VE. Clearance of PAH was calculated and taken as effective renal plasma flow. Clearance of inulin was calculated and taken as glomerular filtration rate.

Statistical Analysis. Data were subjected to analysis of variance to test the overall difference among the groups (13). Multiple comparisons tests were performed to assess the individual differences among the three groups of rats (14). A paired t test was performed to test the differences between the innervated and denervated kidneys within a group. A $P < 0.05$ was considered to indicate statistical significance.

Results

Body Weights, Kidney Weights, and Mean Systemic Blood Pressure. As was expected, the pregnant rats were significantly heavier than the nonpregnant controls (Table I). Kidney weights were not statistically different among the groups of rats. Mean arterial blood

Table I. Body Weight, Kidney Weights, and Mean Blood Pressure in Pregnant and Virgin Rats[a]

	Body wt (g)	Kidney wt (g)		Mean blood pressure (mm Hg)	
		Right	Left	Pre-VE	VE
Virgin (n = 8)	219 ± 3	0.71 ± 0.03	0.70 ± 0.02	103 ± 5	99 ± 7
Pregnant 1 (n = 8)	299 ± 11[b]	0.81 ± 0.04	0.78 ± 0.04	104 ± 4	100 ± 5
Pregnant 2 (n = 7)	302 ± 10[b]	0.79 ± 0.03	0.80 ± 0.04	96 ± 6	94 ± 7

[a] Values represent mean ± SE.
[b] $P < 0.05$ versus virgin.

pressure was not statistically different among the groups under control conditions either before or after VE (Table I). Furthermore, acute volume expansion did not produce a significant change in mean arterial pressure in any of the groups.

Renal Responses to Acute Volume Expansion. There was a significantly greater diuresis and natriuresis from the denervated kidneys than from the contralateral innervated kidneys in all three groups before acute VE (Table II). Diuresis and natriuresis from the intact kidneys in both groups of pregnant rats (Pregnant 1 and Pregnant 2 groups) were significantly smaller than those of control rats (virgin group) before the volume expansion (Table II). There was no significant difference in urine flow or sodium excretion from the denervated kidneys among the groups before the volume expansion. In addition, there were no significant differences in GFR or ERPF among the groups before VE (Table III).

Urine flow and sodium excretion from intact and denervated kidneys of all groups of rats before, during, and after volume expansion are shown in Figures 1 and 2. Acute volume expansion produced an increase in diuresis and natriuresis from both innervated and denervated kidneys in all groups of rats (Figs. 1 and 2). There was significantly smaller diuresis and natriuresis from the intact kidneys of the Pregnant 1 rats than from intact kidneys of virgin rats during VE as assessed by analysis of variance (Figs. 1A and 2A). Cumulative urine flow ((V1-C2) + (V2-C2) + (V3-C2)) was also significantly higher from the intact kidneys of virgin rats (112.5 ± 17.4 μl) compared with Pregnant 1 rats (38.9 ± 17.4 μl). Similarly, cumulative sodium excretion was significantly higher from the intact kidneys of virgin rats (21.8 ± 5.6 μEq) compared with Pregnant 1 rats (6.7 ± 3.6 μEq).

In order to evaluate the contribution of renal nerves in each of the groups, we calculated the difference in urine flow and sodium excretion (Fig. 3, A and B) between the denervated kidneys and the contralateral innervated kidneys in each of the rats. The results indicate a significantly greater contribution of renal nerves in retention salt and water to acute volume expansion in pregnant rats compared with the virgin rats (Fig. 3, A and B).

Cumulative excretion of sodium and water, as percentage of administered load, indicated a significantly lower urine flow (14.5% compared with 33.7%) and sodium excretion (18.6% compared with 43.6%) from the innervated kidneys of Pregnant 2 rats than from control rats. This computation directly compared the virgin and pregnant rats given different volume loads (yet same load relative to their own endogenous volume). There was no significant difference in the diuresis and natriuresis from the denervated kidneys among the three groups during VE (Figs. 1B and 2B). During acute volume expansion, there was a statistically significant increase in ERPF in the Pregnant 1 group compared with virgin rats (Table III).

Table II. Urine Flow and Sodium Excretion from the Intact and Denervated Kidneys of Virgin and Pregnant Rats before Volume Expansion[a]

	Urine flow (μl/min/g kidney wt)		Sodium excretion (μEq/min/g kidney wt)	
	Intact	Denervated	Intact	Denervated
Virgin	8.2 ± 1.7	16.5 ± 4.5[b]	3.1 ± 1.1	3.8 ± 1.1[b]
Pregnant 1	4.6 ± 0.9[c]	31.9 ± 14.0[b]	0.4 ± 0.1[c]	6.7 ± 2.6[b]
Pregnant 2	3.9 ± 0.6[c]	20.1 ± 7.3[b]	0.6 ± 0.2[c]	5.9 ± 2.4[b]

[a] Values represent mean ± SE (n = 7–8).
[b] $P < 0.05$ versus respective intact kidney.
[c] $P < 0.05$ versus virgin.

Discussion

Careful water and electrolyte measurements by various investigators have demonstrated that there is a cumulative sodium chloride retention during the second and third week of pregnancy in rats (1, 2). Although sodium retention has been reported to occur during these 2 weeks of pregnancy, it is the third week of pregnancy (comparable to third trimester in humans) that has been shown to be the prime period of sodium retention (1). It has also been documented that the plasma volume is elevated throughout pregnancy and particularly during the third week of pregnancy (1, 3,

Table III. GFR and ERPF in Intact and Denervated Kidneys of Virgin and Pregnant Rats before and during Volume Expansion[a]

	GFR (ml/min/g kidney wt)		ERPF (ml/min/g kidney wt)	
	Pre-VE	VE	Pre-VE	VE
Intact				
Virgin	0.75 ± 0.14	1.35 ± 0.25	2.38 ± 0.57	3.76 ± 0.88
Pregnant 1	0.64 ± 0.11	1.61 ± 0.27	2.56 ± 0.55	7.66 ± 1.74[b]
Pregnant 2	0.94 ± 0.14	1.91 ± 0.45	2.86 ± 1.07	5.30 ± 1.03
Denervated				
Virgin	0.84 ± 0.15	1.70 ± 0.53	2.36 ± 0.53	5.22 ± 2.15
Pregnant 1	1.32 ± 0.21	1.72 ± 0.34	3.67 ± 0.46	4.81 ± 0.53
Pregnant 2	1.13 ± 0.12	2.84 ± 0.61	3.51 ± 0.72	7.05 ± 1.85

[a] Values represent mean ± SE (n = 7–8).
[b] $P < 0.05$ versus virgin. All VE values were significantly greater than their respective pre-VE values in all groups.

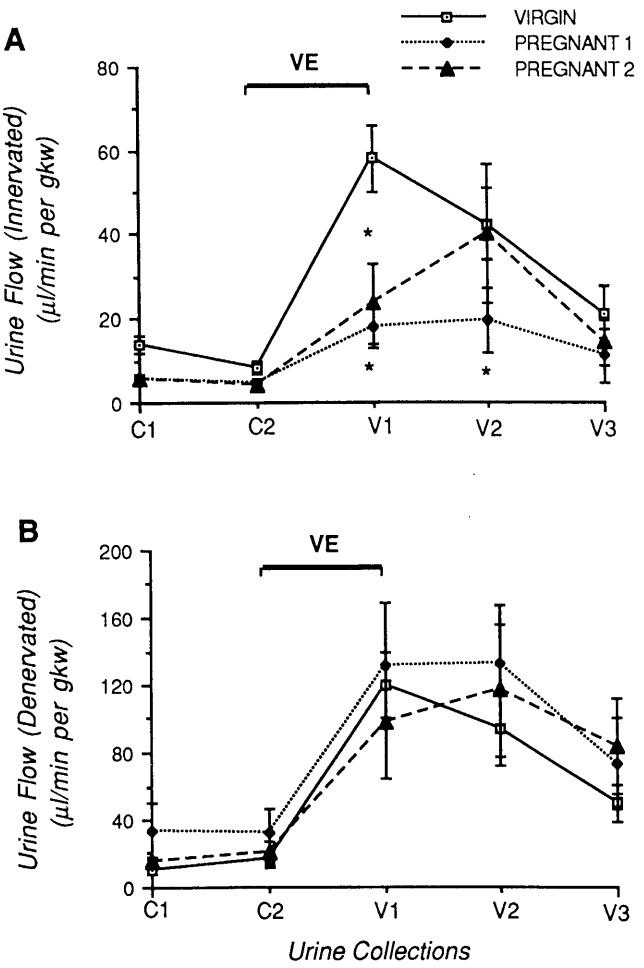

Figure 1. Urine flow (µl/min/g kidney wt) in response to volume expansion from (A) intact and (B) denervated kidneys of virgin, Pregnant 1, and Pregnant 2 rats. Data represent two control periods (C1 and C2), one period during volume expansion (V1), followed by two additional collection periods (V2 and V3). Values represent mean value for each group ± SE (n = 7–8). Asterisk indicates $P < 0.05$ versus virgin rats.

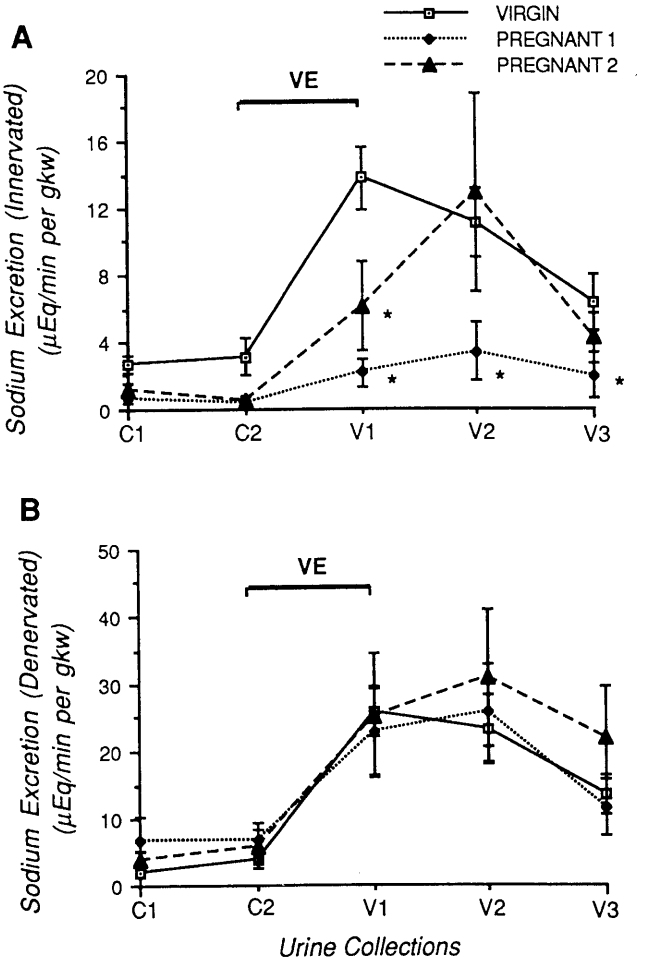

Figure 2. Sodium excretion (µEq/min/g kidney wt) in response to volume expansion from (A) intact and (B) denervated kidneys of virgin, Pregnant 1, and Pregnant 2 rats. Data represent two control periods (C1 and C2), one period during volume expansion (V1), followed by two additional collection periods (V2 and V3). Values represent mean value for each group ± SE (n = 7–8). Asterisk indicates $P < 0.05$ versus virgin rats.

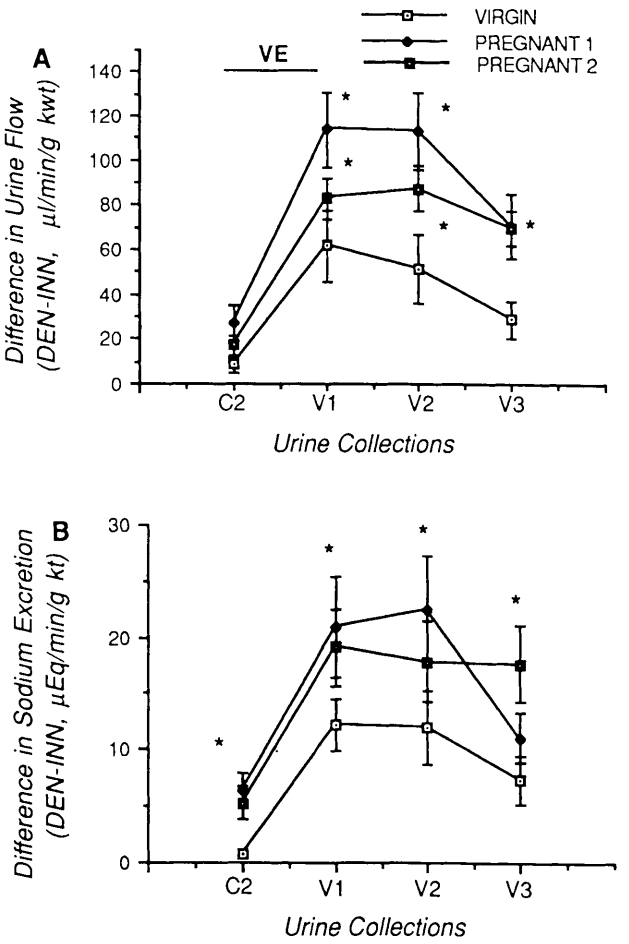

Figure 3. The difference in (A) urine flow (µl/min/g kidney wt) and (B) sodium excretion (µEq/min/g kidney wt) between the denervated kidney and the contralateral innervated kidney in response to acute volume expansion from virgin, Pregnant 1, and Pregnant 2 rats. Data represent one control period (C2), one period during volume expansion (V1), followed by two additional collection periods (V2 and V3). Values represent mean value for each group ± SE ($n = 7$–8). Asterisk, indicates $P < 0.05$ versus virgin rats.

15, 16). In addition, pregnant rats exhibit a decrease in salt and water excretion during volume expansion with an isotonic saline load (4, 5). In spite of the large plasma volume during pregnancy, saline loading, superimposed on the chronic expansion of fluid volume during pregnancy, failed to produce a normal increase in sodium excretion (1, 3–5). The present study confirms these observations. Both groups of pregnant rats showed an impaired ability to excrete the volume load from the innervated kidneys during the 15 min of VE. In addition, even if the volume load was larger (40% increase to account for the increase in blood volume in the pregnant rats), the cumulative urine flow and sodium excretion expressed as a percentage of administered load were significantly smaller from the pregnant rats compared with the virgin rat. More importantly, renal denervation corrects these blunted renal responses to acute volume challenge, indicating that the presence of renal nerves may contribute to the retention of sodium and water observed during pregnancy.

The mechanisms involved in the control of blood volume, volume perception (volume sensing), and regulation during pregnancy remain unclear. Schrier and Durr (17) have raised the concept of "overfill or underfill" of the circulation by blood during pregnancy. If one subscribes to the concept of overfill during pregnancy (17), then it would follow that any amount of volume expansion (assuming an increased pre-existing volume and no change in compliance of the vasculature) should be expeditiously excreted. With this line of thinking, the data in this study would support the concept of underfill during pregnancy (17), since the fixed amount of VE in Pregnant 1 rats was not enough to "fill" the circulation; it produced a reduced amount of excretion (via diuresis and natriuresis) compared with that observed in virgin rats. In addition, the increased amount of VE in Pregnant 2 rats was also not enough (significantly lower diuresis and natriuresis; Figs. 1 and 2) to fill the circulation. In other words, the circulatory systems in the pregnant and the virgin rats do not sense the expanded volume similarly. Thus one would contend that the data in the present study support the concept of underfill during pregnancy. It should be noted that these conclusions are based on the assumption that a curve of size of VE against response (diuresis and natriuresis) does not flatten out at a high pre-existing volume.

The question then arises, "What is responsible for this failure to increase sodium excretion in response to volume expansion?" The results of the present study demonstrate, as others have done previously (4, 5), that acute volume expansion produces a blunted diuresis and natriuresis in pregnant rats (Figs. 1 and 2). The results from the denervated kidneys demonstrate that renal denervation normalizes the diuretic as well as natriuretic responses to volume expansion in pregnant rats. In other words, renal denervation corrects the blunted volume reflex in pregnant rats. These data suggest that renal nerves may also be responsible, in part, for the retention of sodium chloride and water during the third week of pregnancy in rats. This is further supported by the comparison of the effects of renal denervation on both baseline urine flow and sodium excretion. Such a comparison demonstrated that there was a greater change in urine flow and sodium excretion after denervation in pregnant rats than in the virgin rats. These results suggest an activation of renal sympathetic nervous discharge in pregnant rats, which in turn may be responsible for the increased retention of sodium and water. It should be noted, as stated previously, that the preparation used in this study was expected to have increased renal nerve activity to the intact kidney because of both Inactin anesthesia (8) and the renorenal reflex (9). Whether these sympathoexci-

tatory effects would be greater in pregnant rats compared with virgin rats is not known.

The results of the measurements of renal hemodynamics demonstrate that ERPF and GFR were not significantly different between the two groups of rats (virgin compared with pregnant groups), suggesting that these parameters were probably not involved in the altered renal responses observed in the term pregnant rats. The data regarding ERPF are consistent with previous studies that reported no significant differences in renal plasma flow between the virgin and pregnant rats during the third week of pregnancy (3, 18, 19). However, previous reports regarding GFR are inconsistent (5, 18–21). It appears that GFR is increased during the first and second week of pregnancy (15, 20, 21) and decreased or unchanged during the third week of pregnancy (5, 15, 18, 19) in rats. It is now accepted that there is no significant difference in ERPF and GFR by the third week of pregnancy (3, 15, 18, 19). In the present study the differences in GFR observed between the virgin and pregnant rats were not statistically significant. The lack of elevation of GFR in the intact kidneys might be due to an increase in renal sympathetic nerve activity in such an experimental preparation (8, 9). The denervation data support the concept of increased renal sympathetic nerve activity, since in the pregnant rats, GFR were slightly higher in either kidney, although not statistically different, than in denervated kidney in virgin rats. However, this explanation would require a differential effect of the surgical preparation on the pregnant rats compared with the virgin rats. An alternative explanation for the lack of increased GFR in the pregnant rats may be that as the pregnant rats approach term, the GFR returns to normal or below-normal nonpregnant values (5). Therefore, it is not surprising that pregnant rats, which were close to term in the present study, exhibited a GFR that was not statistically different from the GFR of virgin rats. In any case, the decreased sodium excretion to acute volume expansion in term pregnant rats cannot be attributed to changes in GFR.

The roles of hormonal factors such as vasopressin (22) and aldosterone (23–25) have also been examined in the past. Plasma vasopressin and the ability to concentrate urine are similar between late-pregnant and virgin control rats (22). However, in this regard, it is of interest to note that plasma osmolality is lower during the second half of pregnancy (1). A role for aldosterone is also uncertain (23–25). Although increased plasma levels of aldosterone have been observed in late pregnancy (23, 25), surgical adrenalectomy and treatment with spironolactone have consistently failed to alter sodium retention during this stage of pregnancy (24). In preliminary studies, we examined whether the renal responses to atrial natriuretic factor, the most powerful endogenous natriuretic factor in the body, were altered in the pregnant rats. The increase in circulating atrial natriuretic factor levels after acute volume expansion is similar in virgin and pregnant rats (16). The results from our preliminary studies (unpublished) and other studies demonstrate that the diuretic and natriuretic responses to atrial natriuretic factor are not altered in pregnancy (26).

This study confirms the blunted volume reflex observed previously in pregnant rats. Furthermore, it identifies the renal nerves as being primarily responsible for the retention of sodium chloride and water during an acute volume challenge during the third week of pregnancy in rats. One interpretation of these data is that renal nerves also may participate in the retention of salt and water that occurs during the third week of pregnancy in rats.

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