Effects of Endothelium-Derived Nitric Oxide on Renal Hemodynamics and Function in the Sheep Fetus

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ABSTRACT. We investigated the effects of the endothelium-derived nitric oxide system on renal hemodynamics and function during the 3rd trimester in a chronically catheterized fetal sheep preparation. Acetylcholine caused a significant decrease in renal vascular resistance (60% of the baseline value) as compared with aortic constriction (142% of the baseline value). The effects of acetylcholine could be blocked by prior administration of N^{ω}-nitro-L-arginine (renal vascular resistance = 102% of baseline). Sodium nitroprusside also caused a significant drop in renal vascular resistance (63% of baseline), but this could not be blocked by N^{ω}-nitro-L-arginine (77% of baseline). Infusion of N^{ω}-nitro-L-arginine with blood pressure maintained at a constant level resulted in a significant increase in renal vascular resistance (148% of the baseline value) as compared with saline alone (94% of baseline). Glomerular filtration rate increased after saline infusion (156% of the baseline value), but this increase was blocked by N^{ω}-nitro-L-arginine (87% of baseline). Sodium excretion also increased (340%), and this increase was blunted by N^{ω}-nitro-L-arginine (235%). We conclude that basal production of endothelium-derived nitric oxide results in ongoing renal vasodilation in 3rd-trimester fetal sheep, maintaining baseline renal blood flow. The endothelium-derived nitric oxide system can also be stimulated to an increased level of activity, and its blockade partially prevents the homeostatic response of the fetus to volume and salt overload. (*Pediatr Res* 34: 755–761, 1993)

Abbreviations

NO, endothelium-derived nitric oxide
GFR, glomerular filtration rate
EDRF, endothelium-derived relaxing factor

Knowledge of the physiology of fetal renal hemodynamics and function is essential for the development of antenatal treatments of obstructive uropathy. Since Leonardo da Vinci at the beginning of the 16th century recorded his observation that the human fetus produces urine *in utero* [quoted by Needham (1)], there have been increasingly complex investigations of fetal renal physiology. Most have been in the ovine fetus—a well-accepted model (2, 3). Fetal renal blood flow is lower than postnatal values and increases only slightly with fetal age (4–6). Mean arterial blood pressure also increases slightly during gestation; thus, renal

vascular resistance, which is relatively high, remains constant during the 3rd trimester (4, 5). The renin-angiotensin system, arginine vasopressin, and prostaglandins all have been shown to have a role in the control of fetal renal blood flow (7). The importance of NO on fetal renal blood flow has not been investigated.

Filtration fraction and GFR are low during fetal life, but both increase steadily during the 3rd trimester (8, 9). Because the increase in GFR parallels the increase in renal and body weight, the GFR/renal or body weight ratio is virtually constant (8, 10). The factors modulating filtration fraction and GFR in the fetus are complex and not well known, although the GFR increase is accounted for in part by increases in the surface area for filtration and the effective filtration pressure (partly a function of increased blood pressure) (11). Alterations in these parameters in the fetus caused by NO are unknown.

Renal tubular function, as evidenced by sodium chloride metabolism, has been studied extensively. Fractional excretion of sodium decreases from 11% to 5% during the 3rd trimester (9). Multiple factors play a role in this shift: renal tubular maturation (9, 12, 13), relatively large amounts of extracellular fluid (14), the presence of circulating natriuretic factor (15), relative tubular insensitivity to circulating aldosterone (16), prostaglandins (17), the renal kallikrein-kinin system (18), and renal oxygen consumption (19). The proximal tubule participates to a lesser extent in reabsorbing the filtered sodium load. Therefore, a greater proportion of sodium is reabsorbed distally and the distal nephron, under physiologic conditions, plays an important role in glomerulo-tubular balance (20). The effects of NO on sodium excretion in the fetus are unknown.

Furchgott and Zawadzki (21) first demonstrated in 1980 that the vascular relaxation induced by the muscarinic agent acetylcholine was dependent on the presence of a functionally intact endothelium and postulated the release by endothelial cells of a labile humoral factor termed EDRF. Since that study, a variety of agonists have been shown to induce release of EDRF from endothelium of various vascular beds. Comparative pharmacologic studies and direct measurement have provided evidence that EDRF activity results from the release by endothelial cells of NO (22–24). Palmer *et al.* (25) have demonstrated that NO, which is synthesized by endothelial cells, originates from the terminal guanidine nitrogen atom of the amino acid L-arginine. There is evidence that a soluble enzyme is involved in this step, and this enzyme can be inhibited by several L-arginine analogues, N^{G}-monomethyl-L-arginine (26, 27) and N^{ω}-nitro-L-arginine (28). This inhibition is competitive and can be abolished by administration of L-arginine (27, 28). Based on these interactions, increasing evidence in a wide range of organ systems points to the NO system as a major determinant of vascular tone, both in the resting state and in response to various stimuli (29).

The purpose of the present investigation was to study the role and importance of the renal vascular endothelium in the renal hemodynamics and function of the fetus. Chronically catheter-

ized fetal sheep underwent infusions of an endothelium-dependent vasodilator (acetylcholine), an endothelium-independent vasodilator (sodium nitroprusside), and an NO inhibitor (N^ω-nitro-L-arginine) to determine the effects on renal vascular resistance, GFR, and sodium excretion. We hypothesized that the NO system would play a role in baseline fetal renal hemodynamics and function because of the numerous reports of its activity in the mature kidney (30–32).

MATERIALS AND METHODS

Surgical preparation. Pregnant ewes carrying fetuses of 115 d gestation were premedicated with 400 mg ketamine (Ketaset, ketamine HCl, USP, Aveco, Fort Dodge, IA) intramuscularly. Surgical anesthesia consisted of 4 mL of epidural tetracaine hydrochloride (Pontocaine HCl, Breon Laboratories, New York, NY) and s.c. lidocaine hydrochloride (Lidocaine 2%, USP, Elkins-Sinn, Inc., Cherry Hill, NJ) for all maternal (1%) and fetal (0.2%) skin incisions. In addition, ketamine (50 mg i.v.) was given every 10–15 min as necessary to maintain adequate maternal and fetal sedation (maternal ketamine crosses the placenta).

With strictly aseptic technique, a midline incision was made in the ventral abdomen of the ewe and the pregnant horn of the uterus exposed. A 10-cm incision was made in the uterus directly over the hind portion of the fetus. One fetal hind limb was brought through the incision, and polyvinyl catheters (inner diameter 0.8 mm; outer diameter 1.2 mm) were inserted into the fetal dorsalis pedis artery and vein and passed centrally to locate the tip in the fetal descending aorta and inferior vena cava, respectively. The lower portion of the fetus was then exposed and, through a midline abdominal incision just below the umbilical cord, the urachus was ligated several centimeters distal to the bladder. A cuffed silicone catheter (8 F) was placed through the urachus into the bladder and secured with the proximal urachus tightly sealed around it. The urethra was then ligated so that the entire fetal urine output drained through the urachal catheter. (In males, this was accomplished by ligating the penile urethra; in females, a second incision was made above the symphysis pubis and the proximal urethra ligated.) The abdominal wall was closed anatomically. The fetus was then turned laterally and, through a left paravertebral incision, the aorta and the left renal vessels were exposed. The suprarenal aorta was dissected circumferentially and a handmade silicone constrictor placed around it. (This is designed to allow both free flow and graded degrees of vascular obstruction.) A portion of the left renal artery was dissected and an ultrasonic flow transducer (Perivascular Volume Flowsensor, 2R-Series, Transonic Systems, Ithaca, NY) secured around it. The incision was closed anatomically and the fetus replaced in the uterus, which was then closed in a watertight manner. All fetal catheters and two amniotic cavity catheters (one placed to monitor intrauterine pressure and the second to be connected with the fetal urachal catheter) were brought out through the uterus and the ewe's flank and protected by a cloth pouch sewn to the skin. The vascular catheters were filled with heparinized saline and plugged, and the free end of the bladder catheter was connected directly to one amniotic cavity catheter with a 16-gauge connector to ensure free drainage of the urine into the amniotic cavity. The maternal incision was closed in layers and 100 mg of gentamicin (Gentocin, Schering Corporation USA, Kenilworth, NJ) and 1 million U of penicillin (Penicillin G Potassium, USP, Bristol-Meyers Squibb, Princeton, NJ) were administered into the maternal venous circulation and amniotic cavity on the day of the surgery and daily for 5 d.

All protocols were approved by our institution's Committee on Animal Research.

Drug preparation. Acetylcholine chloride (Miochol, Iolab, Claremont, CA) was diluted in 0.9% saline at a concentration of 10 $\mu g/10^{-3}$ L. Sodium nitroprusside (Abbott Laboratories, Chicago, IL) was diluted in 5% dextrose solution at a concentration

of 12.5 $\mu g/10^{-3}$ L. N^ω-nitro-L-arginine (Sigma Chemical Co., St. Louis, MO) was suspended at a 3-mg/10^{-3} L concentration in 0.9% normal saline. All solutions were prepared on the day of study.

General study techniques. Physiologic studies were begun no sooner than 48–72 h postoperatively. During the studies, the status of the fetus was checked before and after each drug application with arterial blood gas and lactate measurements. The ewe had constant access to food and water. One-h fetal urine collections were obtained with the intravesical pressure equal to the amniotic pressure, and a 3- to 5-mL (10^{-3} L) sample was frozen immediately after collection (at $-18°C$ for a maximum of 10 d). Serum from an arterial blood sample obtained 30 min after the beginning of the urine collection was also frozen at $-18°C$ (for a maximum of 10 d). Sodium and creatinine concentrations of the urine and serum were determined with a Cobas Mira Chemistry Analyzer Ion Selective Electrode (Roche Diagnostic Systems, Nutley, NJ).

During the study, continuous measurements of blood pressure in the descending aorta and inferior vena cava and pressure in the amniotic cavity and fetal bladder were recorded on a Gould recorder (Gould 2800S, Gould Inc., Cleveland, OH). Renal blood flow was monitored continuously, with the flow transducer read by the T101D/T201D Animal Research Flowmeter (Transonic Systems) connected to the Gould recorder.

Specific study methods. To determine whether NO could be stimulated to act on the renal circulation, baseline measurements of mean systemic arterial pressure (in the infrarenal aorta), inferior vena cava pressure, amniotic cavity pressure, and left renal blood flow were obtained. With continuous monitoring of the above parameters, three separate interventions were performed in random order: *1*) i.v. infusion into the fetal inferior vena cava of acetylcholine (0.5 $\mu g/kg/min$); *2*) i.v. infusion of sodium nitroprusside (2 $\mu g/kg/min$); and *3*) gradual constriction of the suprarenal aorta. The constriction was performed manually to reproduce the rate and amount of decrease in blood pressure caused by acetylcholine and sodium nitroprusside. The constriction and infusions were stopped when the blood pressure had decreased 10 mm Hg. The acetylcholine, nitroprusside infusions, and constriction were repeated after infusion of N^ω-nitro-L-arginine (1.5 mg/kg/min). The acetylcholine infusion was continued for at least 10 min when no change in mean arterial pressure was observed; nitroprusside was continued until mean arterial pressure decreased 10 mm Hg.

To determine whether NO activity was ongoing, baseline measurements were made at least 30 min after any previous manipulation. With continuous monitoring of hemodynamic parameters as above, N^ω-nitro-L-arginine (1.5 mg/kg/min) was infused into the fetal inferior vena cava. [N^ω-nitro-L-arginine has been shown to be a competitive inhibitor of NO synthesis. The dosage used in this study has been previously reported to inhibit NO synthesis completely (33). This was confirmed by the failure of acetylcholine to induce hypotension in our study and others (34)]. During the infusion, the mean systemic arterial pressure in the lower aorta was kept constant by gradually constricting the suprarenal aorta.

To assess any effect of NO blockade on renal function, a 1-h urine collection was obtained after at least 1 h of continuous infusion for determination of volume, creatinine, and sodium. A blood sample to determine serum creatinine and sodium concentrations was obtained after 30 min of the urine collection. The following day, all parameters were remeasured and, after an infusion of acetylcholine (repeated to ensure complete elimination of N^ω-nitro-L-arginine), an infusion of 0.9% normal saline was begun at the same rate as the N^ω-nitro-L-arginine. Urine and blood samples were obtained at the same time points as after N^ω-nitro-L-arginine.

Measurements. Mean arterial pressure (mm Hg) was calculated by subtracting amniotic pressure from lower aortic pressure. Renal vascular resistance (expressed as mm Hg·kg·min·mL^{-1})

was calculated as follows: (mean arterial pressure − inferior vena cava pressure)/[(left renal blood flow · 2)/fetal body weight]. In practice, vasodilation caused by acetylcholine and sodium nitroprusside resulted in an initial increase in renal blood flow; however, after further vasodilation with steadily decreasing mean arterial pressure, renal blood flow decreased (Fig. 1). The calculated renal vascular resistance would therefore vary with respect to the time point used to determine it. We thus chose to standardize the calculation of the renal vascular resistance by determining it throughout the study at the time when the mean arterial blood pressure had decreased from baseline values by precisely 10 mm Hg.

In fetal sheep, the creatinine clearance is virtually identical with Na-iothalamate or inulin clearance (35, 36) and therefore the GFR (expressed as $mL \cdot min^{-1} \cdot kg^{-1}$) was calculated as follows: (urinary output · urinary creatinine concentration)/(plasma creatinine concentration · fetal body weight). Filtration fraction (%) was calculated by dividing the GFR by twice the left renal blood flow. Sodium excretion (expressed as $mmol \cdot h^{-1} \cdot kg^{-1}$) was calculated as follows: (urinary sodium concentration · urinary output)/fetal body weight. Fractional sodium excretion (%) was calculated as follows: (urinary sodium concentration · serum creatinine concentration)/(serum sodium concentration · urinary creatinine concentration).

Statistical analysis. Baseline values for mean arterial pressure, renal blood flow, and renal vascular resistance between each intervention were compared with repeated measures analysis of variance. The effects of acetylcholine, sodium nitroprusside, suprarenal aortic constriction, and N^{ω}-nitro-L-arginine on the above parameters were compared with their respective baseline values by paired *t* test. Changes in mean arterial pressure, renal blood flow, renal vascular resistance, urine volume, GFR, filtration fraction, fractional excretion of sodium, and sodium excretion after infusion of N^{ω}-nitro-L-arginine and saline were also compared with their respective baseline values by paired *t* test. To differentiate the effects of NO better, the changes in renal vascular resistance after acetylcholine were compared with those after constriction with the paired *t* test, and the changes in mean arterial pressure, renal blood flow, renal vascular resistance, urine volume, GFR, filtration fraction, fractional excretion of sodium, and sodium excretion after N^{ω}-nitro-L-arginine were compared with those after saline, also by paired *t* test.

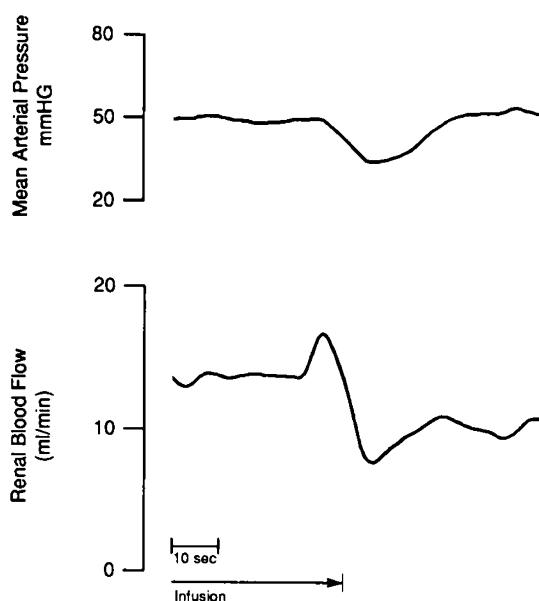All values are presented as mean ± SD. *p* values < 0.05 were considered statistically significant.

RESULTS

All animals were healthy at the time of study, as determined by arterial blood gases and serum lactate (Table 1). Values did not change significantly during the study, except after 1 h of N^{ω}-nitro-L-arginine infusion with the suprarenal aorta constricted, after which there was mild oxygen desaturation and acidosis without change in serum lactate. The oxygen desaturation improved immediately upon releasing the constrictor, although the acidosis persisted. By the next day, the acidosis had resolved spontaneously. There were no significant differences in baseline hemodynamic parameters during the studies.

NO stimulation. Figures 1 and 2 depict tracings of the mean arterial pressure and the renal blood flow after acetylcholine and constriction of the suprarenal aorta, respectively. After infusion of acetylcholine, the renal blood flow slowly increased to a maximum, then decreased with steadily falling mean arterial pressure. Renal blood flow returned to baseline within 2 min, although mean arterial pressure returned to normal within seconds. During aortic constriction (at a slow rate to reproduce the rate of decrease in systemic blood pressure), renal blood flow fell significantly immediately; however, after release of the constrictor a rebound effect was observed. As seen in Figure 3, acetylcholine significantly reduced renal vascular resistance as compared with use of the constrictor ($p < 0.01$). The changes induced by acetylcholine were blocked completely by infusion of N^{ω}-nitro-L-arginine (Table 2); similar changes seen after infusion of sodium nitroprusside were not altered by N^{ω}-nitro-L-arginine.

Baseline NO activity. Infusion of N^{ω}-nitro-L-arginine with blood pressure maintained constant resulted in significant reduction in renal blood flow ($p < 0.005$) (Table 3) and an increase in renal vascular resistance ($p < 0.002$) (Fig. 4). Saline infusion resulted in a significant increase in renal blood flow, but the renal vascular resistance remained stable; the effect on both values was significantly more marked after N^{ω}-nitro-L-arginine ($p < 0.001$).

Renal function changes. Saline infusion resulted in an increase in urine volume ($p < 0.03$) and GFR ($p < 0.02$) and both fractional and total sodium excretion ($p < 0.044$ and 0.008) (Table 3). N^{ω}-nitro-L-arginine, given with saline infusion, blocked the increase in GFR ($p < 0.047$) and total sodium excretion ($p < 0.048$) but had no statistically significant effect on urine volume or fractional sodium excretion.

DISCUSSION

Infusion of N^{ω}-nitro-L-arginine in our chronic fetal sheep preparation resulted in a significant increase in mean arterial pressure. We therefore chose to constrict the suprarenal aorta during the infusion to maintain constant blood pressure in the renal circulation. With this preparation, we have demonstrated a definitive increase in renal vascular resistance during blockade of NO. This suggests ongoing basal NO secretion, which plays a role in regulating the baseline fetal renal vascular tone during the 3rd trimester. In addition, the decrease in renal vascular resistance after infusion of acetylcholine demonstrates that the NO system can be stimulated and may also influence renal vasodilation in the fetus.

Our findings are similar to the results of other investigators studying hemodynamics in the fully developed renal vasculature. Since 1988, numerous investigations with the isolated perfused rat kidney have shown that acetylcholine causes dose-related vasodilation. Inhibition of NO biosynthesis reduces or abolishes the response to acetylcholine without affecting vasodilation due to nitroprusside, an endothelium-independent vasodilator (34). This has been confirmed by several different investigators (5, 30, 37–39). Micropuncture studies have also shown evidence that endothelial cells of the main renal artery modulate intrarenal hemodynamics and renal vascular resistance in the rat (39), and *in vivo* rodent studies have confirmed these observations (40, 41). Other chronic *in vivo* studies in a dog preparation have

Fig. 1. Mean arterial pressure and renal blood flow during infusion of acetylcholine (0.5 μg/kg/min).

Table 1. *Effects of NO blockade on fetal blood gas and lactate levels*

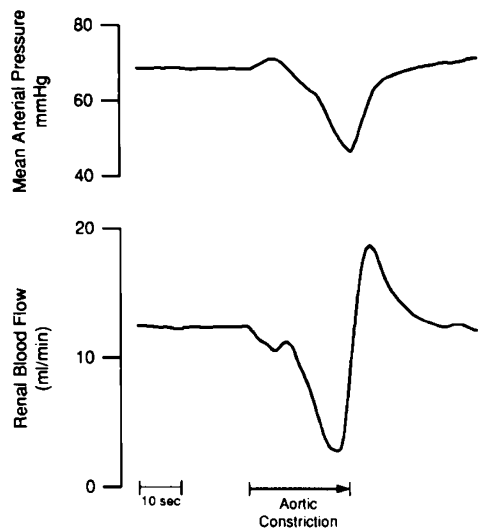|	n	Hb (g/10^{-1} L)	Oxygen saturation (%)	pH	P_{CO_2} (kPa)	P_{O_2} (kPa)	Lactate (mmol/L)
Baseline	8	9.5 ± 2.0	52.6 ± 6.5	7.38 ± 0.01	53.6 ± 4.9	20.7 ± 2.6	1.4 ± 0.4
N$^\omega$-nitro-L-arginine and occluder	8	9.9 ± 1.7	38.2 ± 9.2	7.29 ± 0.02	60.4 ± 3.5	19.3 ± 2.7	1.4 ± 0.4
N$^\omega$-nitro-L-arginine	8	10.6 ± 2.2	46.4 ± 12.2	7.30 ± 0.02	57.5 ± 3.6	22.0 ± 3.9	1.6 ± 0.4
24 h after study	8	9.6 ± 1.7	53.0 ± 6.4	7.38 ± 0.02	54.4 ± 2.2	20.9 ± 2.2	1.6 ± 0.7

Fig. 2. Mean arterial pressure and renal blood flow in response to aortic constriction.
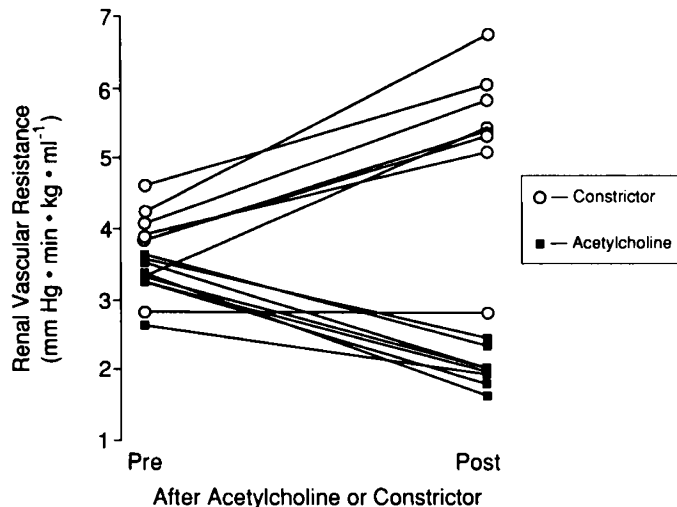

Fig. 3. Comparison of responses in renal vascular resistance to acetylcholine or constriction.

demonstrated hypertension and a rise in renal vascular resistance as a result of blockade of the NO system (31). Whether large or small vessels are involved is unclear from our study; however, a microangiographic study in isolated perfused rat kidneys demonstrated that the vasodilatory action of acetylcholine is spatially homogeneous (42).

Studies of the NO system in the fetus have been limited, most evaluating its role in pulmonary circulation. Almost 30 years ago, Cassin *et al.* (43) showed that acetylcholine dilates the normally high-tone pulmonary vasculature of the fetus. More recent studies have confirmed that the NO system can be stimulated with acetylcholine to modulate pulmonary blood flow in the near-term fetus (44) and that it affects both large and small

pulmonary vessels (45). Studies have shown that stimulation or blockade of NO can have varying effects in different vessels, implying regional differences in vasodilator effects in the same animal (46). Thus, other organ systems are being studied.

In vitro studies by Shaul *et al.* (47) indicate that oxygen might also modulate NO production in fetal sheep pulmonary arteries. Within a normal physiologic range, this modulation is likely to be more prominent in the pulmonary circulation than in other organ systems. In the kidney, it has been shown that severe ischemia—with no flow to the kidney for 25 min—results in an increase in renal vascular resistance (48). This is not comparable to the current study in which infusion of N$^\omega$-nitro-L-arginine and constriction of the aorta led to statistically significant but relatively mild hypoxia. A recent study of mature kidneys in anesthetized dogs demonstrated that hypoxia (50% of the baseline P_{O_2}) and NO inhibition both result in an increase of renal vascular resistance, although their effect in concert was not different from their effect individually (49). In our study, the change from the baseline P_{O_2} induced by NO inhibition and aorta constriction was not statistically significant (94%). This suggests that the results of NO inhibition seen in our study were not altered by the hypoxia.

Because acetylcholine infusion causes significant hypotension, it was not possible to evaluate the renal functional changes associated with acetylcholine stimulation of NO in our *in vivo* preparation. We therefore chose to use NO blockade with N$^\omega$-nitro-L-arginine to determine whether inhibition of basal NO activity affects renal function. Although mean arterial pressure rose during N$^\omega$-nitro-L-arginine infusion, we maintained renal arterial blood pressure at a constant level by constriction of the suprarenal aorta as necessary. Consequently, in our study (unlike other *in vivo* studies reported) renal vascular resistance and renal function could be determined independent of hypertension.

Our baseline GFR determination (1.63 ± 0.45 mL·min^{-1}·kg^{-1}) was similar to that previously reported (1.59 ± 0.15 mL·min^{-1}·kg^{-1}) (50). In our preparation, a 2-h infusion of saline was necessary to study the effects of N$^\omega$-nitro-L-arginine on GFR, resulting in a volume load of 60 mL/h and 9.24 mmol NaCl/h. Thus, we used an infusion of 0.9% normal saline at 60 mL/h (also 9.24 mmol NaCl/h) as a control. After saline infusion, GFR was increased, but this effect was abolished by N$^\omega$-nitro-L-arginine.

Studies of the glomerular microcirculation of the rat kidney during blockade of the NO system demonstrated no change in single-nephron GFR (51). This results from several opposing factors, systemic hypertension and glomerular vasoconstriction and hypoperfusion; efferent resistance was disproportionately increased, resulting in elevated glomerular hydraulic pressure (51). In our study, filtration fraction was unchanged after N$^\omega$-nitro-L-arginine, also suggesting that the NO blockade caused efferent arteriolar vasoconstriction, which maintained filtration despite decreased renal blood flow. The results of previous whole organ studies in mature animals are also generally supportive of our findings. In isolated perfused rat kidneys, stimulation of the NO system with acetylcholine resulted in a dose-dependent increase in GFR that could be blocked by infusion of an NO inhibitor or by arterial denudation by external rubbing of the main renal artery (39, 52). Similarly, an *in vivo* study in dogs undergoing local kidney perfusion with an NO inhibitor dem-

Table 2. Effects of NO on renal vascular resistance*

	n	Acetylcholine	Constrictor	Sodium nitroprusside	Acetylcholine after N^ω-nitro-L-arginine	Sodium nitroprusside after N^ω-nitro-L-arginine
Mean arterial pressure (mm Hg)						
Pre	8	41 ± 4	41 ± 4	42 ± 4	52 ± 5	50 ± 6
Post	8	33 ± 6†	32 ± 4†	32 ± 4†	50 ± 5	41 ± 6†
		(80)	(78)	(76)	(96)	(82)
Renal blood flow (mL·min⁻¹·kg⁻¹)						
Pre	8	12.4 ± 2.2	11.6 ± 2.9	11.1 ± 2.0	11.9 ± 2.2	11.9 ± 2.4
Post	8	16.3 ± 3.4†‡	6.6 ± 2.8†‡	12.9 ± 1.8†	11.7 ± 2.0	12.3 ± 2.1
		(131)	(57)	(116)	(98)	(103)
Renal vascular resistance (mm Hg·min·kg·mL⁻¹)						
Pre	8	3.38 ± 0.39	3.87 ± 0.66	3.88 ± 0.70	4.36 ± 0.93	4.28 ± 0.97
Post	8	2.04 ± 0.32†‡	5.34 ± 1.36†‡	2.51 ± 0.31†	4.28 ± 0.90	3.28 ± 0.73†
		(60)	(138)	(65)	(98)	(77)

* Pre, baseline values; Post, after drug delivery. Numbers in parentheses are % of baseline value.

† Different from baseline, $p < 0.05$.

‡ Change from baseline is different between acetylcholine and constrictor, $p < 0.05$.

onstrated a significant decrease in GFR (53). However, another in vivo study in dogs demonstrated no change in GFR after blocking NO, although hypertension—an invariable response to significant blockade—was not controlled (31). The presence of hypertension might ameliorate the decrease in GFR seen in our study in which blood pressure was maintained at a constant level.

We used sodium excretion as a measure of renal tubular function. In ovine fetuses, both proximal and distal tubular sodium reabsorption is functional; however, the proximal tubule participates to a lesser extent than in mature kidneys, possibly because the fetus is in a relatively "volume-expanded" state or because the kidney is functionally immature (20). Our baseline values (0.31 ± 0.19 mmol·h⁻¹·kg⁻¹) of sodium excretion are comparable to those previously reported for fetal lambs in sodium balance (0.49 ± 0.14 mmol·h⁻¹·kg⁻¹) (54). After saline infusion, urine volume increased significantly, as did both fractional and total sodium excretion. A previous investigation has shown that, although the fetus loses more sodium via lung fluid than through renal excretion, the relationship between the latter and sodium intake is direct (54). Thus, an increase in sodium excretion would not have been surprising because our infusion rate (4.62 mmol·h⁻¹·kg⁻¹) was considerably greater than the normal sodium intake of a fetal lamb in sodium balance (1.39 ± 0.17 mmol·h⁻¹·kg⁻¹) (54).

Although urine volume and fractional excretion of sodium were not affected by N^ω-nitro-L-arginine, total sodium excretion was significantly reduced primarily because of a decrease in filtered sodium load. RIA to determine cGMP, an index of NO production of renal medulla and cortex in the dog kidney, showed the highest concentration in the medulla and a progressive decrease in concentration toward the cortex (55). This suggests a role for NO in the regulation of sodium reabsorption, although the evidence is not strong. Other in vivo studies of sodium excretion in mature kidneys are conflicting. A decrease in total sodium excretion after blockade of the NO system was seen in studies of local infusion of anesthetized dog kidneys (53), as well as in a long-term study with conscious dogs (31). However, the influence of local or systemic hypertension caused by the NO inhibitor was not excluded (31). In another study with anesthetized dogs in which NO blockade caused no hypertension but resulted in a decrease in renal blood flow and an increase in renal vascular resistance, sodium excretion did not change (56). However, stimulation of the NO system with acetylcholine, administered into the renal artery, in a different study (57) resulted in a 259% increase in sodium output. In our investigation, we were only able to study NO blockade, inasmuch as stimulation of the NO system resulted in a significant drop in systemic arterial pressure. Furthermore, our results with NO

blockade may vary from those previously reported because we maintained constant blood pressure.

Why the NO system should be active in the last-trimester fetal kidney is unknown. Clearly, it helps to maintain baseline fetal homeostasis and may help regulate the fetal response to some pathologic processes. Studies in adult rats after bilateral ureteral obstruction have shown decreased availability of arginine for NO synthesis, suggesting that part of the renal ischemia noted after obstruction may be mediated by a lack of NO (58). It has also been shown that renal blood flow initially increases immediately after renal obstruction. This may be prostaglandin mediated, or NO may be involved. Additionally, the fetal kidney during the 3rd trimester responds to partial obstruction with a prolonged increase in renal blood flow, far longer than in postnatal studies (59). Perhaps activity of the nitric oxide/EDRF system accounts for this difference. Further investigation of its role in pathologic processes is essential.

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Table 3. Effects of NO on renal function*

	n	Mean arterial pressure (mm Hg)	Renal blood flow (mL·min⁻¹·kg⁻¹)	Renal vascular resistance (mm Hg·min·kg·mL⁻¹)	Urine volume (mL·h⁻¹·kg⁻¹)	Glomerular filtration rate (mL·min⁻¹·kg⁻¹)	Filtration fraction (%)	Fractional sodium excretion (%)	Total sodium excretion (mmol·h⁻¹·kg⁻¹)
Nω-nitro-L-arginine									
Pre	8	42 ± 5	12.4 ± 4.8	3.71 ± 0.99	8.9 ± 5.2	1.63 ± 0.45	6.63 ± 2.84	2.47 ± 1.44	0.31 ± 0.19
Post	8	42 ± 5	8.4 ± 2.8†	5.52 ± 1.58†	8.4 ± 6.5	1.42 ± 0.65	8.35 ± 4.70	4.27 ± 3.08	0.73 ± 0.60
% of baseline		(100)	(68)	(149)	(94)	(87)	(126)	(173)	(235)
(Post-Pre)	8		−4.0 ± 2.7	1.80 ± 1.10	−0.5 ± 9.4	−0.21 ± 0.70	1.71 ± 4.54	1.79 ± 4.54	0.41 ± 0.56
Sodium chloride									
Pre	8	40 ± 3	14.2 ± 4.2	3.17 ± 0.99	7.1 ± 5.7	1.37 ± 0.36	4.77 ± 2.20	2.26 ± 2.02	0.30 ± 0.20
Post	8	42 ± 3	15.5 ± 4.5‡	3.16 ± 1.23	12.9 ± 10.6‡	2.14 ± 0.88‡	6.77 ± 3.78	3.49 ± 1.70‡	1.02 ± 0.01†
% of baseline		(105)	(109)	(99)	(182)	(156)	(142)	(154)	(340)
(Post-Pre)	8		1.35 ± 1.12	−0.01 ± 0.45§	5.8 ± 5.9	0.77 ± 0.72¶	2.00 ± 2.48	1.23 ± 1.42	0.71 ± 0.54¶

* Pre, baseline values; Post, after drug delivery.
† p < 0.01 vs baseline.
‡ p < 0.05 vs baseline.
§ p < 0.01 vs Nω-nitro-L-arginine.
¶ p < 0.05 vs Nω-nitro-L-arginine.
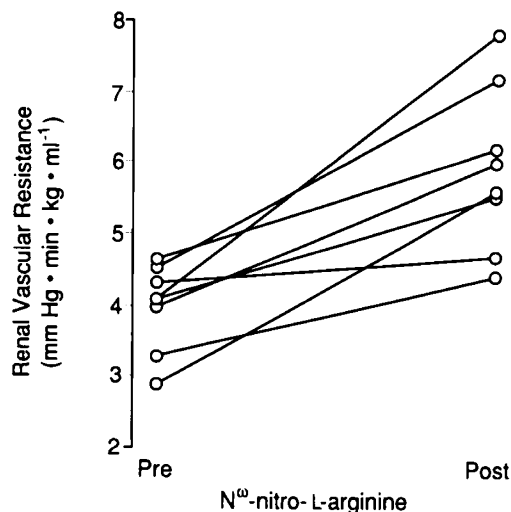
Fig. 4. Response of renal vascular resistance to infusion of Nω-nitro-L-arginine.

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