In Vitro Prostacyclin Production by Ovine Uterine and Systemic Arteries

Effects of Angiotensin II

Ronald R. Magness, Kwabena Osei-Boaten, Murray D. Mitchell, and Charles R. Rosenfeld

Departments of Pediatrics, Obstetrics and Gynecology, and Biochemistry, and The Green Center for Reproductive Biology Sciences, The University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, Texas 75235

Abstract

Normal pregnancy is associated with reduced systemic pressor responses to infused angiotensin II (ANG II); furthermore, the uterine vascular bed is even less responsive to vasoconstriction by ANG II than the systemic vasculature overall. The mechanism(s) for this refractoriness remains unknown. To determine if vessel production of prostacyclin may be responsible, uterine and omental artery segments were obtained from four groups of sheep, nonpregnant (NP), pregnant (P; 131±4 d), early postpartum (2.2±0.4 d), and late postpartum (16±2 d), and incubated in Krebs-Henseleit alone or with ANG II in the absence or presence of Saralasin. Prostacyclin was measured as 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$). Synthesis of 6-keto-$PGF_{1\alpha}$ was *de novo,* since aspirin inhibited its formation. P and early uterine arteries produced more 6-keto-$PGF_{1\alpha}$ than NP and late vessels ($P < 0.05$): 386±60 ($\bar{X}$±SE) and 175±23 vs. 32±5 and 18±4 pg/mg · h, respectively. A similar relationship was observed for omental arteries: 101±14 and 74±14 vs. 36±10 and 22±4 pg/mg · h, respectively. Furthermore, synthesis by arteries from P and early animals was greater in uterine than omental vessels ($P < 0.05$); this was not observed in NP or late vessels. ANG II increased 6-keto-$PGF_{1\alpha}$ production 107±20% and 92±16% in P and early uterine arteries only; the threshold dose was between 5×10^{-11} and 5×10^{-9} M ANG II. This ANG II-induced increase in 6-keto-$PGF_{1\alpha}$ by uterine arteries was inhibited by Saralasin, which by itself had no effect. During pregnancy, the reduced systemic pressor response to ANG II and the even greater refractoriness of the uterine vascular bed may be reflective of vessel production of the potent vasodilator, prostacyclin. Furthermore, in the uterine vasculature, this antagonism may be potentiated by specific ANG II receptor-mediated increases in prostacyclin.

Introduction

Normal pregnancy is associated with the development of relative vascular refractoriness to the systemic pressor effects of infused angiotensin II (ANG II)[1] in women (1–3), sheep (4, 5), and several other species (6, 7). This refractoriness is lost in women who develop pregnancy-induced hypertension (preeclampsia), and they become exquisitely sensitive to the pressor effects of infused ANG II (3, 8). The mechanism(s) responsible for the development of this relative vascular refractoriness remains unclear. Elevated concentrations of the vasodilating prostaglandins have been found in the circulation of normal pregnant women and sheep (9–11), whereas decreased production rates of the potent vasodilator prostacyclin have been reported in women with pregnancy-induced hypertension (9). Furthermore, the treatment of normal near-term pregnant women (12) or sheep (13) with prostaglandin synthetase inhibitors has been reported to increase the systemic pressor responses to infused ANG II, while the addition of prostaglandin precursors to the diet of male rats results in diminished vascular responses to either renin or ANG II (14). Thus, it appears that prostaglandins may modify vessel reactivity to infused vasoconstrictors such as ANG II during normal pregnancy.

We have demonstrated that the chronically instrumented sheep may serve as an excellent animal model in which to study the normal cardiovascular changes that occur in pregnancy (5, 15). In these studies, the uterine vascular bed of late pregnant sheep was even less responsive to infused ANG II than the systemic vascular bed overall (16–18). Furthermore, doses of ANG II that did not significantly alter ovine uterine blood flow resulted in decreases in blood flow of 50% or greater to nonreproductive tissues such as the kidney, adrenal gland, and perirenal and greater omental fat (17). Thus, specific vascular beds respond differently to infused ANG II during normal ovine pregnancy. It is possible that prostaglandins may be important in this differential vascular responsiveness since it has been shown that a high venous-arterial concentration difference of vasodilating prostaglandins exists across the gravid uterus (10).

Terragno et al. (19, 20) have reported that bovine mesenteric arteries have the capacity to synthesize prostaglandins, and that the principal prostaglandin produced is prostacyclin. In addition, the uterine (21), renal (22), and splenic (23) vascular beds have been shown to produce various prostaglandins in response to infused ANG II. Thus, it would appear that during normal pregnancy the systemic vascular refractoriness to ANG II and the even greater refractoriness of the uterine vascular bed to ANG II could be due to increased vessel production of prostaglandins, specifically prostacyclin. The objectives of the present experiments therefore were to examine in vitro the production of prostacyclin by ovine uterine and systemic arteries and to determine the effect that ANG II might have on this synthesis.

Methods

Experiment 1. Postpartum ewes were assigned randomly to be killed either on days 1–4 after delivery (early, n = 10, 2.2±0.4 d, $\bar{X}$±SE) or after 7 d (late, n = 7, 15.9±2.3 d). Six parous, nonpregnant ovariectomized

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Address correspondence and reprint requests to Dr. Rosenfeld, Department of Pediatrics.

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1. *Abbreviations used in this paper:* ANG II, angiotensin II; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$.

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sheep were also assigned to this study. Animals were killed with an intravenous bolus dose of pentobarbital sodium (70 mg/kg). The uterine mesometrium, containing a portion of the main uterine artery and several generations of uterine artery, and samples of the greater omentum were rapidly removed (10–12 min) and placed in oxygenated Krebs-Henseleit solution (25°C) which had been freshly prepared and bubbled with 95% O_2/5% CO_2. This solution had a Po_2, Pco_2, and pH of 680 mmHg, 45 mmHg, and 7.435, respectively. The chemical composition of Krebs-Henseleit was KCl (4.8 mM), $CaCl_2$ (2.0 mM), KH_2PO_4 (1.2 mM), $MgSO_4$ (1.2 mM), dextrose (11 mM), NaCl (118 mM), and $NaHCO_3$ (25 mM). The dissection of arteries was performed in separate petri dishes filled with continuously oxygenated Krebs-Henseleit solution maintained at room temperature. Since the main uterine artery is unlikely to act as a resistance vessel, we obtained third or fourth order generation vessels; these were dissected free from the mesometrium and cut into individual segments. Omental arteries, representative of systemic arteries, were dissected free from surrounding adipose tissue and veins and divided into segments. Each arterial segment was blotted once on a tissue, weighed, and immediately returned to the media. Weights for both groups of vessel segments were similar, ranging from 30 to 50 mg.

The uterine and omental artery segments were placed sequentially into polypropylene chambers containing 4.0 ml of oxygenated Krebs-Henseleit (37°C). Each chamber was bubbled continuously with 95% O_2/5% CO_2 throughout the study. Individual chambers contained 0, 5×10^{-9} M, 5×10^{-8} M, 5×10^{-7} M, or 5×10^{-6} M ANG II (Hypertensin [Val^5-angiotensin II amide)–Ciba-Geigy Corp., Summit, NJ) mixed in isotonic saline and administered in volumes that did not exceed 2% of the chamber volume. At the end of 1 h, 2.0 ml of the incubate were removed and placed in tubes containing 20 μl of an aspirin (acetylsalicyclic acid) solution (5.0 mg/ml). For nonpregnant vessels the media was completely removed, the chamber refilled, and the vessels retreated with ANG II; a second sample was obtained at the end of the second hour. Tubes were immediately frozen on dry ice and stored at −20°C until the time of assay.

Experiment 2. In a second set of experiments we sought to determine the role of the ANG II vascular receptors in the ANG II-induced production of prostacyclin using Saralasin (Saralasin acetate [Sar^1, Val^5, Ala^8-angiotensin II], Eaton Laboratories, Norwich, NY), the specific ANG II receptor antagonist. Early (n = 6, 2.2±0.6 d) and late postpartum (n = 5, 13.0±1.0 d) animals were randomly assigned to be killed and the uterine and omental arteries were collected as described above. Incubation chambers were treated with 0, 5×10^{-9} M, 5×10^{-8} M, 5×10^{-7} M, or 5×10^{-6} M ANG II as described above, but in the presence or absence of 5×10^{-7} M Saralasin. After 1 h of incubation the media was completely evacuated from each chamber; the chamber was refilled with Krebs-Henseleit, and retreated with ANG II and/or Saralasin; a second sample was collected at the end of the second hour of incubation. Samples were immediately frozen and stored at −20°C until the time of radioimmunoassay for prostaglandins.

Experiment 3. In this study, seven pregnant sheep were killed at 131±4 d of gestation (term 144±5 d) and the uterine and omental arteries were collected as described above. Incubation chambers were treated with 0, 5×10^{-11} M, 5×10^{-10} M, 5×10^{-9} M, and 5×10^{-8} M ANG II in the presence or absence of Saralasin (5×10^{-7} M). Additional doses of ANG II comparable to exps. 1 and 2 could not be studied because of the limited number of available incubation chambers. After 1 h of incubation the vessel segments were transferred into another chamber containing Krebs-Henseleit and the drug treatment repeated; another sample was collected at the end of the second hour of incubation. All samples were immediately frozen and stored at −20°C until assayed for prostaglandins.

Radioimmunoassay procedures. Samples of the incubate were assayed for the stable metabolite of prostacyclin, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), by radioimmunoassay using the methods previously described and validated in these laboratories (24). Briefly, the direct assay procedure used standard (0–500 pg) and unknown quantities of 6-keto-$PGF_{1\alpha}$ mixed in Krebs-Henseleit plus 0.1 M phosphate saline gelatin buffer (1:1) prepared in duplicate 0.2-ml aliquots. Antiserum (0.1 ml, 1:1,800 final titre) and 0.01 ml 6-keto-[5,8,11,12,14,15-H^3] $PGF_{1\alpha}$ (150 Ci/mmol) label (~6,100 dpm) were added successively and tubes were incubated at 4°C for 8–12 h. Bound and free ligand were separated with dextran-coated charcoal. The sensitivity of the assay was consistently ≤4 pg. The Krebs-Henseleit buffer had a blank value of 2.7±1.4 pg/ml. Intraassay coefficients of variation at 45 and 634 pg/ml were 6.8 and 7.6%, respectively. Accuracy of recovered standard amounts of 6-keto-$PGF_{1\alpha}$ and parallelism of diluted unknown samples yielded correlation coefficients of 0.995 and 0.999, respectively. We also attempted to quantify the amounts of PGE_2 and $PGF_{2\alpha}$ in the media obtained from incubated arteries; however, values were near the sensitivity of the assay (4 and 6.6 pg/tube, respectively), and thus, no consistent results can be reported. All values are reported as pg/mg · h, unless otherwise noted, to adjust for the wet weight and the time of incubation.

Validation procedures. To determine if the arterial production of 6-keto-$PGF_{1\alpha}$ represented *de novo* synthesis, uterine and omental arteries obtained from nonpregnant and early postpartum sheep were incubated in the presence and absence of aspirin (50 μg/ml). In separate studies we also boiled uterine and omental artery segments for 15 min before incubation. To assess if substrate availability was rate limiting within the time-span of our experiments, uterine and omental arteries from early animals were incubated for 2 h and the media exchanged at 15-min intervals.

Statistical analysis. Data were analyzed by split plot analysis of variance. Treatments were days postpartum (early or late), nonpregnant, or pregnant; vessels (uterine and omental) were nested within treatment; drug treatments were subplots within each vessel type. In exps. 2 and 3, results from the first and second hour of incubation were analyzed as subsamples (25). Means were compared by Student Newman-Keuls multiple range test using the proper error term for the degree of interaction from the analysis of variance table. Data are presented as the mean and standard error.

Results

Validation. As seen in Fig. 1, the production of 6-keto-$PGF_{1\alpha}$ remained constant for both early uterine and omental vessels over a 2-h incubation, averaging 3.93±0.13 and 1.19±0.13 pg/mg · min, respectively. Therefore, there was no evidence of limited substrate availability in the paradigm used. The addition of aspirin to the incubation media significantly inhibited prostacyclin production by both uterine and omental segments obtained from early postpartum (n = 18) and nonpregnant sheep (n = 11) (Table I). Additionally, boiling uterine and systemic arteries (n = 12) from early animals decreased prostacyclin production from 163±30 and 60±5.3 to 2.0±0.4 and 1.7±0.6 pg/mg · h, respectively ($P < 0.01$). Thus, prostaglandin production was *de novo*. It is also of note (Table I) that the early uterine

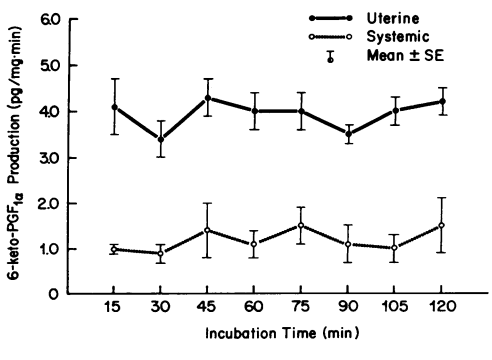

Figure 1. In vitro production of 6-keto-$PGF_{1\alpha}$ by early ovine uterine and omental (systemic) arteries during 2 h of incubation.

*Table I. Effects of Aspirin on the In Vitro Production of 6-Keto-$PGF_{1\alpha}$ (pg/mg · h) by Uterine and Systemic Arteries Obtained from Early Postpartum and Nonpregnant Sheep**

	Early postpartum	Nonpregnant
Uterine vessels		
Control	165±34 ‡	32±5.2 ‡
Treated	10±1	10±2.7
Systemic vessels		
Control	79±22 ‡	36±10 §
Treated	9±3	16±5.0

* Values are mean±SE.
‡ $P < 0.01$.
§ $P < 0.05$.

arteries produced substantially more 6-keto-$PGF_{1\alpha}$ than early omental arteries and nonpregnant uterine and omental arteries.

Experiment 1. The effects of ANG II on the in vitro production of 6-keto-$PGF_{1\alpha}$ by uterine and omental vessels obtained from early and late postpartum ewes and nonpregnant sheep are presented in Table II. In the absence of ANG II, early uterine and omental arteries produced substantially more 6-keto-$PGF_{1\alpha}$ than comparable vessels from late postpartum or nonpregnant sheep ($P < 0.05$). Furthermore, the amount of 6-keto-$PGF_{1\alpha}$ produced by control early uterine arteries was nearly twice that produced by the early omental vessels, 165±34 vs. 79±22 pg/mg · h ($P < 0.01$), respectively; this difference in vessel production of 6-keto-$PGF_{1\alpha}$ was not found in arteries obtained from nonpregnant and late postpartum ewes. The addition of various concentrations of ANG II to the media resulted in increased 6-keto-$PGF_{1\alpha}$ production only by early uterine arteries ($P < 0.05$); however, no dose-response was observed over the dose range studied and a mean increase to 255±24 pg/mg · h was obtained. The addition of aspirin (50 μg/ml) inhibited the ANG II-induced

*Table II. Effect of ANG II on In Vitro Production of 6-Keto-$PGF_{1\alpha}$ (pg/mg · h) by Uterine and Systemic Arteries Obtained from Postpartum (Early and Late) and Nonpregnant Sheep**

	Dose of ANG II (*M*)				
	0	5×10^{-9}	5×10^{-8}	5×10^{-7}	5×10^{-6}
A Uterine arteries					
Early ($n = 10$)	165±34	272±48‡	229±47‡ §	278±56‡	239±47‡
Late ($n = 7$)	13±7	18±6	23±8	24±5	15±3
Nonpregnant ($n = 11$)	32±5	40±6	43±5	52±14	46±8
B Systemic arteries					
Early ($n = 10$)	79±22	80±28	57±14 §	62±13	50±13
Late ($n = 7$)	27±7	22±7	20±3	13±4	16±4
Nonpregnant ($n = 11$)	36±10	29±5	34±4	40±12	41±9

* Values are mean±SE.
‡ Different from control, $P < 0.05$.
§ All values different from early, $P < 0.01$.

*Table III. Effect of Saralasin (5×10^{-7} M) and ANG II on In Vitro Production of 6-Keto-$PGF_{1\alpha}$ (pg/mg · h) by Uterine Arteries Obtained from Early and Late Postpartum Sheep**

	Dose of ANG II (*M*)				
	0	5×10^{-9}	5×10^{-8}	5×10^{-7}	5×10^{-6}
Postpartum days 1–4 ($n = 12$)					
Control	183±33	363±76‡ §	307±65‡ §	393±100‡ §	260±61‡ §
Saralasin	199±41	173±35	202±38	193±37	168±36
>7-d postpartum ($n = 10$)					
Control	19±5	24±6	26±6	27±5	15±2
Saralasin	22±5	25±6	14±3	17±3	22±4

* Values are mean±SE.
‡ Different from no ANG II, $P < 0.05$.
§ Different from control, $P < 0.05$.

rise in 6-keto-$PGF_{1\alpha}$, a reflection of the inhibition of the basal production of prostacyclin.

Experiment 2. As in exp. 1, in the absence of ANG II, uterine arteries obtained from early postpartum ewes produced greater amounts of 6-keto-$PGF_{1\alpha}$ than vessels obtained from late postpartum animals (Table III). Furthermore, as noted above, ANG II specifically augmented the synthesis of prostacyclin by early uterine arteries ($P < 0.05$) and no dose-response was detected. The addition of Saralasin (5×10^{-7} M) to the incubate had no affect on the basal production rate of prostacyclin; however, the specific increase in 6-keto-$PGF_{1\alpha}$ production by early uterine arteries treated with ANG II was inhibited by Saralasin ($P < 0.05$). Prostacyclin production by late postpartum uterine arteries (Table III), as well as early or late omental arteries (Table IV), was unaffected by the addition of either ANG II or ANG II plus Saralasin. Again, note in Table IV that early omental arteries produced more 6-keto-$PGF_{1\alpha}$ than late postpartum vessels.

Experiment 3. When vessels obtained from near-term pregnant sheep were examined (Table V), the basal production of 6-keto-$PGF_{1\alpha}$ by uterine arteries was nearly fourfold greater than

*Table IV. Effect of Saralasin (5×10^{-7} M) and ANG II on In Vitro Production of 6-Keto-$PGF_{1\alpha}$ (pg/mg · h) by Systemic Arteries Obtained from Early and Late Postpartum Sheep**

	Dose of ANG II (*M*)				
	0	5×10^{-9}	5×10^{-8}	5×10^{-7}	5×10^{-6}
1–4 Postpartum days ($n = 10$)					
Control	68±18	60±20	58±17	58±8	51±7
Saralasin‡	66±12	62±13	64±14	66±21	75±12
>7-D postpartum ($n = 10$)					
Control	16±3	23±5	20±3	14±3	15±3
Saralasin‡	10±3	14±4	16±3	14±5	18±5

* Values are the mean±SE.
‡ Saralasin (5×10^{-7} M) not significantly different from controls ($P > 0.05$).

*Table V. Effect of Saralasin (5×10^{-7} M) and ANG II on In Vitro Production of 6-Keto-$PGF_{1\alpha}$ (pg/mg · h) by Uterine and Systemic Arteries from Pregnant Sheep (131±4 d)**

	Dose of ANG II (*M*)				
	0	5×10^{-11}	5×10^{-10}	5×10^{-9}	5×10^{-8}
A Uterine arteries (n = 14)					
Control	386±60	406±63	466±78 §	775±94‡ §	762±145‡ §
Saralasin	288±49	344±50	314±55	329±60	356±56
B Systemic arteries (n = 12)					
Control	101±14	106±16	107±23	140±29	144±25
Saralasin	113±23	123±12	124±14	133±30	121±21

* Values are mean±SE.
‡ Different from no angiotensin II, $P < 0.01$.
§ Different from control, $P < 0.05$.

that by systemic arteries ($P < 0.01$). In an attempt to define a dose-response to ANG II, the minimum dose used in these studies was two orders of a magnitude lower (5×10^{-11} M vs. 5×10^{-9} M) than that employed in exps. 1 and 2. As with early uterine arteries, ANG II specifically augmented prostacyclin synthesis by uterine arteries; however, this occurred in a dose-dependent manner, with a threshold dose between 5×10^{-11} M and 5×10^{-9} M ANG II. Although the addition of Saralasin (5×10^{-7} M) to the incubation media did not alter basal synthesis of prostacyclin, the ANG II-induced increases in 6-keto-$PGF_{1\alpha}$ production by uterine arteries were inhibited ($P < 0.05$). The production of 6-keto-$PGF_{1\alpha}$ by omental arteries tended to increase at 5×10^{-9} M and 5×10^{-8} M ANG II; this, however, was not significant. There was no effect of Saralasin or ANG II plus Saralasin on omental arteries.

A comparison of the basal production rates of 6-keto-$PGF_{1\alpha}$ obtained in exps. 1–3 is presented in Fig. 2. Although the production of 6-keto-$PGF_{1\alpha}$ by nonpregnant and late postpartum uterine arteries was not different, these values were 10-fold less ($P < 0.01$) than that obtained for respective pregnant and early postpartum uterine arteries. Furthermore, vessels from pregnant sheep produced twice the amount of 6-keto-$PGF_{1\alpha}$ observed for early postpartum arteries, 386±60 vs. 175±23 ($P < 0.01$), respectively. As with the uterine vessels, nonpregnant and late postpartum omental arteries produced amounts of 6-keto-$PGF_{1\alpha}$ that were similar, but significantly less than that produced by either pregnant or early postpartum arteries. In contrast to that seen with uterine arteries, however, there was no significant difference in 6-keto-$PGF_{1\alpha}$ production by the latter two groups of arteries, 101±14 and 74±14, respectively. When the responses of uterine arteries to ANG II were compared at similar doses (5×10^{-9} M and 5×10^{-8} M), the synthesis of 6-keto-$PGF_{1\alpha}$ was increased 107±20% and 92±16% by arteries from pregnant and early postpartum sheep, respectively. Although the percent change in prostacyclin production by the two groups of uterine arteries was not different ($P > 0.05$), the absolute change was significantly greater in vessels from pregnant sheep, 350±80 vs. 113±26 pg/mg · h ($P < 0.01$), respectively.

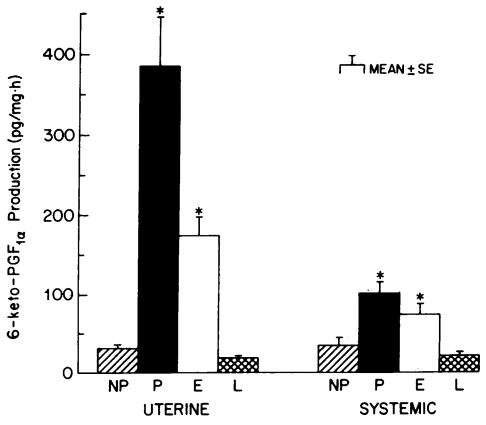

Figure 2. Comparison of the in vitro basal production of 6-keto-$PGF_{1\alpha}$ by nonpregnant (NP), pregnant (P), early postpartum (E), and late postpartum (L) uterine and omental (systemic) arteries. *$P < 0.05$, values different from NP and L.

Discussion

Interest in determining the mechanism(s) responsible for the attenuated vasoconstrictor response to ANG II that occurs during normal pregnancy is obtained from the observation that not only is this refractoriness lost in women who develop pregnancy-induced hypertension, but that they also become even more sensitive to ANG II than normal nonpregnant subjects (3, 8). An understanding of this phenomenon of normal pregnancy might help in determining the pathophysiology of pregnancy-induced hypertension.

Although numerous hypotheses have been advanced concerning the development of this relative vascular refractoriness, few have been proven. Theories have included down-regulation of ANG II receptors secondary to high circulating levels of ANG II found in pregnancy (26), differences in cardiac responses between pregnant and nonpregnant subjects (27), an effect of the steroid hormones of pregnancy (7, 28), alterations in vessel wall mechanics, and increased production of vasodilating prostaglandins (9–13). Since pregnant sheep develop refractoriness to the pressor effects of infused ANG II similar to that seen in women (5), we have used this chronically instrumented animal model to investigate many of these theories in vivo. Although relative vascular underfilling occurs in both normal human and ovine pregnancy, resulting in elevated circulating levels of ANG II, down-regulation of ANG II receptors does not appear to be the predominant mechanism responsible for development of

vascular refractoriness (29, 30). We also have shown that neither changes in cardiac responses (31) nor alterations in the metabolic clearance rate of ANG II (32) appear to be important. Therefore, at least two possible considerations remain, i.e., alterations in the vessel wall itself, which may be related to the elevation in steroid hormones found in normal pregnancy, or production of a prostaglandin(s) that might serve to antagonize the vasoconstricting properties of infused ANG II (12, 13).

Little is presently known about changes in the mechanics of the vessel; however, evidence for a role for prostaglandins in the development of vascular refractoriness in pregnancy is obtained from several observations, e.g., high circulating levels and an increased production rate of vasodilating prostaglandins in both normal ovine and human pregnancy (9–11), and significantly enhanced systemic pressor responses to infused ANG II in pregnant women and sheep after pretreatment with either aspirin or indomethacin, respectively (12, 13). Therefore, we chose to examine the in vitro production of prostaglandins, especially prostacyclin, in arteries obtained from nonpregnant and pregnant sheep and from animals in the early and late peurperium.

It is clear from the results obtained that arteries from the uterine vasculature and greater omentum of sheep have the capacity to release newly synthesized prostaglandins from endogenous substrate stores, and that this synthesis is not substrate limited for up to 2 h of incubation. Furthermore, the primary prostaglandin produced by these vessels was 6-keto-$PGF_{1\alpha}$, the stable metabolite of prostacyclin, since values for PGE_2 and $PGF_{2\alpha}$ were at or below the sensitivity of our assays. The observation that prostacyclin is the primary prostaglandin produced by these vessels is consistent with that of Terragno et al. (20), who reported similar results in studies of bovine mesenteric vessels.

Although omental arteries from all animals produced 6-keto-$PGF_{1\alpha}$, vessels obtained from pregnant and early postpartum sheep produced considerably more than arteries obtained from nonpregnant animals or sheep killed remote from parturition (late). This is consistent with the observations of Lewis et al. (33) that normal pregnant (>28 wk) and early postpartum women have similar plasma concentrations of 6-keto-$PGF_{1\alpha}$, and that these levels are greater than that found in nonpregnant and early pregnant (<12 wk) subjects. This increased production of prostacyclin during normal pregnancy may serve to modify vascular responsiveness and thus the systemic pressor responses to infused ANG II and possibly other vasoconstrictors. Support for this is obtained from several other observations: the systemic vasoconstrictor response to ANG II is enhanced in women and sheep after treatment with prostaglandin synthetase inhibitors (12, 13); prostacyclin is a potent vasodilator in vivo (34); prostacyclin inhibits in vitro vascular smooth muscle constriction in response to KCl-induced depolarization (35); and finally, women with pregnancy-induced hypertension have been reported to have decreased production of prostacyclin (9).

In considering any cardiovascular changes that occur in pregnancy, it is important to examine the uterine circulation, since alterations in this vascular bed are peculiar to pregnancy. We have demonstrated in unanesthetized, near-term pregnant sheep that although ANG II acts only as a vasoconstrictor in this vascular bed, as in other vascular beds (16, 17, 36), the uterine vasculature is even less responsive to infused ANG II than the systemic vasculature overall (16–18). Because of this differential sensitivity to the vasoconstricting effects of ANG II, uterine blood flow may be observed to rise during the systemic infusion of physiologic doses of ANG II, a finding that has caused some to report that this vasoconstrictor might be a uterine vasodilator (21, 37). However, this increase in uterine blood flow is reflective of the substantial rise in perfusion pressure at a time when the increase in uterine vascular resistance is significantly less than that of systemic vascular resistance, thus reflecting the interaction between perfusion pressure, vascular resistance, and blood flow to this vascular bed.

To determine whether vessel production of prostaglandins, specifically prostacyclin, might be important in the even greater refractoriness of the uterine vascular bed to infused ANG II than the systemic vasculature, we also studied uterine arteries obtained from nonpregnant, pregnant, early postpartum, and late postpartum sheep. Not only did pregnant and early postpartum uterine arteries produce 5- to 10-fold more 6-keto-$PGF_{1\alpha}$ than similar vessels obtained from late postpartum and nonpregnant sheep, but they also produced substantially more than that by omental (systemic) arteries from all animals. This difference in the ability to produce prostacyclin could explain the differential sensitivity to infused ANG II previously observed between the uterine and systemic vascular beds (16–18). Moreover, we also observed that when ANG II was added to the incubate of uterine arteries from pregnant and early postpartum sheep, there was a nearly 100% increase in 6-keto-$PGF_{1\alpha}$ production that was specific to these vessels and mediated through the ANG II receptor. Thus, it is even more likely that the substantial uterine refractoriness to infused ANG II is reflective of not only the greater basal production rate of prostacyclin, but also the ANG II-induced increases in prostacyclin. Similar ANG II-stimulated increases in prostaglandin production have been shown to occur in the kidney (22, 38) and spleen (23), and as in the present study, this was blocked by specific ANG II receptor antagonists. Satoh et al. (38) have reported that a differential effect of ANG II, similar to that reported in the present investigation, exists for renal and femoral arteries, and that this ANG II receptor-mediated phenomenon in renal arteries is associated with the action of phospholipase A_2.

Although we could not demonstrate a dose-response for the ANG II-induced increases in 6-keto-$PGF_{1\alpha}$ by uterine arteries from early postpartum sheep, a dose-response was observed when subsequent studies were performed with arteries from pregnant sheep using doses of ANG II two orders of magnitude lower. In these studies, maximum stimulation of vascular production of 6-keto-$PGF_{1\alpha}$ occurred at 5×10^{-9} M ANG II, and no further increases occurred at higher doses. The presence of a dose-response is consistent with the observations of others studying renal arteries (38). It is noteworthy that although the relative ANG II-induced increase in 6-keto-$PGF_{1\alpha}$ production by uterine arteries from pregnant and early postpartum ewes was not different, ~100%, the absolute increase by pregnant arteries was three times that of early postpartum vessels, 350±81 vs. 113±26 pg/mg · h, respectively. This difference likely reflects the ability of the pregnant uterine arteries to produce greater amounts of prostacyclin; however, an explanation for this is not yet apparent.

In these studies, we also have demonstrated that systemic and uterine arterial production of 6-keto-$PGF_{1\alpha}$ rises 3- and 10-fold, respectively, from the nonpregnant state to near-term pregnancy, and that it gradually falls during the puerperium, attaining production rates similar to that seen in vessels from nonpregnant animals by 2 wk postpartum. Although quantitatively different, pregnancy also has been shown to modify the effects of estrogen-induced vasodilation (39, 40); that is, estrogen-induced vaso-

dilation of the uterine vasculature is maximum in nonpregnant sheep, progressively decreases during pregnancy, and gradually returns to nonpregnant responses by 10–14 d postpartum. We also have demonstrated in the present studies that the ANG II-induced increase in uterine arterial production of 6-keto-$PGF_{1\alpha}$ is absent in nonpregnant and late postpartum uterine vessels, but present in vessels from near-term pregnant sheep and within 1 to 4 d after parturition. These observations are supportive of the view that some factor peculiar to pregnancy, likely a hormone, is responsible for the pattern of these vascular alterations. In this regard, Dadak et al. (41) have suggested that a prostacyclin stimulating factor is present in the plasma of pregnant and postpartum women. It is possible that this factor may be estrogen, since prostacyclin production by vascular endothelium (42) and smooth muscle (43, 44) may be stimulated by this steroid hormone. Moreover, we have shown that estrogenization of nonpregnant castrated sheep will result in refractoriness to the pressor effects of ANG II (28).

From the results obtained in these studies, it is reasonable to suggest that prostacyclin production by both uterine and omental arteries may serve to antagonize the vasoconstrictor responses to infused ANG II in normal pregnancy. Moreover, the greater refractoriness to infused ANG II of the uterine vascular bed as compared with the systemic vasculature overall very likely reflects not only an increased capacity of the uterine arteries to produce prostacyclin, but also the ANG II receptor-mediated increases in prostacyclin production. This difference between the uterine and systemic vascular beds could be reflective of an adaptative mechanism that is necessary for the maintenance of uteroplacental perfusion and fetal well being, since ANG II normally increases in pregnancy and likely increases even further, although intermittently, during the course of normal daily activities.

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