Prostacyclin Produced by the Pregnant Uterus in the Dog May Act as a Circulating Vasodepressor Substance

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A B S T R A C T   Uterine production of PGI_2 (prostacyclin) was quantitated in late pregnant dogs to evaluate if PGI_2 could act as a circulating vasodepressor substance in pregnancy. In five anesthetized, laparotomized dogs, the uterine venous plasma concentration of 6-keto $PGF_{1\alpha}$ (the in vitro hydrolysis product of PGI_2) was 6.7 ± 1.9 ng/ml and the arterial plasma concentration was 2.6 ± 0.8 ng/ml. In four nonpregnant female dogs the arterial plasma concentration of 6-keto $PGF_{1\alpha}$ was consistently below 0.2 ng/ml. In eight pregnant dogs we also evaluated the ability of the pregnant uterus to inactivate PGI_2 by comparing the hypotensive response to increasing doses of PGI_2 infused into the uterine artery to the hypotensive response to increasing doses of PGI_2 infused into the inferior vena cava. In addition, the effect of PGI_2 infused into the uterine artery on uterine blood flow and intraamniotic fluid pressure was evaluated. The dose-response curves of intrauterine and intravenous PGI_2 in causing systemic hypotension were identical suggesting that the pregnant uterus does not inactivate infused PGI_2. Intrauterine PGI_2 had no consistent effect on uterine hemodynamics although it did increase intraamniotic fluid pressure significantly. These data demonstrate that the pregnant uterus has the capacity to produce large quantities of PGI_2 which is not inactivated in the uterus and therefore can appear in the arterial blood to exert a systemic vasodepressor effect.

INTRODUCTION

Despite salt and water retention and a large increase in cardiac output, pregnancy is associated with a slight decrease in mean arterial pressure (1). This decrease in arterial pressure is secondary to an even larger decrease in total peripheral vascular resistance. The mediator of the decreased peripheral vascular resistance is not known, but prostaglandins have been implicated to play a role in this effect. We have recently reported that the late pregnant uterus in the dog is capable of synthesizing large amounts of prostaglandin E_2 (PGE_2)[1] which is a potent vasodepressor agent (2). Even though the uterine venous levels of PGE_2 were in the nanogram per milliliter range, the arterial PGE_2 levels were too low to detect. Because of the large capacity of the lungs to metabolize PGE_2, it is unlikely that PGE_2 could have a systemic role in controlling blood pressure from a distant organ like the pregnant uterus. However, prostacyclin (PGI_2), unlike PGE_2, travels through the lung intact making it a good candidate for a circulating depressor hormone (3).

The present study was undertaken to evaluate the capacity of PGI_2 production by the pregnant uterus in the dog and to study the ability of the pregnant uterus to inactivate PGI_2.

METHODS

(A). Eight mongrel dogs, weighing between 16–28 kg, in late pregnancy but before labor, were anesthetized using pentobarbital, 30 mg/kg, and placed on a positive pressure respirator pump. One femoral artery and one femoral vein were catheterized for blood pressure monitoring, and drug and fluid administration, respectively. Through a low abdominal incision, the pregnant uterus was exposed and one of the uterine veins was cannulated from a smaller venous branch. The right main uterine artery was identified and a noncannulating electromagnetic flow probe was placed around the artery to measure continuously uterine blood flow via a flowmeter (Statham Instruments, Inc., Oxnard, Calif.). The uterine artery was then punctured with a curved 27-gauge needle connected to a polyethylene tube for the intrauterine infusion of PGI_2. Amniotic fluid pressure was continuously monitored on the right

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[1] *Abbreviations used in this paper:* 6-keto $PGF_{1\alpha}$, 6-keto prostaglandin $F_{1\alpha}$; PGE_2, prostaglandin E_2; PGI_2, prostacyclin.

side through a floating catheter in the amniotic sac attached to a pressure transducer. After the surgery, we allowed ~45 min for stabilization.

In five of the animals 30 ml of uterine venous and 30 ml of arterial blood were withdrawn into plastic syringes containing 0.1 vol of 3.8% sodium citrate and 1 mM indomethacin for the analysis of the plasma for 6-keto $PGF_{1\alpha}$, the acid hydrolysis product of PGI_2.

To evaluate the hemodynamic and uterine contractile effects of PGI_2, plus the ability of the pregnant uterus to inactivate PGI_2, we infused PGI_2 first into the uterine artery and then into the inferior vena cava at doses of 3, 10, 30, and 100 ng/kg per min. Mean arterial pressure, uterine blood flow, and intraamniotic fluid pressure were continuously monitored. Between doses of PGI_2, a 30-min stabilization period was introduced to allow all the hemodynamic parameters to return to base line.

(B). Four nonpregnant female dogs weighing between 18–25 kg were anesthetized with 30 mg/kg pentobarbital i.v. and placed on a positive pressure respirator pump. The femoral artery and vein were catheterized as with the pregnant dogs. Through a midabdominal incision the nonpregnant uterus was identified and manipulated to a similar degree as with the eight pregnant animals in section A. The incision was then closed and the animals were allowed to stabilize for 45 min. When the animals were hemodynamically stable, 30 ml of arterial blood was collected in plastic syringes containing 0.1 vol of 3.8% sodium citrate and 1 mM indomethacin for the subsequent analysis of the plasma for the measurement of 6-keto $PGF_{1\alpha}$.

(C). Analysis of 6-keto $PGF_{1\alpha}$ concentration in the plasma. 500 ng of tetradeutero 6-keto $PGF_{1\alpha}$ (a gift of Dr. John Pike, Upjohn Co., Kalamazoo, Mich.) was added to the plasma as the internal standard and 100,000 cpm of tritiated 6-keto $PGF_{1\alpha}$ (New England Nuclear, Boston, Mass.) was added to the plasma to facilitate the isolation and purification of the prostaglandin. The prostaglandin was extracted into 3 vol of ethyl acetate after acidification of the plasma to pH 3 with 1 N HCl. The extract was initially purified over an open silicic acid column and eluted with chloroform (96)/methanol (4)/acetic acid (0.1) (vol/vol/vol). The extract was further purified and separated by using two high pressure liquid chromatography steps using the fatty acid column and μPorasil columns (Waters Associates, Inc., Milford, Mass.) sequentially. The purified 6-keto $PGF_{1\alpha}$ was then derivatized to the methoxime, methyl ester, trimethylsilyl derivative.

The derivatized prostaglandin was analyzed by gas chromatography-mass spectrometer under electron impact conditions (70 eV). The gas chromatography was performed with a silanized glass column containing 3% OV 101 on Gas-Chrom 120/140 (Applied Science Labs., Inc., State College, Pa.) at an oven temperature of 240–250°C. The prostaglandin was analyzed on a Finnigan 3200 quadrupole mass spectrometer using selected ion monitoring under control of a Finnigan 6100 data system (Finnigan Corp., Sunnyvale, Calif.). The ion pairs (mass/energy) 598 and 602 were monitored for estimating the ratio of D_0/D_4 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) in the biologic samples. The principles and details of these procedures have been recently described by Green et al. (4). The detection limit for estimating 6-keto $PGF_{1\alpha}$ plasma concentrations using 15 ml of plasma was 200 pg/ml.

This method measures both PGI_2, which hydrolyzes to 6-keto $PGF_{1\alpha}$ during the work-up of the plasma, as well as preformed 6-keto $PGF_{1\alpha}$, which is in plasma at the time of sampling. In vivo, PGI_2 is thought to be metabolized predominately to 15-keto PGI_2 and then to 6,15 diketo $PGF_{1\alpha}$ and 6-keto $PGF_{1\alpha}$ is probably not a major in vivo product of PGI_2 (5, 6).

TABLE I

Arterial and Uterine Venous Concentrations of 6-keto $PGF_{1\alpha}$ in Pregnant and Nonpregnant Dogs

Pregnant		Nonpregnant
Arterial	Uterine vein	Arterial
ng/ml		
3.42	9.26	<0.2
1.46	1.77	<0.2
1.33	11.77	<0.2
1.28	2.74	<0.2
5.62	7.86	
2.62±0.85	6.68±1.92	

Measurement of 6-keto $PGF_{1\alpha}$ in plasma, therefore, most likely reflects primarily circulating PGI_2.

Statistics. A paired Student's t test was used to analyze the changes in uterine blood flow, uterine vascular resistance, intraamniotic fluid pressure, and mean arterial pressure secondary to intrauterine PGI_2 infusion. An unpaired Student's t test was used to compare the hypotensive effect of intrauterine PGI_2 infusion to intravenous PGI_2 infusion. A P value of <0.05 was considered significant.

RESULTS

In the five pregnant dogs, the mean uterine vein and arterial plasma concentrations of 6-keto $PGF_{1\alpha}$ were 6.68±1.92 and 2.62±0.85 ng/ml, respectively. In the nonpregnant female dogs, the arterial plasma concentrations of 6-keto $PGF_{1\alpha}$ were below the detection limit of our assay in all four of the animals (<200 pg/ml) (Table I).

Infusion of increasing concentrations of PGI_2 resulted in a progressive decrease in mean arterial pressure. There was no difference in the hypotensive response to PGI_2 whether the prostaglandin was infused intravenously or into the pregnant uterus (Fig. 1). This suggests that very little if any of the PGI_2 traveling through the uterus is metabolized to inactive compounds. Since PGI_2 is not inactivated by the lungs, PGI_2 produced by the pregnant uterus would be expected to travel to the systemic circulation intact. Our data showing high uterine venous as well as arterial plasma 6-keto $PGF_{1\alpha}$ concentrations are consistent with the lack of the lung metabolism of PGI_2. Although the metabolism of intrauterine-generated PGI_2 may be different than the metabolism of infused PGI_2, this difference has not been demonstrated for either PGI_2 or other prostaglandins. Using the above infusion technique we have previously shown that the liver is the major organ to inactivate PGI_2 (3).

The local hemodynamic effects of intrauterine infusion of PGI_2 were variable. Neither uterine blood flow
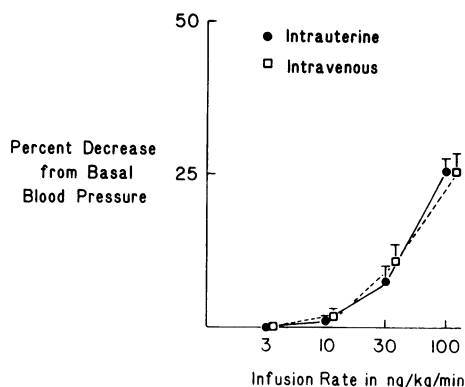
FIGURE 1 The systemic hypotensive effect of intrauterine and intravenous infusion of PGI_2. The abscissa represents increasing infusion rate of PGI_2 and the ordinate represents the percent decrease in mean arterial pressure from the control.

nor uterine vascular resistance were significantly altered when the intrauterine infusion of PGI_2 reached a steady state. In all of the animals, however, we could observe a transient increase in uterine blood flow at the start of the PGI_2 infusion, but by 3 min these changes were not significant. This is in contrast to the effects of PGI_2 on other vascular beds where the increases in organ blood flows were maintained for the duration of the PGI_2 infusion (7, 8). The hypotensive effect of the higher doses of PGI_2 was maintained for the duration of the PGI_2 infusion suggesting that the vascular resistance in organs other than the uterus remained below control levels.

The two highest doses of intrauterine PGI_2 infusion significantly increased intraamniotic fluid pressure,

suggesting a contractile effect of the prostaglandin on the pregnant myometrium (Table II). Whether this was a direct effect of PGI_2 or an indirect effect as a consequence of systemic hypotension was not elucidated. Since increased intrauterine pressure is associated with an increase in uterine vascular resistance (9), it is possible that the true vascular dilatory effect of PGI_2 was camouflaged by the increase in uterine vascular resistance as a consequence of the increased intrauterine pressure. This dynamic relationship between uterine blood flow and intrauterine pressure was well demonstrated in one of the dogs, where the intrauterine infusion of 100 ng/kg per min of PGI_2 caused a transient increase in blood flow followed by a marked decrease coincident with a large increase in intraamniotic fluid pressure (Fig. 2).

DISCUSSION

The role that prostaglandins play during pregnancy is unclear. Prostaglandins are thought to be important in maintaining a patent ductus in the fetus (10), in initiating labor (11), and maintaining some uterine myo-
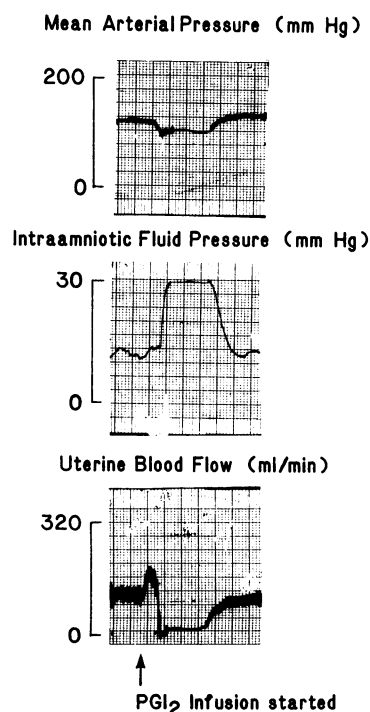

FIGURE 2 The effect of intrauterine PGI_2 infusion (100 ng/kg per min) on mean arterial pressure, intraamniotic fluid pressure, and uterine blood flow in one of the dogs that had an excessive response to PGI_2. The uterine blood flow shows a transient increase in flow followed by a sustained decrease concomitant with the large increase in intraamniotic fluid pressure. Each vertical line represents 2 min.

TABLE II
Hemodynamic Effects of Intrauterine PGI_2 Infusion

	Mean uterine blood flow	Uterine resistance	Intraamniotic pressure	Mean arterial pressure
ng/kg/min	ml/min	mm Hg/ml/min	mmHg	mmHg
Control	146±27	1.03±0.24	7.7±0.7	112±4
3	153±27	0.93±0.18	7.8±0.8	112±4
Control	150±26	1.00±0.23	7.9±0.8	113±5
10	150±25	0.93±0.17	8.6±1.0	111±4
Control	148±24	1.00±0.14	7.9±0.6	112±4
30	155±28	0.90±0.17	9.2±0.9*	103±5*
Control	153±25	0.96±0.17	7.9±0.6	116±5
100	103±25*	0.97±0.10	12.7±2.1*	83±4*

* $P < 0.05$ paired t test.

metrial tone (2, 12). Another possible role of prostaglandins in pregnancy is maintaining a low peripheral vascular resistance. For a prostaglandin to fit the role of a systemic vasodilator in pregnancy, the following criteria need to be met. The pregnant uterus has to have the capacity to make the prostaglandin, and the prostaglandin has to escape inactivation by both the uterus and the lung to appear in the arterial blood to exert an effect. We have previously demonstrated that PGE_2, a potent vasodilator, was produced by the pregnant uterus of the dog. Even though the mean uterine venous level of PGE_2 was found to be 1.6 ng/ml, the arterial level of PGE_2 was consistently not detectable suggesting extensive pulmonary metabolism. Our present data demonstrate that PGI_2, measured as the acid hydrolysis product of 6-keto $PGF_{1\alpha}$, is also produced in generous quantities by the pregnant uterus of the dog, but unlike PGE_2, recirculates to appear in the arterial blood. In addition, when PGI_2 was infused into the uterine artery, it proved to be as potent as vasodepressor agent as when it was infused intravenously, thus demonstrating that PGI_2 escaped inactivation by the pregnant uterus.

Although we were measuring 6-keto $PGF_{1\alpha}$ concentration in the plasma which is a composite of both intact PGI_2 (hydrolyzed to 6-keto $PGF_{1\alpha}$ ex vivo) and already present 6-keto $PGF_{1\alpha}$, the uterus does not synthesize 6-keto $PGF_{1\alpha}$ but produces PGI_2, which is then nonenzymatically hydrolyzed to 6-keto $PGF_{1\alpha}$. Thus, the uterine venous minus arterial concentration of 6-keto $PGF_{1\alpha}$ most likely represents net PGI_2 synthesis. Since the half-life of PGI_2 dissolved in blood is 3 min (13), and the lung does not metabolize PGI_2, a significant portion of the uterine synthesized PGI_2 could reach the arterial circulation unchanged. The reports that angiotensin II stimulation of PGI_2 synthesis in the canine lung (14) and kidney (15) results in a platelet effect also strongly argues in favor of PGI_2 being released intact from organs.

The hypothesis that PGI_2 may be an important circulating vasodepressor hormone in pregnancy is attractive from several viewpoints. Pregnancy is associated with a decline in blood pressure and peripheral vascular resistance, the mechanism of which is unclear. Indeed the mean blood pressure in our anesthetized, pregnant dogs was significantly lower than in the four anesthetized, nonpregnant controls (112 ± 4 vs. 141 ± 9 mm Hg; $P < 0.05$, unpaired t test). The possibility that circulating PGI_2 is partially responsible for the decrease in peripheral vascular resistance in pregnancy gains substance from our data. In addition, pregnancy is associated with elevated plasma renin activity and vascular angiotensin resistance (16). This vascular insensitivity to angiotensin is analogous to that seen in Bartter's syndrome, where prostaglandin inhibition can ameliorate the vascular defects of the syndrome (17). Thus, it is conceivable that the high concentration of circulating PGI_2 is responsible for both the elevated plasma renin activity as well as angiotensin insensitivity in pregnancy.

The actual amount of PGI_2 secreted by the pregnant uterus in the unanesthetized, uninstrumented dog could be several fold less than in the anesthetized and laparotomized animal. Both the stress of anesthesia and laparotomy has been shown to increase prostaglandin production by the kidney (18). Nonetheless, in the four nonpregnant dogs that also underwent anesthesia and laparotomy the arterial plasma concentrations of 6-keto $PGF_{1\alpha}$ were below the sensitivity of our assay suggesting that indeed the capacity of the pregnant uterus to make PGI_2 must be very high and that anesthesia, per se, cannot explain our results.

We can conclude from our data that PGI_2, measured as 6-keto $PGF_{1\alpha}$, is produced by the pregnant uterus in the dog and that the arterial concentration of 6-keto $PGF_{1\alpha}$ in pregnant animals is elevated above that of the nonpregnant dogs. In addition, although the pregnant uterus makes PGI_2, probably does not play any role in inactivating it. Thus, uterine PGI_2 is a likely mediator to contribute to the lowered systemic vascular resistance in pregnancy.

ACKNOWLEDGMENTS

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