

## Evidence against the hypothesis that prostaglandins are the vasodepressor agents of pregnancy. Serial studies in chronically instrumented, conscious rats.

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### Research Article

Renal hemodynamics increase dramatically during pregnancy, and pressor responsiveness to exogenous administration of vasoconstrictors is attenuated. We investigated whether or not vasodilatory prostaglandins mediate these phenomena. Trained, chronically instrumented, conscious pregnant rats were used. Control values of glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were elevated at midgestation (P less than 0.01 and P = 0.05 from prepregnant means, respectively), and effective renal vascular resistance was decreased (P = 0.05). Indomethacin (4.5-6.5 mg/kg body weight [BW]) failed to decrease renal hemodynamics at this stage of pregnancy; in fact, it raised GFR somewhat further (P less than 0.05). Systemic pressor responsiveness to bolus administration of norepinephrine and angiotensin II (All) was significantly attenuated by at least gestational day 20. Neither indomethacin (7 mg/kg BW) or meclofenamate (6 mg/kg BW) affected the refractory response. The renal vasculature was also relatively unresponsive to an intravenous infusion of All (5 ng X kg<sup>-1</sup> X min<sup>-1</sup>) during late gestation (day 19); in particular, the fall in ERPF in response to All (16 +/- 3%) was markedly less than that observed in the prepregnant condition (34 +/- 3%; P less than 0.05). Indomethacin (6 mg/kg BW) failed to restore this blunted response, and further attenuation was evident, despite the presence of the inhibitor (gestational day 21). We conclude that vasodilatory prostaglandins do not [...]

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# Evidence against the Hypothesis That Prostaglandins Are the Vasodepressor Agents of Pregnancy

## Serial Studies in Chronically Instrumented, Conscious Rats

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### Abstract

Renal hemodynamics increase dramatically during pregnancy, and pressor responsiveness to exogenous administration of vasoconstrictors is attenuated. We investigated whether or not vasodilatory prostaglandins mediate these phenomena. Trained, chronically instrumented, conscious pregnant rats were used. Control values of glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were elevated at midgestation ( $P < 0.01$  and  $P = 0.05$  from prepregnant means, respectively), and effective renal vascular resistance was decreased ( $P = 0.05$ ). Indomethacin (4.5–6.5 mg/kg body weight [BW]) failed to decrease renal hemodynamics at this stage of pregnancy; in fact, it raised GFR somewhat further ( $P < 0.05$ ). Systemic pressor responsiveness to bolus administration of norepinephrine and angiotensin II (AII) was significantly attenuated by at least gestational day 20. Neither indomethacin (7 mg/kg BW) or meclofenamate (6 mg/kg BW) affected the refractory response. The renal vasculature was also relatively unresponsive to an intravenous infusion of AII ( $5 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) during late gestation (day 19); in particular, the fall in ERPF in response to AII ( $16 \pm 3\%$ ) was markedly less than that observed in the prepregnant condition ( $34 \pm 3\%$ ;  $P < 0.05$ ). Indomethacin (6 mg/kg BW) failed to restore this blunted response, and further attenuation was evident, despite the presence of the inhibitor (gestational day 21). We conclude that vasodilatory prostaglandins do not appear to mediate the rise in renal hemodynamics, and the attenuation of the systemic and renal pressor responsiveness observed during pregnancy, insofar as these phenomena were unaffected by acute cyclooxygenase inhibition in unstressed, conscious rats.

### Introduction

Dramatic alterations in renal and cardiovascular hemodynamics occur during gestation in humans. Glomerular filtration rate (GFR)<sup>1</sup> and effective renal plasma flow (ERPF) increase in the

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1. *Abbreviations used in this paper:* AII, angiotensin II; BW, body weight; ERPF, effective renal plasma flow; ERVR, effective renal vascular resistance; GFR, glomerular filtration rate; MAP, mean arterial pressure; NE, norepinephrine; PAH, sodium aminohippurate; PG, prostaglandin.

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first trimester by as much as 50%; this increment is sustained at least until late pregnancy, when renal hemodynamics may decline toward prepregnant values (1–3). Cardiac output also rises dramatically (4), and blood pressure falls by  $\sim 10$  mmHg (5). Taken together, these data indicate that renal and systemic vascular resistance are decreased during much of gestation.

We have recently reported that the chronically instrumented, conscious rat demonstrates alterations of renal hemodynamics and blood pressure during pregnancy which resemble those observed in human gestation (6). (Our study corroborated several, but not all, previous investigations performed in various strains of acutely prepared, pregnant rats [for pertinent reviews, see References 6 and 7].) The application of a chronically prepared, conscious animal preparation to the study of renal and cardiovascular function during pregnancy provides three distinct advantages: (a) The perturbations of renal hemodynamics and of the hormonal and cardiovascular systems, which are brought about by anesthesia and surgical stress (8–18) as well as by alterations of plasma volume (19, 20), are avoided. This advantage may be particularly apropos to pregnancy, in which the underlying physiology is radically altered from the nonpregnant condition; that is, even before an experimental variable of interest is implemented, the anesthesia and surgical stress may have affected pregnant and nonpregnant animals differently, such that the latter no longer serves as an adequate control. (b) Each animal can be used as her own control—the same rat can be studied longitudinally (before, during, and after pregnancy). (c) Inasmuch as the changes in renal hemodynamics and blood pressure observed in the chronically instrumented, conscious pregnant rat resemble those of human gestation (6), it is possible that results regarding mechanisms can be extrapolated to humans.

Several investigators have postulated a role for vasodilating prostaglandins (PGs) in the control of systemic vascular resistance and blood pressure during pregnancy (21, 22). Pedersen and co-workers (23) have demonstrated an increase in urinary excretion of PGE<sub>2</sub> during human pregnancy, as assessed by radioimmunoassay. Using gas chromatography-mass spectrometry, Goodman and associates (24) observed a rise in urinary excretion of two metabolites of PGI<sub>2</sub>. Venuto and Donker (25) and Paller (26) have reported increases of urinary PGE<sub>2</sub> excretion in pregnant rabbits and rats, respectively. In the present study, we tested whether or not PGs serve as vasodepressor agents in pregnancy. Our study, carried out in chronically instrumented, conscious rats, included (a) the effects of cyclooxygenase inhibition on GFR, ERPF, and effective renal vascular resistance (ERVR); (b) the change in mean arterial pressure (MAP) produced by bolus administration of angiotensin II (AII) and norepinephrine (NE), before and after inhibition of prostaglandin synthesis; and (c) the influence of an intravenous infusion of AII on renal hemodynamics and MAP, before and after cyclooxygenase inhibition. The general approach, therefore, was to assess the contribution of PGs (if any) to the control of renal and cardiovascular

function during pregnancy by acute interruption of PG synthesis. Clarification of the role that these hormones may have in normal pregnancy could facilitate investigation of the pathogenesis of preeclampsia. Indeed, several investigators (23, 24, 27) have postulated that a relative deficiency of vasodilating PGs may be causative.

## Methods

### Animal preparation

Female Long-Evans rats (Charles River Breeding Laboratories, North Wilmington, MA) fed a normal diet (Charles River RMH 3000 formula) were used. They were ~14 wk old at the beginning of the study. All animals underwent training in the experimental cage (28) prior to surgery (3–4 h/d for at least 7 d). Details of the surgical preparation and post-operative care have been previously described (6, 28). Aseptic technique was employed, and animals were allowed 7–10 d of recovery from surgery before the first experiment was run.

### Experimental procedures

**Effects of indomethacin on renal function.** Animals were studied twice before mating with 4 d allowed between studies. After conception (day 1 of gestation was documented by the presence of spermatozoa in the vaginal lavage), the same rats were again examined on gestational days 12 and 20 (total gestation being 22 d). For each study, the rat was first placed in the experimental cage. A special cage was designed to accommodate the enlarging abdominal girth during days 16–20 of pregnancy. The femoral artery catheter was connected to a Statham pressure transducer (model P23 ID, Statham Instrument Div., Hato Rey, PR) and a chart recorder (model 5D, Grass Instrument Co., Quincy, MA; or model ICT-2H, Gilson Medical Electronics, Inc., Middleton, WI). This catheter was also used for collection of blood samples, and administration of vehicle or indomethacin. The femoral venous catheter was attached to a Sage infusion pump (model 355, Orion Research, Inc., Cambridge, MA) for the delivery of polyfructosan (Inutest, Laevosan-Gesellschaft, Linz-Donau, Austria), and PAH (sodium aminohippurate, Merck, Sharp, and Dohme, West Point, PA) in Ringer's solution. Finally, the bladder cannula was extended with a polyethylene tube (Intramedic, Clay Adams, Parsippany, NJ), and timed urine collections were made. (This technique of urine collection has proven to be reliable; after achieving steady-state conditions, the excretion of polyfructosan and PAH virtually matched their infusion rates— $98 \pm 5\%$  and  $97 \pm 4\%$ , respectively,  $n = 9$  rats.) The clearances of polyfructosan and PAH provided a measure of GFR and ERPF, respectively.

After the infusion was started ( $1.0 \text{ mg} \cdot \text{min}^{-1} \cdot 100 \text{ g body weight (BW)}^{-1}$  for polyfructosan, and  $0.075$  or  $0.1 \text{ mg} \cdot \text{min}^{-1} \cdot 100 \text{ g BW}^{-1}$  for PAH) at  $24 \mu\text{l}/\text{min}$ , an equilibration of at least 60 min was allowed. Vehicle ( $0.05 \text{ M Na}_2\text{CO}_3$ ) was administered over a 10-min interval early in the equilibration period. Then, two 30-min urine collections were obtained with midpoint blood samples (each sample 200–250  $\mu\text{l}$ ). Indomethacin ( $6 \text{ mg}/\text{kg BW}$ ) was next administered over a 10-min period. (In several of the earlier experiments, indomethacin was given as a  $3 \text{ mg}/\text{kg BW}$  bolus, followed by an infusion of  $2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  ( $3.0 \mu\text{l}/\text{min}$ ), or in divided doses— $4 \text{ mg}/\text{kg BW}$ , followed 15 min later by  $2 \text{ mg}/\text{kg BW}$ .) 45–60 min after the start of indomethacin administration, two or three more 30-min renal clearances were performed with midpoint blood samples. After centrifugation of blood samples and separation of plasma from cells, the latter were resuspended in Ringer's solution, and returned to the rat.

**Time-control studies.** Two types of time-control experiments were conducted in nonpregnant rats. (a) Periodic studies were performed in the same animals over a period ranging from 5 to 40 d after surgical preparation. Each experiment was conducted as follows. After the start of an infusion of polyfructosan and PAH in Ringer's solution, an equilibration period of 1 h was allowed. Then, two 30-min renal clearances were performed. (b) Renal function and MAP were assessed in rats over a period of 4.5 h while the animals were in the experimental cage. After

a 60-min equilibration period with polyfructosan and PAH in Ringer's solution, five 40-min renal clearances were obtained.

**Effects of AII and NE on blood pressure before and after indomethacin or meclofenamate.** Because urine collection was unnecessary in these studies, bladder cannulae were not implanted. Three types of experiments were performed. (a) Rats were studied twice before mating, with 4 d allowed between studies. After a 60-min equilibration period in the experimental cage, AII (3.12, 6.25, 12.5, 25, and 50  $\text{ng}/\text{kg BW}$ ) and NE (50, 100, 200, and 400  $\text{ng}/\text{kg BW}$ ) were administered intravenously in random order, and the change in MAP recorded. 10 min were allowed between each bolus (200  $\mu\text{l}$  each). (In each experiment, a 200- $\mu\text{l}$  bolus of vehicle [5% dextrose] was also administered. The vehicle yielded an average increment in MAP of 2.5 mmHg.) Then, indomethacin ( $7 \text{ mg}/\text{kg BW}$ ) or meclofenamate ( $6 \text{ mg}/\text{kg BW}$ ) was given intravenously over a 10-min period. After an equilibration of 45–60 min, the vasoconstrictors were again administered. After mating, the same rats were studied in an identical fashion on gestational days 12, 16, and/or 20. Several of them were also examined on postpartum day 5–6. (b) To enhance the accuracy of our study, experiments were performed as described above, except that only one dosage of AII (25  $\text{ng}/\text{kg BW}$ ) and NE (200  $\text{ng}/\text{kg BW}$ ) was given, each in triplicate, before and after meclofenamate. (c) In order to assess the potential effects of recovery from acute surgical preparation and ether anesthesia, as well as lack of training to the experimental cage (26), we performed studies similar to those in (a) and (b), except that rats (untrained) were initially mated, and catheters implanted under ether anesthesia on gestational days 15–16. The incision was  $\leq 1 \text{ cm}$ , no blood loss was incurred, catheters were secured with ties and exteriorized directly from the site of incision. The wound was then sutured together around the catheters. Saline (0.9% NaCl; 0.5% of BW) was given during surgery (26). After surgical preparation, the animals were placed in the experimental cage, and allowed to recover for  $\geq 60$  min before studies began. Age-matched, virgin female rats prepared in the same fashion, served as controls.

**Effects of AII on renal hemodynamics before and after indomethacin.** Rats were studied before mating. Two control renal clearances were obtained (30 min each), followed by administration of vehicle ( $0.05 \text{ M Na}_2\text{CO}_3$ ) over a 10-min period, and a continuous intravenous infusion of AII ( $5 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  at  $3 \mu\text{l}/\text{min}$ ). After a 45-min equilibration, three 30-min renal clearances were performed. These animals were then mated, and the same experiment was repeated on gestational days 19 and 21, except that indomethacin ( $6 \text{ mg}/\text{kg BW}$ ) was administered on day 21, instead of vehicle.

### Preparation of drugs

All solutions were sterilized through millipore filters (Millex-GS, Millipore, Bedford, MA). Indomethacin (Sigma Chemical Co., St. Louis, MO) was freshly prepared for each experiment. It was dissolved over a 3-min period in  $0.05 \text{ M Na}_2\text{CO}_3$  heated to  $40^\circ\text{C}$ . Then, HCl ( $1.0 \text{ M}$ ) was added to achieve a final pH of  $\sim 8.0$ . A solution of  $\text{Na}_2\text{CO}_3$  ( $0.05 \text{ M}$ , titrated to pH 8.0) served as vehicle. Meclofenamate (Warner-Lambert, Ann Arbor, MI) was also freshly prepared for each experiment. It was dissolved in  $0.1 \text{ M NaCl}$ . It has been shown that the dosages of indomethacin (ranging from 4.5 to  $7.0 \text{ mg}/\text{kg BW}$ ) and meclofenamate ( $6 \text{ mg}/\text{kg BW}$ ) employed in these studies effect significant inhibition of renal prostaglandin production (29–31). For verification, urine samples obtained from a few of the studies performed in the very first experimental procedure described above were analyzed for urinary  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  excretion (32). Indomethacin reduced the urinary excretion of  $\text{PGE}_2$  and/or  $\text{PGF}_{2\alpha}$  by  $>90\%$  ( $n =$  three observations in two nonpregnant rats, and  $n =$  two observations in two pregnant rats). In a day-14 pregnant rat, meclofenamate decreased the urinary excretion of  $\text{PGE}_2$  by 74%.

AII (5-ILE AII or Hypertensin II; Sigma Chemical Co.) was freshly prepared every 2 mo as stock solution in 5% dextrose ( $100 \mu\text{g}/\text{ml}$ ), and kept in 1.0-ml aliquots at  $-20^\circ\text{C}$ . Final dilutions were carried out with Ringer's solution or 5% dextrose. NE (Levophed, Winthrop Laboratories, New York) was prepared as stock solution in 5% dextrose ( $800 \text{ ng}/\text{ml}$ ), and kept in 1.0-ml aliquots at  $-20^\circ\text{C}$ . Final dilutions were also made in 5% dextrose.

Table I. Influence of Indomethacin on Renal Function in Nonpregnant Rats

	MAP	GFR	ERPF	ERVR	
	mmHg	$\mu\text{l}/\text{min}$	$\mu\text{l}/\text{min}$	$\text{mmHg} \cdot \text{ml} \cdot \text{min}^{-1}$	
Vehicle	110±1	2,902±77	9,641±224	6.51±0.18	
Indomethacin	111±1	2,827±83	9,135±240*	6.87±0.21	
	$\dot{V}$	$U_{\text{Na}} \cdot \dot{V}$	$C_{\text{Na}}/C_{\text{In}}$	$U_{\text{K}} \cdot \dot{V}$	$C_{\text{K}}/C_{\text{In}}$
	$\mu\text{l}/\text{min}$	$\mu\text{eq}/\text{min}$	%	$\mu\text{eq}/\text{min}$	%
Vehicle	20.3±1.2	2.45±0.28	0.59±0.06	3.21±0.14	26.24±0.98
Indomethacin	22.7±1.6	3.78±0.35‡	0.95±0.08‡	2.27±0.09‡	19.47±0.62‡

Values are means±SEM. The data were obtained from 21 chronically instrumented, conscious rats. MAP, mean arterial pressure; GFR, glomerular filtration rate; ERPF, effective renal plasma flow; ERVR, effective renal vascular resistance (MAP-5/ERBF);  $\dot{V}$ , urinary flow;  $U_{\text{X}} \cdot \dot{V}$ , urinary excretion rate of solute;  $C_{\text{X}}/C_{\text{In}}$ , fractional excretion of solute. \*  $P < 0.05$ . ‡  $P < 0.001$  indomethacin vs. vehicle.

### Analytical techniques

Polyfructosan in plasma and urine was determined by the anthrone technique (33), and PAH, by the method of Bratten and Marshall as modified by Smith et al. (34). Sodium and potassium in plasma and urine were measured by flame photometry (model 343, Instrumentation Laboratory, Inc., Lexington, MA). Urine volume was determined gravimetrically. All clearance data have been expressed per whole animal.

### Statistical analysis

Except for Table I, which was analyzed by the paired  $t$  test, data on each measure were subjected to a univariate analysis of variance. Measures for which there were no missing observations were analyzed in a randomized block or split-plot design (in the event of a between-subject factor) with "rat" as the blocking variable. Measures for which there were missing observations were analyzed in a partially balanced incomplete block design; in the latter case, effects were tested against the rat by effect interaction. Comparisons between means were performed using Dunnett or Tukey tests, with corrections made for unbalanced cell sizes if necessary. These comparisons were performed only to explore significant effects unless planned by a priori hypotheses. Separate error terms were computed for contrasts in which assumptions about variance were not met by the data. The reference for all analytical procedures was Kirk (35). A value of  $P < 0.05$  was taken to be significant.

### Results

**Effects of indomethacin on renal function.** Indomethacin did not alter MAP or GFR in nonpregnant rats (Table I). ERPF was decreased, and ERVR (MAP-5/ERBF) increased, slightly—both by ~5% ( $P < 0.05$  and  $P < 0.1$ , respectively). Time-control studies (Table II) performed in nonpregnant rats showed no significant changes in any of these variables, although ERPF tended to decrease, and ERVR to increase by ~5%. Thus, indomethacin had little, if any effect on renal hemodynamics. (The percent changes of ERPF and ERVR between indomethacin and time-control studies were not significant—both  $P > 0.1$ .)

As shown in Table I, the urinary excretion rate of sodium ( $U_{\text{Na}} \cdot \dot{V}$ ) and fractional excretion of sodium ( $C_{\text{Na}}/C_{\text{In}}$ ) were apparently augmented by indomethacin. Examination of the time-control studies (Table II), on the one hand, shows that similar increments occurred. Indomethacin, therefore, most likely had no overall effect on renal handling of sodium. (The percent changes of  $U_{\text{Na}} \cdot \dot{V}$  and  $C_{\text{Na}}/C_{\text{In}}$  between indomethacin and time-control studies were not significant—both  $P > 0.1$ .) On the other hand (Table I), the drug did effect significant decrements in po-

Table II. Time-Control Studies Performed during a 4½-h Period in Nonpregnant Rats

	Time (min)				
	60–140*	150–190	190–230	230–270	150–270‡
MAP (mmHg)	111±2	112±2	112±2	112±2	112±2
GFR ( $\mu\text{l}/\text{min}$ )	2,763±148	3,035±216	2,867±108	2,850±157	2,920±138
ERPF ( $\mu\text{l}/\text{min}$ )	9,162±519	9,365±734	8,386±466	8,630±511	8,794±525
ERVR ( $\text{mmHg} \cdot \text{ml} \cdot \text{min}^{-1}$ )	6.87±0.40	6.82±0.47	7.47±0.40	7.23±0.36	7.17±0.37
$\dot{V}$ ( $\mu\text{l}/\text{min}$ )	20.8±2.9	32.3±5.6	35.4±6.5	33.5±6.2	33.8±5.7
$U_{\text{Na}} \cdot \dot{V}$ ( $\mu\text{eq}/\text{min}$ )	1.75±0.34	3.60±0.68§	4.08±0.73	3.39±0.57	3.69±0.59§
$C_{\text{Na}}/C_{\text{In}}$ (%)	0.46±0.09	0.84±0.13	1.01±0.17	0.85±0.13	0.90±0.12§
$U_{\text{K}} \cdot \dot{V}$ ( $\mu\text{eq}/\text{min}$ )	3.02±0.29	3.43±0.46	3.06±0.33	2.70±0.30	3.06±0.35
$C_{\text{K}}/C_{\text{In}}$ (%)	27.9±2.5	29.6±2.5	28.2±2.3	25.3±2.0	27.7±2.1

Values are means±SEM. Time controls were performed in nine chronically instrumented, conscious animals. For abbreviations, see Table I.

\* The first two 40-min renal clearances were averaged. ‡ Average of last three 40-min renal clearances. §  $P < 0.05$ . ||  $P < 0.01$  vs. 60–140-min period. N.B. Although analysis of variance revealed a significant main effect of time on  $\dot{V}$  ( $P = 0.05$ ), application of Dunnett tests failed to show significant differences between the control (60–140 min) and subsequent time periods.

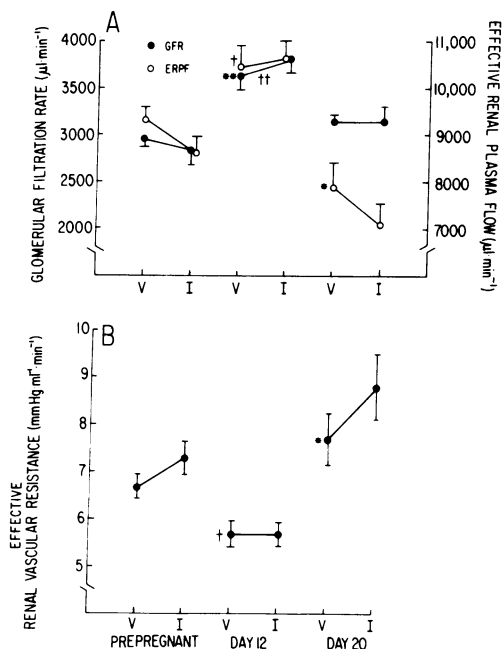


Figure 1. (A and B) Influence of indomethacin on renal hemodynamics in conscious, pregnant rats. After being each studied twice, nine rats were mated. Seven were subsequently examined on gestational day 12, and eight on day 20. V, vehicle; I, indomethacin. † $P < 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$  pregnant vs. prepregnant; †† $P < 0.05$  vehicle vs. indomethacin.

tassium excretion ( $U_K \cdot \dot{V}$ ) and fractional excretion of potassium ( $C_K/C_{In}$ ), which were not observed in time-control experiments. Finally, whereas urinary flow rate ( $\dot{V}$ ) increased in time control studies (Table II,  $P = 0.05$ ), no such increment was observed in the experiments involving administration of indomethacin (Table I).

Of the 21 nonpregnant rats tested with indomethacin, nine were subsequently mated, and examined on gestational days 12 and 20. The prepregnant values for renal function and MAP before and after indomethacin (Fig. 1 A and B; Table III) were not different from those obtained from the larger group of 21

rats discussed above. As we observed previously (6), base-line values of GFR and ERPF were significantly elevated on gestational day 12 (Fig. 1 A), when compared to prepregnant means ( $P < 0.01$  and  $P = 0.05$ , respectively), and ERVR was decreased (Fig. 1 B,  $P = 0.05$ ). In order to further verify that this increment in renal hemodynamics is specific to pregnancy, time-control studies were performed in nonpregnant animals over a period of 5–40 d after surgical preparation (Table IV). Analysis of variance showed a significant main effect of time only on BW ( $P < 0.001$ ).

Indomethacin did not decrease renal hemodynamics during midgestation. In fact, GFR was enhanced further ( $P < 0.05$ ). The tendency for ERPF to fall, and ERVR to rise, as seen in time-control studies (Table II), and in experiments using indomethacin in nonpregnant rats (Table I; Figs. 1 A and B) was not observed on gestational day 12. By day 20, GFR had returned towards prepregnant levels, and ERPF was actually less than, and ERVR greater than, prepregnant means (both  $P < 0.05$ ; Fig. 1 A and B). Although indomethacin did not alter GFR at this stage of pregnancy, it did seem to further depress ERPF, and augment ERVR, albeit not significantly. These changes were not statistically different from those obtained in the prepregnant or time-control studies.

Data of renal sodium and potassium excretion from the pregnant rats are portrayed in Table III. (The prepregnant data are similar to those presented in Table I, which were discussed above.) On gestational days 12 and 20,  $U_{Na} \cdot \dot{V}$  and  $C_{Na}/C_{In}$  did not increase significantly after indomethacin, as we had observed in the nonpregnant rats that received the inhibitor (Tables I and III), and in the time-control studies (Table II). After administration of indomethacin, potassium excretion decreased on both gestational days.

*Effects of AII and NE on blood pressure before and after indomethacin or meclofenamate.* The response of MAP to boluses of AII and NE before and after administration of indomethacin (7 mg/kg BW) is shown in Fig. 2 A. In the nonpregnant condition, the inhibitor did not potentiate the blood pressure response by the vasoconstrictors. The control dose-response curves for AII and NE were generally unchanged during pregnancy until day 20, when the curve was shifted significantly downward. Indomethacin failed to affect the attenuated responses obtained on

Table III. Influence of Indomethacin on Renal Sodium and Potassium Handling during Pregnancy

Rat	$U_{Na} \cdot \dot{V}$	$C_{Na}/C_{In}$	$\dot{V}$	$U_K \cdot \dot{V}$	$C_K/C_{In}$
Prepregnant	$\mu\text{eq}/\text{min}$	(%)	$\mu\text{l}/\text{min}$	$\mu\text{eq}/\text{min}$	(%)
Vehicle	$2.24 \pm 0.44$	$0.53 \pm 0.11$	$19.9 \pm 1.7$	$3.17 \pm 0.19$	$26.19 \pm 1.27$
Indomethacin	$3.20 \pm 0.44 \ddagger$	$0.81 \pm 0.11 \ddagger$	$21.5 \pm 2.3$	$2.18 \pm 0.10 \ddagger$	$19.41 \pm 1.03 \ddagger$
<i>Pregnancy: day 12</i>					
Vehicle	$2.15 \pm 0.54$	$0.41 \pm 0.09$	$21.4 \pm 2.0$	$3.84 \pm 0.19 \S$	$26.39 \pm 2.11$
Indomethacin	$2.35 \pm 0.33$	$0.46 \pm 0.07$	$15.4 \pm 0.9$	$2.96 \pm 0.09 \ddagger \S$	$19.73 \pm 1.34 \ddagger$
<i>Pregnancy: day 20</i>					
Vehicle	$2.07 \pm 0.37$	$0.48 \pm 0.08$	$20.7 \pm 2.4$	$3.52 \pm 0.28$	$27.25 \pm 2.35$
Indomethacin	$2.27 \pm 0.52$	$0.58 \pm 0.16$	$19.5 \pm 3.2$	$2.98 \pm 0.15^* \S$	$24.90 \pm 2.83$

Values are means  $\pm$  SEM. Nine prepregnant rats were each studied twice before mating. Of these animals, seven were studied on day 12, and eight rats on day 20 of pregnancy. For abbreviations, see Table I. \*  $P < 0.05$ . †  $P < 0.01$  indomethacin vs. vehicle. §  $P < 0.05$  pregnant vs. prepregnant.

Table IV. Time Control Studies Performed during a 40-d Period in Nonpregnant Rats

	Days after surgery			
	5-10 Nine expts. in five rats	11-20 10 expts. in six rats	21-30 Eight expts. in five rats	31-40 Seven expts. in four rats
BW (g)	241±8	251±8*	269±7*	271±9*
MAP (mmHg)	105±2	106±1	109±3	107±2
GFR (μl/min)	2,487±107	2,562±134	2,717±160	2,776±95
ERPF (μl/min)	8,700±418	8,795±401	8,802±464	9,331±608
ERVR				
ERVR (mmHg·ml·min <sup>-1</sup> )	6.97±0.31	7.01±0.29	6.94±0.20	6.42±0.43

Values are means±SEM. For abbreviations, see Table I. \*  $P < 0.01$  vs. the 5-10-d period.

gestational day 20. During the postpartum period, they spontaneously returned to prepregnant values.

That cyclooxygenase inhibition does not influence the downward shift of the dose-response curves for AII and NE observed on gestational day 20, was corroborated by studies using meclofenamate (6 mg/kg BW). Shown in Fig. 2 *B* is the response of MAP to boluses of AII and NE before and after this inhibitor. The increments in MAP were significantly less on day 20 of pregnancy. Analysis of variance showed a significant main effect of pregnancy for AII and NE,  $P < 0.001$  and  $< 0.005$ , respectively. These attenuated responses were unaffected by meclofenamate (no significant main effect of inhibitor was demonstrated).

To improve the accuracy of our study, we administered only one dosage of AII (25 ng/kg BW) and NE (200 ng/kg BW), each in triplicate, before and after meclofenamate (rather than infusing different dosages, each only once, so as to obtain a complete dose-response curve). Using this protocol (Fig. 2 *C*), the pressor responses to AII and NE were significantly decreased by gestational days 20 and 12, respectively. Note that the curves connecting the mean values obtained after giving meclofenamate are essentially parallel to or overlapping the control curves—indicating that cyclooxygenase inhibition did not affect the blunted response to exogenous vasoconstrictors observed during pregnancy. Again, analysis of variance showed no significant main effect of inhibitor.

Presented in Table V is the pressor responsiveness in acutely prepared, virgin and pregnant rats (gestational days 15-16), which had recovered for 60 min from implantation of catheters under ether anesthesia. The initial control responses (60-110 min after surgery) of these animals were significantly less than those for chronically instrumented rats (all  $P < 0.05$ ; see Fig. 2 *C* for comparison). Overall, acutely prepared, pregnant rats demonstrated lower responses than virgins ( $P < 0.05$  for NE and AII). Meclofenamate appeared to significantly augment the control responses in pregnant and virgin animals—a finding clearly different from results obtained in chronically prepared rats (Fig. 2 *A-C*). The increase in the responses, however, may not have been due solely to meclofenamate. They tended to recover spontaneously, particularly in virgin animals (Table V): control pressor responses obtained 120-140 min postimplantation of catheters were greater than those elicited 60-110 min after surgical preparation. Finally, the inhibitor did not restore responses of virgin and pregnant rats to the same level—the latter were still somewhat attenuated.

*Effects of AII on renal hemodynamics before and after in-*

*domethacin.* In that systemic pressor responsiveness was blunted during late gestation, we tested whether or not the renal circulation also developed an attenuated pressor response. In nonpregnant animals (Table VI), AII infusion elicited a significant rise in MAP and ERVR, and fall in GFR and ERPF (all  $P < 0.01$ ). The reduction in ERPF exceeded that of GFR (34±3% and 15±2%, respectively). During late gestation, increments of MAP and ERVR in response to AII were less than those observed in nonpregnant animals. The decrement in ERPF and GFR were also attenuated. These attenuated responses observed during late pregnancy (day 19) were not restored by indomethacin. In fact, they tended to be further depressed on gestational day 21, despite administration of indomethacin.

## Discussion

Based on these studies performed in conscious unstressed rats, we are unable to support the hypothesis that PGs function as vasodepressor agents in the renal and systemic vasculature during pregnancy. That is, acute inhibition of cyclooxygenase with indomethacin and/or meclofenamate did not reverse: (a) the rise in GFR and ERPF, and fall in ERVR; (b) the attenuated systemic pressor response to an infusion of AII, or boluses of AII and NE; and (c) the attenuated renal vascular response to an AII infusion.

To our knowledge, only one other group of investigators addressed the potential role of PGs in the control of renal hemodynamics during pregnancy (25). In that study, administration of indomethacin for 3 d (10 mg·kg<sup>-1</sup>·d<sup>-1</sup>) to rabbits failed to prevent the gestational rise in creatinine clearance. In our study, acute administration of indomethacin (4.5-6.5 mg/kg BW) to pregnant rats (gestational day 12) did not decrease GFR or ERPF, or increase ERVR toward prepregnant levels (Fig. 1 *A* and *B*).<sup>2</sup> Paradoxically, GFR was further elevated ( $P < 0.05$ ), and the tendency for ERPF to fall, and ERVR to rise, as demonstrated by nonpregnant animals that received indomethacin (Table I; Fig. 1 *A* and *B*) and by time-control studies (Table II), was not observed. Because we did not perform time-control studies during midgestation, we cannot exclude the possibility

2. Naden and co-workers (77) have recently reported the effects of infusions of indomethacin upon uterine vascular resistance in unstressed, pregnant sheep. When PGE<sub>2</sub> concentrations were observed to be decreased by the drug in systemic venous, vena cava, or uterine venous plasma, uterine vascular resistance was not significantly different from control (pre-indomethacin) values.

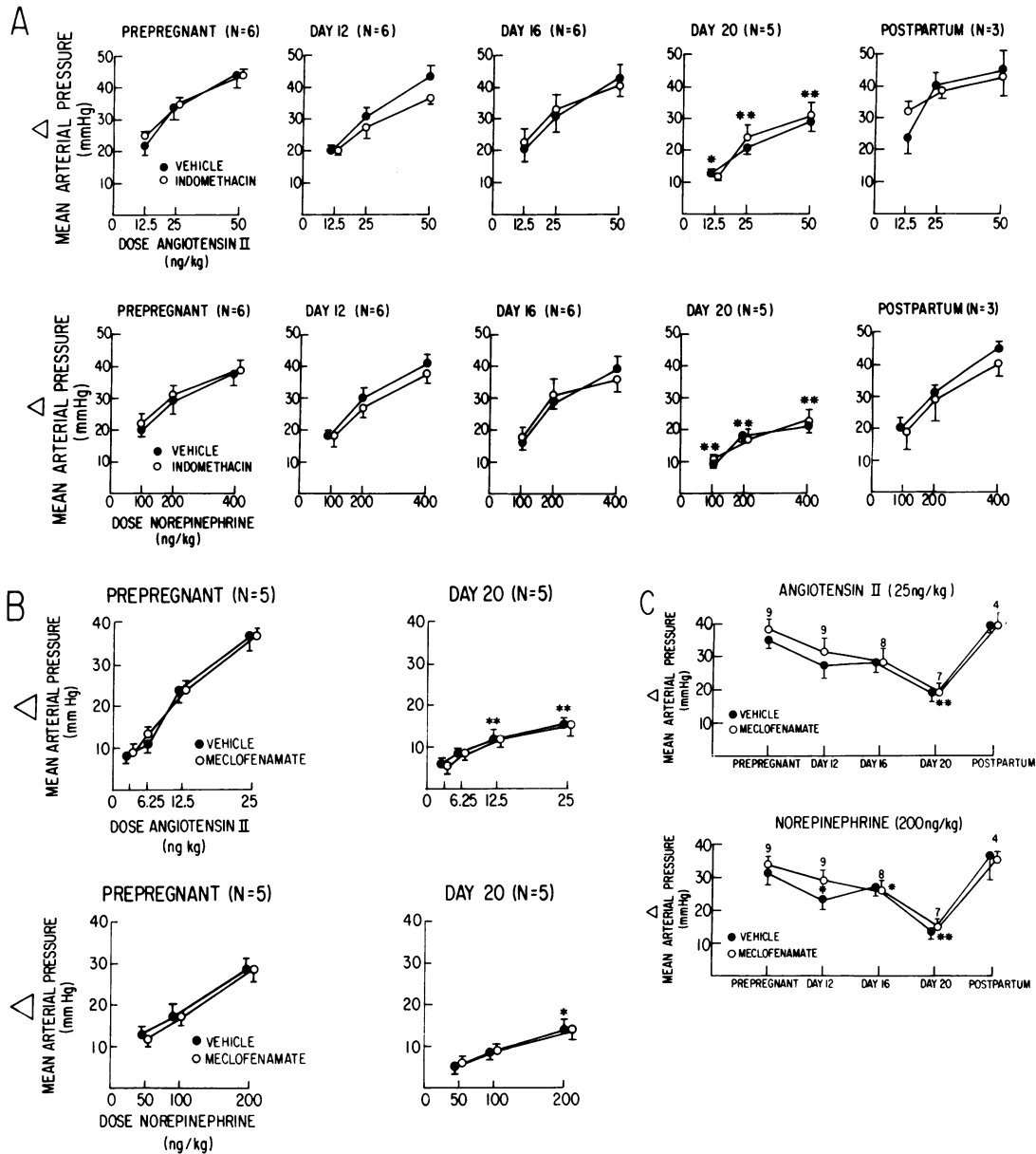


Figure 2. (A–C) Systemic pressor responsiveness in conscious, pregnant rats. Increments of MAP in response to boluses of AII and NE before and after administration of indomethacin or meclofenamate. \* $P < 0.05$ ; \*\* $P < 0.01$  from pre-pregnant mean.

that similar findings would have occurred in the absence of indomethacin. Alternatively, other potential effects of the inhibitor (36, 37) could have caused the additional increment of GFR.

During late pregnancy (day 20), renal hemodynamics may have shown a dependency on vasodilating PGs (Fig. 1 A and B), in that indomethacin seemed to depress ERPF and to raise ERVR more precipitously. But these changes were not statistically different from those observed in the same animals before conception (Fig. 1 A and B), or in time-control studies (Table II).

Briefly, our data obtained from nonpregnant rats (see Results; Tables I and II) confirm previous investigations in conscious, unstressed animals (30, 38–41) that cyclooxygenase inhibition has little or no effect on renal hemodynamics. Because the excretion of sodium increased in nonpregnant animals, whether

or not indomethacin was given (Tables I–III), cyclooxygenase inhibition apparently did not effect renal handling of sodium. (The excretion of sodium increased spontaneously, probably because we were infusing solutions that contained the ion, albeit at a relatively low rate [ $<3.5 \mu\text{eq}/\text{min}$ ].) The influence of cyclooxygenase inhibitors on sodium excretion is controversial (30, 41, 42; for a pertinent review, see Reference 40). Because the increment in sodium excretion was absent during pregnancy (Table III), indomethacin may be antinatriuretic in this condition. Most likely, however, time-control studies performed in pregnant animals would reveal the same finding. Because extracellular water is expanded (by as much as 50%; Reference 43), the infusion would have less impact. Additionally, pregnancy is a relatively sodium-avid condition, in that the ion is accumulated throughout its course (43); perhaps on this basis, the infused

**Table V. Pressor Responsiveness in Acutely Prepared, Virgin Female and Pregnant Rats (Gestational Days 15–16)\***

	Virgin rats (n = 5)		
	Δ MAP‡		
	Control (60–110 min)	Control (120–140 min)	Meclofenamate (185–245 min)
	mmHg	mmHg	mmHg
AII (25 ng/kg BW)	21±3	30±3	38±2 <sup>  </sup>
NE (200 ng/kg BW)	23±2	32±2 <sup>§</sup>	35±2 <sup>  </sup>
	Pregnant rats (n = 6)		
	Δ MAP‡		
	Control (60–110 min)	Control (120–140 min)	Meclofenamate (185–245 min)
	mmHg	mmHg	mmHg
AII (25 ng/kg BW)	15±2	20±3	26±4 <sup>  ¶</sup>
NE (200 ng/kg BW)	17±2	22±2	29±3 <sup>  ¶</sup>

Means±SEM.

\* The dosages of AII and NE were each administered in triplicate during the 60–110-min interval after surgical preparation. They were again administered once during the 120–140-min period. Meclofenamate (6 mg/kg BW) was given, and the vasoconstrictors were subsequently retested, each in triplicate (185–245 min postsurgical preparation).

‡ Δ MAP, change in mean arterial pressure.

§ *P* < 0.05 control (120–140 min) vs. control (60–110 min).

|| *P* < 0.01 meclofenamate vs. control (60–110 min).

¶ *P* < 0.05 meclofenamate vs. control (120–140 min). N.B. Although analysis of variance showed a significant main effect of group (pregnant vs. virgin, *P* < 0.05), Tukey tests failed to demonstrate significance between groups at any one time period.

sodium was retained. The observation that urine flow increased in time-control studies (Table II; probably as a result of our infusion of 24 μl/min), and was unchanged in nonpregnant and pregnant rats given indomethacin (Tables I and II), suggests that cyclooxygenase inhibition is antidiuretic. This conclusion agrees with some, but not all previous reports (30, 42; for a pertinent review, see Reference 40). Finally, the pronounced antikaliuretic effect of the inhibitor that we observed (Tables I and II) disagrees with several previous investigations in conscious animals (30, 40, 42). On the other hand, infusion of PGs into conscious dogs promoted kaliuresis (44), and a strong correlation exists between urinary PG and K<sup>+</sup> excretion in some conditions (45, 46). Indomethacin may promote antikaliuresis indirectly, by inhibition of the renin-angiotensin aldosterone axis (37).

The attenuated systemic pressor response to exogenous vasoconstrictors that develops during pregnancy has been observed in many species (26, 47–52, present study). The mechanism(s) is not clearly defined, and prereceptor, receptor, and/or postreceptor phenomena could contribute. For example, increased production of enzymes that inactivate the administered vasoconstrictors (53) or enhanced sensitivity of the baroreflex (54) could result in apparent attenuated vascular responsiveness; down-regulation, decreased affinity, or prior occupancy of receptors by high circulating levels of hormone could effect a blunted response (49); alteration of vessel wall constituents (55),

**Table VI. Effects of Angiotensin Infusion before and after Administration of Indomethacin on Renal Hemodynamics during Late Gestation**

	Prepregnant			
	Change from control			
	GFR	ERPF	ERVR	MAP
	%	%	%	%
	–15±2	–34±3	+90±12	+21±4
	Late gestation			
	Change from control			
	GFR	ERPF	ERVR	MAP
	%	%	%	%
Day 19	–2±3*	–16±2*	+37±11*	+12±5
Day 21 (Indomethacin)	+7±5‡	–9±3‡	+21±9‡	+8±4*

Values are means±SEM. The effects of AII on renal hemodynamics were assessed in five rats before conception. The same animals were studied during late gestation (day 19). On gestational day 21, indomethacin was administered, in addition to the infusion of AII (see Methods). Control values for prepregnant rats were: GFR (μl/min) 2,968±270; ERPF (μl/min) 10,638±657; ERVR (mmHg·ml·min<sup>–1</sup>) 5.87±0.15; MAP (mmHg) 111±3. Control values for day 19 pregnant animals were: GFR 2,729±177, ERPF 7,956±594, ERVR 8.15±0.84, MAP 100±0. Control values for day 21 pregnant animals were: GFR 2,528±118, ERPF 6,999±321, ERVR 8.47±0.60, MAP 94±2.

\* *P* < 0.05.

‡ *P* < 0.01 pregnant vs. control.

lumen radius (56), smooth muscle membrane potential (57), or production of vasodilators (47) could oppose vasoconstriction. We tested the hypothesis that enhanced synthesis of prostaglandins mediates the attenuated systemic pressor response. In four, separate studies (Fig. 2 A–C; Table VI), using either indomethacin or meclofenamate, we could not reverse the attenuated pressor response to AII and NE, which had developed during late gestation. We conclude, therefore, that mechanism(s) other than a vasodilatory action of PGs must be operative in the rat.

Several investigators (26, 47, 50–52) have published data that favor a role for PGs. However, other interpretations of these studies are possible. Gant and colleagues (47) gave indomethacin (or aspirin) to pregnant women on a subacute basis (14 h of treatment). If PGI<sub>2</sub> and PGE<sub>2</sub> contribute toward the activation of the renin-angiotensin system during pregnancy (58, 59), then reduction of these PGs could decrease circulating levels of AII. A decrease for 14 h could promote up-regulation of the receptor, and consequently, a restoration of the pressor response. In fact, Siddiqi et al. (49) have shown that a 24-h infusion of enalapril (3 μg·kg<sup>–1</sup>·min<sup>–1</sup>), a converting enzyme inhibitor, to chronically instrumented, conscious pregnant sheep restored the refractory response of AII towards nonpregnant levels. Importantly, a 30-min infusion of captopril (1 μg·kg<sup>–1</sup>·min<sup>–1</sup>) or enalapril did not effect a restoration. This latter study suggests that down-regulation of the AII receptor during pregnancy may mediate the blunted pressor response. O'Brien and Pipkin (52) have demonstrated that an essential fatty acid-deficient diet enhanced



the AII pressor response in pregnant rabbits. The drawback of their study is that the effects of the diet were not examined in nonpregnant rabbits—it is possible that the pressor response would have been enhanced by a similar degree. As well, surgical stress of tracheal intubation, and the anesthetic agent may have rendered the pressor response PG-dependent in those pregnant animals maintained on a regular diet—not unlike the PG dependency of renal blood flow which develops in dogs as a consequence of surgical preparation and/or anesthesia (for pertinent reviews, see References 16 and 40).

Our results conflict with those of Paller (26), who reversed the attenuated pressor response of rat pregnancy with meclofenamate. The major difference between his study and ours lies in the preparation; whereas we used trained, chronically instrumented, conscious rats that were allowed 7–10 d of recovery from surgery, Paller employed untrained, conscious rats given 60 min for recovery from implantation of catheters under ether anesthesia. In an attempt to reproduce Paller's work, we followed his protocol (26). As demonstrated in Table V, control pressor responsiveness of pregnant rats (gestational days 15–16) was less than that of virgins, and after meclofenamate, it was significantly enhanced in pregnant animals ( $P < 0.05$ ). This much of the study is in accordance with the data of Paller. We further showed that a similar potentiation occurred in virgin rats, although pressor responsiveness tended to recover spontaneously, particularly in the virgin animals (Table V). Because ether anesthesia and surgical stress dramatically increase plasma renin activity (11, 14), and noradrenaline levels (18), much of the attenuation may have been secondary to prior occupancy of AII and NE receptors, or even receptor down-regulation (60) by high levels of circulating hormones. A degree of spontaneous recovery would then be expected to occur, as the hormone levels decreased. We have previously shown that renal hemodynamics are also perturbed for at least 2–3 h after implantation of catheters under ether anesthesia (10). Moreover, because AII and NE may augment PG synthesis (61–63), pressor responsiveness could become PG-dependent in this acute rat preparation. A further possibility is that PG production may be differentially activated by stress in pregnant and virgin animals: in an effort to defend a low vascular resistance during stressful conditions, thereby ensuring adequate blood flow and oxygen delivery to the fetoplacental unit, pregnant animals may recruit vasodilatory substances, such as PGs, to a greater degree. (The above discussion of AII and NE applies to arginine vasopressin, which Paller also used to elicit pressor responses.)

Venuto and colleagues have provided the most convincing data that PGs modify the pressor response during pregnancy, at least in rabbits (50, 51). Meclofenamate was given acutely to conscious, pregnant rabbits (48–72 h were allowed for recovery from surgery), and partial restoration of the pressor response to NE was observed (compare their Figs. 1 and 2 in Reference 50). The same inhibitor significantly decreased the dose of AII required to raise diastolic pressure by 20 mmHg in pregnant rabbits, although the amount of AII needed to achieve this rise in blood pressure was not significantly different from that in nonpregnant animals before meclofenamate was given (51). McLaughlin and associates (64) demonstrated that the threshold concentration required to raise blood pressure by 4 mmHg was increased for AII, but not for barium chloride in the perfused hind limb of pregnant rabbits. Hart (65, 66) has shown that aortic arterial and portal venous strips from pregnant rats display a reduction in barium-induced increments of tension. These

studies (50, 51, 64–66), including our own, perhaps suggest that pressor responsiveness in pregnant rabbits and rats are modified by different mechanisms.

Our investigations suggest that the renal vasculature also develops an attenuated pressor response to an intravenous infusion of AII during late gestation (Table VI). This finding is consonant with studies of pulmonary vascular reactivity in pregnant rats (67) and dogs (68), in which the response to hypoxia and to infusions of AII or  $\text{PGF}_{2\alpha}$  was depressed. In the pregnant dogs (68), meclofenamate failed to reverse the blunted hypoxic pressor response. Likewise, in our experiments, indomethacin failed to restore the attenuated effect of AII on ERPF observed during late gestation (Table VI). Based on these data (Table VI, present study; see also Reference 68) we suggest that mechanism(s) other than the vasodilatory action of PGs offset the imposed vasoconstrictor stimuli.

It seems unlikely that the amount of indomethacin employed in our experiments was insufficient to inhibit cyclooxygenase—at least in the kidney. (The arguments which follow, also apply to meclofenamate.) First, the dosage is consistent with (or exceeds) those reported to have significantly reduced urinary excretion of  $\text{PGE}_2$  and/or  $\text{PGF}_{2\alpha}$  (29–31). Secondly, urine analyzed from our experiments demonstrated a significant reduction in the excretion rates of  $\text{PGE}_2$  and/or  $\text{PGF}_{2\alpha}$  in both nonpregnant and pregnant animals (>90% for indomethacin and 74% for meclofenamate; see Methods). Of course, we cannot be sure whether the drug inhibited synthesis by glomeruli, and the adjacent microvasculature (presumably the major sites that regulate renal hemodynamics), or by the systemic vasculature, because these compartments probably do not contribute greatly to urinary levels—the urinary excretion of  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  may predominantly reflect renal medullary production (69 [at least in nonpregnant rats]). Some investigators have reported circulating levels of PGs during pregnancy (21, 22, 25, 70), which perhaps originate from the fetoplacental unit (22, 70). These PGs could then gain access to the urine (61), presumably by filtration and secretion. Because indomethacin can cross the placenta in late, but probably not midgestation in the rat (71, 72), this potential site of production may or may not have been inhibited in our studies. Whether or not vasoactive PGs, such as  $\text{PGE}_2$  and  $\text{PGI}_2$ , circulate in pregnancy in high enough concentrations to exert an effect on renal and cardiovascular function is controversial.  $\text{PGI}_2$  probably does not (73, 74). They are thought to have short half-lives in the circulation (75, 76), and interpretation of measurements obtained from plasma or serum is complicated by the fact that blood collection can lead to artifactual production of large amounts of hormone (75, 76).

In conclusion, our studies fail to support the hypothesis that prostaglandins serve as vasodepressor agents in the renal and systemic vasculature during rat gestation. In order to explain the vasorelaxation observed in pregnancy, other potential mechanisms require investigation.

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