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Role of Endogenous Atrial Natriuretic Peptide in Chronic Anemia in the Ovine Fetus: Effects of a Non-Peptide Antagonist for Atrial Natriuretic Peptide Receptor

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ABSTRACT

Chronic fetal anemia causes polyhydramnios and fetal hydrops and is associated with increased fetal diuresis and natriuresis. To determine the role of atrial natriuretic peptide (ANP) in the renal adaptation to chronic fetal anemia we studied the effects of HS-142-1 (HS), a specific inhibitor of the guanylate cyclase-linked ANP receptor (ANP-GC), in two groups of chronically instrumented unanesthetized sheep fetuses. Seven fetuses were made anemic by serial isovolemic hemorrhage over 1 wk, and five fetuses served as nonanemic controls. Over the 7 d of hemorrhage ANP concentrations increased (45 ± 7 to 234 ± 15 fmol/mL). Hematocrit and arterial blood oxygen content were significantly lower in the anemic compared with the nonanemic fetuses (13.8 ± 0.7 *versus* 34.6 ± 2.3% and 0.7 ± 0.1 *versus* 2.6 ± 0.2 mmol/L). Before HS urine flow rate, urinary sodium excretion, fractional excretion of sodium, and renal blood flow were increased in the anemic fetuses, and the extracellular fluid volume (inulin space) was increased (674 ± 94 *versus* 497 ± 71 mL/kg). However, GFR was not different between the groups. HS caused a significant increase in the central venous pressure of the anemic fetuses (0.49 ± 0.03 to 0.70 ± 0.05 kPa). Urinary excretion of cGMP was considered to be a marker of endogenous ANP renal effect and was measured before and after a single bolus of HS (5.2 ± 0.30 mg/kg). HS decreased urinary cGMP excretion to 50 and 37% of baseline levels in anemic and nonanemic fetuses, respectively. Urine flow decreased in both nonanemic and anemic fetuses (0.48 ± 0.13 to 0.25 ± 0.06 and 1.30 ± 0.66 ± 0.06 mL/min). Sodium excretion decreased in both groups after HS (19 ± 5 to 9 ± 2 and 83 ± 16 to 39 ± 5 μmol/min). GFR decreased after HS (3.0 ± 0.8 to 2.4 ± 0.5 and 3.6 ± 0.3 to 2.6 ± 0.2 mL/min. Fraction excretion of sodium also decreased in both groups after HS (4.6 ± 2.7 to 2.7 ± 0.5 and 16.1 ± 2.4 to 11 ± 1.6). Percent decreases in urine flow, sodium excretion, GFR, and fractional excretion of sodium observed in the anemic fetuses were not statistically different from the nonanemic fetuses. Urine flow and sodium excretion did not decrease to control levels after HS, suggesting that factors in addition to ANP contribute to the natriuresis seen with chronic anemia. After HS a transient increase in renal blood flow was observed in the nonanemic fetuses. An immediate and sustained further increase in renal blood flow was observed in the anemic fetuses (336 ± 37 to 436 ± 58 mL/min/100 g of kidney). Decreasing GFR and increasing renal blood flow suggests HS may alter the renal microcirculation by reversing ANP-induced constriction of the glomerular efferent arteriole. We conclude that sustained increases of the central venous pressure suggest that ANP inhibition results in decreased fluid movement into perivascular tissue. Endogenous ANP may help to maintain basal renal function in the normal fetal kidney and participates in the renal adaptation to chronic fetal anemia. ANP may promote urine flow and sodium excretion by its effects on both the renal microcirculation and the sodium reabsorptive capacity of the nephron. (***Pediatr Res* 38: 722–728, 1995**)

Abbreviations

ANP, atrial natriuretic peptide
ANP-GC, guanylate cyclase-linked ANP receptor
HS, microbial polysaccharide HS-142-1

Alterations in fetal kidney function may play an important role in stabilizing the fetus stressed by prolonged anemia. In contrast to the renal effects of acute hemorrhage, urine flow, sodium excretion, and renal blood flow are increased in chronically anemic fetuses (1, 2). Enhanced fetal natriuresis is linked

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to the excretion of organic acids and the resulting increased amniotic fluid may provide a sink for hydrogen ions (3). Thus increased sodium excretion and polyhydramnios may indirectly help to maintain normal fetal plasma pH in the face of increased lactate production (1). The mechanism for the renal adaptation to chronic anemia is incompletely understood. One possibility is that decreased oxygen availability might stimulate hormonal factors which promote sodium excretion. In this regard, tissue hypoxia is a potent secretegogue of ANP in the fetus (4). Circulating ANP concentrations are elevated in human fetuses with chronic anemia due to isoimmunization (5), as well as in experimental models of fetal hydrops and polyhydramnios (6), and ANP has been implicated in the pathogenesis of these conditions because it promotes vascular permeation of fluid and protein in the ovine fetus (7). The observation that exogenously administered ANP causes increased sodium excretion when administered to otherwise normal fetuses (8) suggests that this hormone may also enhance the natriuresis in prolonged fetal anemia.

Morishita *et al.* (9) recently identified a polysaccharide of microbial origin, HS, which selectively inhibits the binding of ^{125}I-ANP to the ANP-GC found in renal cortex. Zhang *et al.* (10) independently confirmed that HS is a potent and specific antagonist of natriuretic peptides *in vitro* and *in vivo*. Urinary cGMP excretion has been assumed to be a biologic marker for renal ANP activity (11). HS-induced decreases in urinary cGMP have implicated endogenously produced ANP in a variety of conditions, including the natriuresis caused by volume expansion (12), in renal compensation for hypertensive states (13), in experimental congestive heart failure (14), and in the pathogenesis of diabetic glomerular sclerosis (15). The effect of antagonism of the ANP-GC receptor on the activity of endogenous ANP has not been studied in the fetus. We tested the hypothesis that stimulation of the ANP system contributes to diuresis and natriuresis in prolonged fetal anemia by determining the hemodynamic and renal effects of HS in two groups of chronically instrumented, unanesthetized late gestation fetal lambs. The first group of fetuses was subjected to 7 d of isovolemic hemorrhage before HS administration. The second group served as nonanemic controls.

METHODS

Surgical preparation. All care and procedures were approved by the Oregon Health Sciences University Animal Care and Use Committee. Surgery was performed in 12 ewes (group 1, $n = 7$; group 2, $n = 5$) at 116–125-d gestation (term, 146 d). General anesthesia was induced using i.v. diazepam and ketamine, the ewe was intubated, and anesthesia was maintained with halothane and 50% N_2O-50% O_2. After the uterus was exposed through a midline abdominal incision, an incision in a cotyledon-free area of the uterine wall permitted access to the fetal legs and abdomen. Polyvinyl catheters (1.0 mm inside diameter) were inserted into the femoral vein and were positioned so that their tips were in the abdominal vena cava for the purposes of HS infusion and measurement of central venous pressure. Catheters were inserted into the femoral artery to measure arterial blood pressure and to withdraw blood, and a catheter was positioned in the amniotic space for reference pressure measurements. In eight fetuses (four fetuses in each group) a flank incision was made, and the left kidney was exposed. The renal artery was isolated *in situ* with care taken to preserve the renal nerves, and a Doppler flow meter flow probe (Transonic 2S, Transonic Systems, Ithaca, NY) was placed around the artery. A catheter (1.4 mm inside diameter) with a Silastic tip was placed in the fetal bladder. At the end of surgery, 1,000,000 U of penicillin G were administered directly into the amniotic fluid, and the catheters and the flow probe wire were exteriorized through a s.c. tunnel and placed in a cloth pouch on the ewe's flank. The vascular catheters were filled with a heparinized saline solution (1 U/mL) and were flushed either once or not at all before the day of the experiment. After recovery from anesthesia, the ewes were kept in a restricted area and fed a standard diet.

Experimental protocol. Both groups were permitted to recover for a period of 4 or 5 d. The hematocrit was reduced in seven anemic fetuses by daily hemorrhage of 30–115 mL and replacement with an equal volume of isotonic saline. Gradual isovolemic hemorrhage over a period of days was aimed at achieving a femoral arterial oxygen content that approximated 0.7 mmol/L because renal blood flow and urine flow are nearly double at this degree of chronic fetal anemia. Before each day's hemorrhage, 24 h after the previous day's hemorrhage, 2 mL of fetal arterial blood were obtained to determine the circulating ANP concentration. On the day of the experiment, arterial blood was collected anerobically in heparinized plastic syringes, and pH, P_{CO_2}, and P_{O_2} (IL 1312 blood analyzer calibrated to 39°C) were measured, as well as oxygen content (IL 482 CO-Oximeter). The hematocrit was determined in duplicate. Mean arterial and central venous pressures were measured continuously (referenced to amniotic fluid pressure) using Transpac transducers (Abbott Critical Care Systems). Renal blood flow, mean arterial pressure, central venous pressure, and amniotic fluid pressure were recorded on a Beckman polygraph (R611) and mean values stored on-line to a computer every 20 s. The heart rate was calculated from the cardiotachometer signal triggered from the femoral arterial pressure. The cardiotachometer signal was continuously recorded (30 Hz) and averaged every 20 s. GFR was determined as the renal clearance of inulin as previously described for the sheep fetus (1). Briefly, fetal arterial blood and urine samples were obtained for background measurements just before a bolus of inulin (150 mg/kg of estimated fetal weight; Sigma Chemical Co., St. Louis, MO) in 4 mL saline was injected through a fetal venous catheter followed by a 2-mL saline flush. The inulin was injected approximately 1 h before the first clearance period, and the precise time was noted to aid in determining the inulin space. The fetal bladder catheter was allowed to drain for at least 50 min. Renal function was measured during three successive 10-min clearance periods before and three successive 10-min periods beginning 15 min after a single i.v. bolus of HS. HS kinetics have not been specifically studied, and the compound has never before been administered to either adult or fetal sheep, therefore the dosage used in the present investigation (~5 mg/kg) was determined

on the basis of previous studies in rats (0.5–8 mg/kg) (13). The arterial blood samples were taken at the mid-point of each clearance period. Blood samples were centrifuged at 4°C, and the plasma was stored at −80°C with the urine samples until analyzed. The extracellular fluid volume was measured as the inulin space (16) by semilog extrapolation of the slow decay portion of the plasma inulin curve to the zero-time inulin concentration. The inulin space per kg of fetal body weight was calculated from the formula:

$$\text{Inulin space} = \frac{\text{amount of inulin injected}}{\text{plasma inulin concentration at zero time}} \div \text{fetal body weight}$$

Electrolytes were measured by ion-selective electrode (Nova 10 electrolyte analyzer). Blood bicarbonate levels were calculated from the equation (17):

$$[HCO_3^-] = 0.294 P_{CO2} \times 10^{(-4.9911 + 0.6576pH + 0.0262pH2)}$$

Assays. Blood samples for measurement of ANP were immediately centrifuged at −20°C, and plasma supernatants were stored at −80°C. The ANP RIA has been described previously (18). Briefly, purified ^{125}I-labeled human α ANP was diluted in assay buffer to a concentration of 10,000 cpm per 100 μL. Rabbit antiserum to human α ANP (Amersham Corp., Arlington Heights, IL) was also diluted in assay buffer to a concentration such that 100 μL of the solution were bound to 35% of the total counts in the absence of standard. The standard curve was constructed by serial dilution of synthetic human α ANP from 162 to 0.6 fmol per 100 μL. Before assay, plasma samples were extracted using C-18 SepPack cartridges and eluted with 1% triflouoroacetic acid. ANP anti-serum and standards or plasma samples were incubated together for 24 h at 4°C after which ^{125}I-labeled human α ANP was added to incubate for an additional 16 h, also at 4°C. The free and bound fractions were separated by precipitation with a second antiserum (goat-anti-rabbit, Peninsula Laboratory) in the presence of cold 15% polyethylene glycol 8000. The mixture was incubated for 30 min at 4°C and then centrifuged. The supernatant or free fractions were aspirated and discarded; the precipitate or bound fraction was counted in a gamma counter, and a standard curve was calculated. All samples were measured in a single assay. The intraassay variability was 3.5%. Urinary cGMP concentrations were determined by RIA after succinylation as previously described (19). Inulin concentrations were measured by spectrophotometry by using a modification of the method of Waugh (20).

Statistical analysis. All data are expressed as mean ± SE. All statistical analyses were performed on a Macintosh SE computer using the Statistica program (StatSoft, Tulsa OK). The initial ANP concentrations in the fetuses destined to become anemic were compared with the pre-HS values in the nonanemic group using an unpaired *t* test. Daily changes in ANP concentrations through the period of isovolemic hemorrhage were assessed by repeated measure analysis of variance and Dunnett's test. Values for urine flow rate and sodium excretion were calculated as the means of three successive 10-min clearance periods both before and after HS administration. The effects of both chronic anemia and HS administration on hemodynamics, urine flow, and sodium excretion values before and after HS were analyzed using multivariate analysis of variance for repeated measures. Paired contrasts were used to determine within group differences when a significant interaction was demonstrated. As an aid to the analysis of renal blood flow, the data were binned into three epochs: pre-HS (baseline), post-HS (0–20 min), and post-HS (20–40 min). The values were averaged for each epoch and analysis of variance with repeated measures over time was performed, after which polynomial contrasts were fitted. Unless otherwise specified, any responses referred to as increases or decreases are significant at $p < 0.05$ or better.

RESULTS

Comparison of anemic and nonanemic fetuses before HS. The initial circulating ANP concentrations in the fetuses destined to become anemic were not significantly different from those of the nonanemic control fetuses (45 ± 7 *versus* 39 ± 6 fmol/mL). Over the 1-wk period of isovolemic hemorrhage, ANP concentrations increased significantly (234 ± 15 fmol/mL; Fig. 1). On the day of the experiment, gestational age was slightly less in the nonanemic fetuses compared with anemic fetuses (128 ± 0.5 *versus* 130 ± 1). Fetal weight was also lower in the nonanemic fetuses (2.7 ± 0.3 *versus* 3.3 ± 0.1 kg), but there was no significant difference in the kidney weights between the two groups (19.5 ± 2.6 *versus* 21.7 ± 1.6 g). Within each group the right and left kidney weights were similar for the fetuses with renal artery flow probes (11.2 ± 2.9

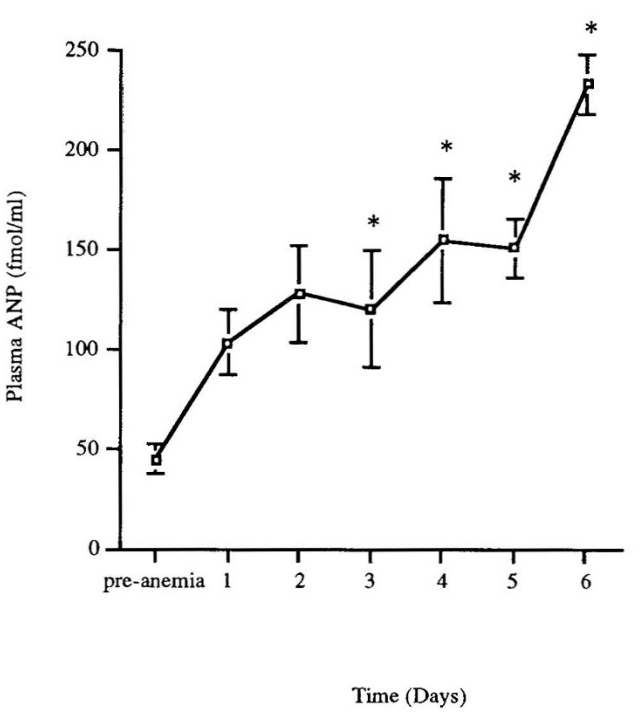

Figure 1. Plasma ANP concentration before daily isovolemic hemorrhage in progressively anemic ovine fetuses. Data are mean ± SE. Analysis was accomplished using repeated measures analysis of variance and Dunnett's test *$p < 0.05$ compared with control.

versus 10.8 ± 2.1 and 10.2 ± 3.6 *versus* 9.4 ± 2.6 g, anemia group and nonanemia group, respectively). As anticipated, the hematocrits and the blood oxygen contents were significantly lower in the anemic fetuses (Table 1). Extracellular fluid volume (inulin space) before HS was significantly increased in the anemic compared with the nonanemic fetuses (674 ± 94 *versus* 497 ± 71 mL/kg). Before HS administration, arterial blood gases, central venous pressure and mean arterial pressure were not different between the groups (Table 2). Heart rate was significantly increased in the anemic fetuses. Renal blood flow was higher and renal vascular resistance was lower in the anemic fetuses compared with nonanemic fetuses. Urinary flow rate (both absolute value and per g of kidney), sodium excretion, and fractional excretion of sodium were significantly higher in the anemic fetuses compared with the control fetuses. Baseline GFR (both as an absolute value and per g of kidney) was not different between the two groups before HS administration.

Effect of HS on blood gases and hemodynamic values (Table 1). The HS dose was similar in the nonanemic and anemic fetuses (5.7 ± 0.55 *versus* 5.0 ± 0.44 mg/kg). HS had no effect on hematocrit. After HS, arterial blood pH decreased slightly but significantly in the anemic fetuses but not in the control fetuses. Arterial blood Po_2 decreased significantly in the anemic fetuses and tended to decrease in the nonanemic fetuses. HS did not cause a change in the mean arterial pressure or heart rate in either group. However, central venous pressure increased in the anemic fetuses. During the pre-HS period (epoch 1) renal blood flow was stable in both anemic and nonanemic fetuses but was significantly elevated in the anemic fetuses (200 ± 14 *versus* 336 ± 47 $mL \cdot min^{-1} \cdot 100$ g of $kidney^{-1}$; Fig. 2). During the first post-HS period (0–20 min, epoch 2) an immediate and significant increase in renal blood flow was observed in the anemic fetuses. A transient and relatively small increase in renal blood flow occurred in the nonanemic fetuses after HS administration. In all nonanemic fetuses renal blood flow returned to baseline within 15 min of HS administration. During the second post-HS period (20–40 min, epoch 3) the further renal blood flow increases were maintained in the anemic group. In the nonanemic fetuses renal blood flow rate during epoch 3 was not different from the nonanemic group's baseline values.

Effect of HS on urine flow, sodium excretion, and urinary cGMP (Table 2). HS administration caused a 45 ± 6% decrease in urine flow of the anemic fetuses which was not different from the 44 ± 9% decrease observed in the nonanemic fetuses. The absolute decrease in the urine flow tended to be greater in the anemic fetuses compared with the nonanemic fetuses (0.64 ± 0.17 versus 0.23 ± 0.09 mL/ min). Similar trends were observed with regard to fractional excretion of sodium, filtration fraction, and sodium excretion rate. In response to HS, GFR decreased (both absolute value and per g of kidney) in both groups. Excretion of cGMP tended to be higher in the anemic versus nonanemic fetuses. The excretion of cGMP (both absolute value and per g of kidney) decreased after administration of HS in both groups.

DISCUSSION

During prolonged fetal anemia, plasma lactate levels increase without a change in blood pH or bicarbonate levels (2). Furthermore, phosphate and lactate excretion are coupled to sodium transport (3). Inasmuch as phosphate is the principal buffer of excretable acid, it seems likely that mechanisms that stimulate natriuresis also contribute to the maintenance of acid-base balance in the setting of chronic fetal anemia. Polyhydramnios may represent a sink for excretable hydrogen ion (1). The principle finding of the present study is that inhibition of the guanylate cyclase-linked ANP receptor with HS causes decreases in urine flow, GFR, sodium excretion, and fractional excretion of sodium in anemic fetuses with elevated circulating ANP concentrations, as well as in nonanemic fetuses with normal ANP concentrations. HS also decreased urinary cGMP excretion in both groups. Because urinary cGMP is thought to be a biologic marker of the renal effects of ANP (11), these findings suggest that endogenous ANP plays an important role both in maintaining basal kidney function in normal fetuses and in the renal adaptation to chronic fetal anemia.

The effect of HS on hemodynamics. HS had no effect on heart rate or mean arterial blood pressure. However, a sus-

Table 1. *Effect of HS and chronic anemia on fetal arterial blood gases and hemodynamic data*

	Nonanemic		Anemic		*p* Value		
	Pre-HS	Pos-HS	Pre-HS	Pos-HS	HS effect	Anemia effect	Interaction
pH	7.35 ± 0.01	7.34 ± 0.01	7.32 ± 0.02	7.30 ± 0.01	0.046	0.090	0.55
po_2 (kPa)	2.21 ± 0.25	2.45 ± 0.23	2.03 ± 0.16	1.64 ± 0.11*	0.38	0.07	0.004
pco_2 (kPa)	6.99 ± 0.12	6.99 ± 0.09	6.91 ± 0.16	7.12 ± 0.20	0.274	0.885	0.274
Oxygen content (mmol/L)	2.6 ± 0.2	2.5 ± 0.3	0.7 ± 0.1	0.6 ± 0.1	0.505	<0.001	0.892
HCO_3^- (mmol/L)	28.0 ± 0.9	27.0 ± 0.2	26.0 ± 1.12	25.2 ± 1.1	0.237	0.146	0.886
Hematocrit (%)	34.6 ± 2.2	32.5 ± 3.8	13.8 ± 0.7	13.2 ± 0.7	0.12	<0.001	0.36
Heart rate (beats/min)	162 ± 3	160 ± 6	182 ± 5	189 ± 5	0.62	0.007	0.26
Central venous pressure (kPa)	0.37 ± 0.09	0.37 ± 0.08	0.49 ± 0.03	0.70 ± 0.05*	0.002	0.030	0.003
Mean arterial pressure (kPa)	5.81 ± 0.13	5.95 ± 0.09	5.60 ± 0.29	5.58 ± 0.217	0.416	0.388	0.234
Renal blood flow ($mL \cdot min^{-1} \cdot 100$ g $kidney^{-1}$)	200 ± 14	200 ± 16	336 ± 47	437 ± 58*	0.028	0.006	0.029
Renal vascular resistance ($dyne \cdot s^{-1} \cdot cm^5 \cdot 100$ g $kidney^{-1}$)	17.6 ± 1.4	18.4 ± 1.5	11.5 ± 1.6	8.5 ± 1*	0.013	0.006	0.005

Data are expressed as mean ± SE; $n = 5$ nonanemic and 7 anemic fetuses, except $n = 4$ for both groups for renal blood flow and renal vascular resistance. Pos-HS represents the mean of three values measured between 15 and 45 min after an i.v. bolus of HS (~5 mg/kg).

* $p < 0.05$ *vs* pre-HS anemic fetuses. Conversion: 1 kPa = 7.5006 torr.

Table 2. *Effect of HS and chronic anemia on fetal renal function**

	Nonanemic		Anemic		*p* Value		
	Pre-HS	Pos-HS	Pre-HS	Pos-HS	HS effect	Anemia effect	Interaction
Urine flow (mL/min)	0.48 ± 0.13	0.25 ± 0.06	1.30 ± 0.18	0.66 ± 0.06	0.002	0.002	0.087
Glomerular filtration rate (mL/min)	3.0 ± 0.8	2.4 ± 0.5	3.6 ± 0.3	2.6 ± 0.2	0.005	0.55	0.40
Glomerular filtration rate ($mL \cdot min^{-1} \cdot g\ kidney^{-1}$)	0.15 ± 0.02	0.12 ± 0.01	0.17 ± 0.03	0.12 ± 0.01	0.016	0.647	0.387
Plasma Na^+ (mmol/L)	143 ± 0.6	143 ± 0.6	142 ± 0.6	141 ± 0.8	0.53	0.102	0.274
Na^+ excretion (μmol/min)	19 ± 5	9 ± 2	83 ± 16	39 ± 5	0.01	0.003	0.073
Fractional Na^+ excretion (%)	4.6 ± 0.5	2.7 ± 0.5	16.1 ± 2.4	11 ± 1.6	0.019	<0.001	0.245
Filtration fraction (%)	19.6 ± 2.7	12.0 ± 2.0	16.8 ± 3.6	8.2 ± 1.4	0.007	0.12	0.627
cGMP excretion (mmol/min)	18.4 ± 6.6	6.6 ± 2	37.9 ± 10.3	19.4 ± 6.5	0.007	0.144	0.441

Data are expressed as mean ± SE; $n = 5$ nonanemic and 7 anemic fetuses, except $n = 4$ for both groups for filtration fraction.
* Post HS data were obtained beginning 15 min after HS administration and for each fetus the mean of three successive 10-min collections was determined.

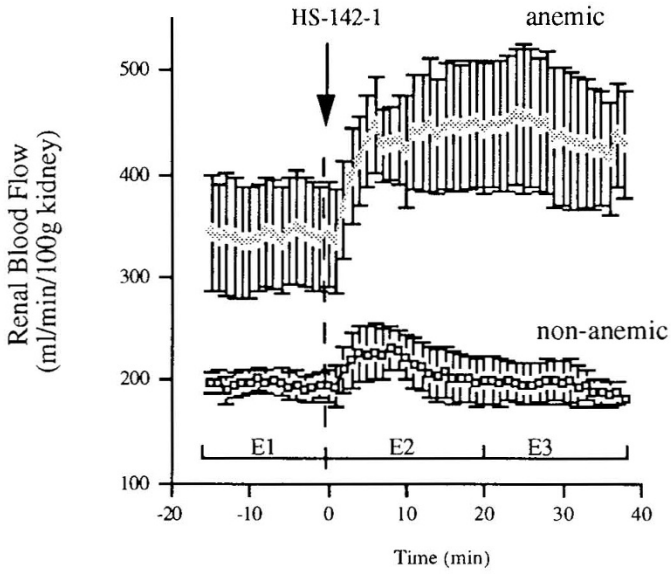

Figure 2. Renal blood flow before and after a single bolus injection of the guanylyl cyclase-linked ANP receptor antagonist HS in chronically anemic and nonanemic ovine fetuses. Renal blood flow in the anemic fetuses increased significantly ($p < 0.005$) but the transient increase (*E2*) in the nonanemic fetuses was not significant. *E* = epoch in which values were averaged before analysis of variance with repeated measures over time. Data are mean ± SE.

tained increase in central venous pressure occurred shortly after HS. The most likely explanation for this effect is that ANP receptor blockade increases venous pressure by inhibiting ANP-caused fluid movement from the microcirculation into perivascular tissue (7). Previous studies from our laboratory have demonstrated that exogenously administered ANP causes a significant reduction of blood volume and produces transudation of radiolabeled albumin, particularly in fetal skin, but also in muscle, adrenal, bone, kidney, and gut (7). ANP-induced increases in vascular permeation independent of renal effects have been identified in adult animal models as well (21–23). The correlation between increased ANP concentrations and significantly expanded extracellular fluid volume in the anemic fetuses is consistent with the hypothesis that ANP may directly or indirectly increase capillary filtration of fluid. Blood volume expansion due to post-HS oliguria is not likely to produce lasting elevation of venous pressure, because even after relatively large and rapid fluid boluses, prompt equilibration with the extravascular compartment due to high fetal capillary filtration coefficient results in modest and transient increases in venous pressure (24). By the same token, increased venous pressure caused by enhanced venous tone is unlikely to be sustained without a simultaneous decrease in the capillary filtration coefficient.

The present report confirms previous observations from our laboratory that chronic fetal anemia, unlike acute isovolemic hemodilution or acute anemia with volume depletion, is associated with increased renal blood flow (1, 2). ANP receptor blockade in anemic fetuses resulted in a sustained further increase in renal blood flow and concomitant decrease in renal vascular resistance. A brief increase in renal blood flow occurred in the nonanemic fetuses as well. Consistent with these findings, Robillard *et al.* (8) observed a decrease in renal blood flow and increasing renal vascular resistance in ovine fetuses receiving exogenous ANP. Furthermore, our findings are consistent with previous studies in adult models: ANP increased renal vascular resistance in the isolated perfused rat kidney (25), and in some reports, but not others (26), ANP administered *in vivo* decreased renal blood flow in the euvolemic adult rat. ANP has potent effects on the renal microvasculature, acting as both an afferent arteriolar vasodilator and an efferent arteriolar vasoconstrictor (27). Each of these effects would cause an increase in glomerular hydrostatic pressure and GFR. Our present observation that HS increased renal blood flow and decreased GFR is consistent with inhibition of ANP-induced efferent arteriolar vasoconstriction coupled with continued vasodilation of the preglomerular vessels. The mechanism of this apparent site-specific ANP effect could be related to differential receptor type, density, or sensitivity of the efferent and afferent arteriole and is the subject of ongoing investigations in our laboratory.

HS-induced decreases in GFR are possibly independent of the effects of HS on arteriolar resistances. Rat glomeruli contain a high density of guanylate cyclase-linked receptors (28, 29), suggesting that HS might antagonize an ANP-mediated increase in the glomerular ultrafiltration coefficient (K_f). Alternatively, HS blockade of the ANP-GC receptor might lead to redistribution of intrarenal blood flow to nonfiltering cortical glomeruli causing decreasing GFR. However, the distribution of glomerular blood flow does not appear to play a role in neonatal natriuresis (30). In any case, there is evidence that

exogenous ANP does not participate in the intrarenal redistribution of single nephron GFR (31).

Role of ANP in maintenance of fetal sodium and water excretion. Other investigators have demonstrated differing effects of exogenous ANP on fetal renal function. There is evidence that ANP increases urine flow (32, 33) and/or sodium excretion and fractional excretion of sodium (8). Taken together these data indicate that exogenous ANP probably increases sodium and water excretion in the normal euvolemic fetus. Our study demonstrates for the first time that blockade of endogenous ANP has marked effects on sodium and water excretion in the fetus under normal, as well as pathologic conditions.

We observed an absolute fall in sodium and water excretion that was greater in the anemic fetuses compared with the nonanemic fetuses. Nonetheless, in the anemic fetuses urine flow and sodium excretion after HS continued to be greater than the pre-HS baseline values in the nonanemic fetuses, suggesting that factors in addition to ANP-GC receptor stimulation contribute to increased renal excretion in the chronically anemic fetus. Tissue hypoxia in chronic anemia, in addition to stimulating ANP secretion (34) limits the Na^+-K^+ ATPase pump, thereby blunting tubular reabsorption of sodium (35). Other possible natriuretic mechanisms might include the effect of dopamine (35), changes in the medullary osmolar gradient (washout phenomenon) (31), stimulation of the so-called ANP silent receptor which is not linked to guanylate cyclase, or the effect of other cleavage products of the atrial peptide prohormone (ANP_{1-126}) (36, 37). The acute effects of phlebotomy through the course of the experiment might have contributed to the slight but significant decrease in Po_2 and pH which occurred after HS in the anemic fetuses could have influenced urine flow. However, hematocrits did not decrease, and furthermore, hypoxemia or acidosis-induced decreases in urine flow would not be expected to be associated with increasing renal blood flow and falling renal vascular resistance. Perhaps more importantly, the HS-induced decrease in pH lends support to the notion that ANP-caused increases in sodium excretion are potentially linked to the excretion of fixed acid and the maintenance of blood pH (2).

In summary, we have created chronic anemia in the late gestation ovine fetus through serial isovolemic hemorrhages over a period of days. The results confirm our earlier work that in the ovine fetus, chronic anemia, unlike acute anemia, increases sodium excretion, urine flow, and renal blood flow and extends these observations by demonstrating for the first time that the extracellular fluid volume under chronically anemic conditions is expanded as well. Our observations that circulating ANP levels are significantly elevated in chronic fetal anemia, that blockade of the ANP-GC receptor causes sustained elevation of the central venous pressure, as well as our previous demonstration that exogenous ANP promotes the movement of fluid from the vascular space into a variety of fetal tissues suggests a linkage between anemia-induced expansion of the extracellular fluid volume and ANP-induced alteration of capillary filtration. That the administration of the specific ANP-GC receptor antagonist HS attenuates urine flow and sodium excretion in both the anemic and nonanemic fetus provides new evidence that endogenous ANP participates both in the day-to-day regulation of normal fetal kidney function and in the renal adaptation during prolonged fetal anemia.

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