Hemodynamic effects of platelet-activating factor in nonpregnant and pregnant sheep

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PLATELET-ACTIVATING FACTOR (PAF) is a low molecular weight phospholipid with a broad array of physiological actions. Classically, PAF has been regarded as mediator of inflammation that is produced by platelets, neutrophils, and macrophages. More recently, however, evidence has accumulated to show that PAF can have potent vasoactive effects and is synthesized by a number of nonimmune cell types such as endothelial (4), vascular smooth muscle (35), renal glomerular (7, 24), and amnion cells (10, 22). The effects of PAF on the vasculature appear to be somewhat complex in that this compound has demonstrated abilities as both a vasoconstrictor and vasodilator, seemingly dependent on dose, specific vascular bed, and species (2, 11, 16). In general, PAF appears to produce hypotension and decreased cardiac output; however, intrarenal infusion of PAF causes vasodilation in the rat kidney (1, 16, 33, 40).

Another important aspect of the actions of PAF is its ability to increase microvascular permeability, both systemically and within the kidney. In this regard, infusion of PAF is associated with both hemococoncentration and the development of proteinuria (18, 30). Although the mechanism for this effect is not entirely clear, it does appear to involve specific receptor-mediated events, because specific antagonists of PAF receptors have been shown to prevent PAF-induced protein extravasation across the systemic, pulmonary, and glomerular microvasculatures (6, 9, 26, 31).

Studies have shown that the production and metabolism of PAF is significantly influenced by changes in estrogen, progestins, and cytokines, all of which are central to the progression of pregnancy (36). This suggests that PAF may participate importantly in normal pregnancy as well as in certain complications of pregnancy. To begin to investigate the role of PAF in the regulation of cardiovascular hemodynamics in pregnancy, we sought to evaluate the actions of exogenously administered PAF on blood flow and vascular resistance as well as on microvascular permeability. The present studies were designed to assess the dose-related effects of PAF on systemic, renal, and uterine hemodynamics in nonpregnant sheep and to evaluate how pregnancy might alter these responses.

MATERIALS AND METHODS

Animals. Eight nonpregnant ewes (50–70 kg) and 13 pregnant ewes (110–115 days gestation) were purchased from Russell (Williamsburg, OH) and Morris (Reisterstown, MD), respectively. All animals were of similar mixed breed. Animals were housed in individual portable stainless steel cages in a temperature- and light-controlled, American Association for Accreditation of Laboratory Animal Care accredited facility with free access to food and water.

Surgical procedures. Animals were sedated with pentobarbital sodium (15 mg/kg iv), and anesthesia was maintained by ventilation with a mixture of 2–3% isoflurane and oxygen. Under sterile conditions, the right kidney was exposed by a small retroperitoneal flank incision, and a transit-time Doppler flow probe (Transonic Systems, Ithaca, NY) was positioned on the renal artery for subsequent monitoring of unilateral renal blood flow (RBF). After a 1-wk recovery period, ewes were again anesthetized for abdominal preparation. In nonpregnant sheep, chronic indwelling polyvinyl catheters were implanted in the femoral artery and vein and advanced to the level of the distal aorta and vena cava, respectively. Through a 15-cm lower abdominal incision, transit-time Doppler flow probes were placed bilaterally on the main uterine arteries for measurement of uterine blood flow (UBF). Nonpregnant ewes were ovariolectomized to control exposure to sex steroids. Pregnant sheep were similarly instrumented with maternal...
femoral artery and vein catheters as well as with flow probes on the uterine arteries. Additionally, catheters were placed in the fetal femoral artery and vein to monitor fetal blood gases for assessment of fetal well-being. A large-bore catheter was also placed in the amniotic sac of some pregnant animals to monitor intrauterine pressure. In all animals, catheters and flow-probe cables were exteriorized through the respective incision site, passed subcutaneously to the ewe's flank, placed in a cloth pouch, and secured to the ewe's side. A 1-wk recovery period was allowed after surgery in all animals before study.

Antibiotics (3 ml im, Combiotics, Hanford Manufacturing, Syracuse, NY) were administered on the day of and 3 days after surgery. Maternal catheters were flushed daily with sterile saline and filled with sodium heparin (1,000 USP units/ml, Elkins-Sinn; Cherry Hill, NJ) to maintain patency. Fetal catheters were flushed daily with bacteriostatic-free sterile saline and filled with sodium heparin (500 USP units/ml). Daily, fetal arterial blood samples were collected anaerobically into heparinized syringes and fetal blood gas values (PaO2, P aCO2, and pH) were determined with a blood-gas analyzer (model 288, Ciba-Corning Diagnostics; Norwood, MA) to verify fetal well-being. To prevent uterine atrophy, nonpregnant ovariotomized sheep received estradiol-benzoate (3 mg im, Combiotics, Hanford Manufacturing, Wood, MA) to verify fetal well-being. To prevent uterine atrophy, nonpregnant ovariotomized sheep received estradiol-benzoate (3 mg im, Combiotics, Hanford Manufacturing, Wood, MA) to verify fetal well-being. To prevent uterine atrophy, nonpregnant ovariotomized sheep received estradiol-benzoate (3 mg im, Combiotics, Hanford Manufacturing, Wood, MA) to verify fetal well-being.

Experimental protocol. After surgical recovery, baseline measurements were obtained over a 1-h equilibration period. PAF-C16 (Cayman Chemical; Ann Arbor, MI) was diluted into sterile isotonic saline containing 0.25% bovine serum albumin (Sigma Chemical; St. Louis, MO). Cumulative dose-response curves to PAF were then generated by systemic infusion at 10, 30, and 100 ng·kg⁻¹·min⁻¹ into the femoral vein. Infusion at each dose was maintained for a 15-min period. Mean arterial pressure (MAP), heart rate, and total UBF and RBF were recorded continuously throughout the control and experimental periods. In pregnant sheep, intra-amniotic pressure was measured in addition to the above parameters. At the end of the control period and at the end of each PAF dose infusion period, reflex urination was stimulated by gently stroking the perineum and urine samples were collected by clean catch and stored for determination of urinary protein excretion. Arterial blood samples were collected at these same time points, and hematocrit was recorded. Vehicle infusion as well as time controls were performed to confirm that there was no effect of these variables on baseline measurements.

Analytic methods. Vascular resistances [systemic, uterine (UVR), and renal (RVR)] were calculated as MAP divided by the respective blood flow rate. To measure urinary protein concentration, urine samples were extracted with 72% TCA and 0.15% sodium deoxycholate to precipitate proteins. Protein pellets were resuspended in 5% SDS. Urinary protein concentration was then determined by a modification of the Lowry method (Bio-Rad DC Protein Assay). Creatinine concentration for each urine sample was measured with a standard alkaline picrate colorimetric assay, and protein concentration was expressed per unit of creatinine to normalize for changes in glomerular filtration rate.

Statistical analysis. Results are presented as the group means ± SE. Multiple comparisons were conducted by two-way ANOVA for repeated measures. Mean differences were determined by the Student's-Newman-Keuls test. The 0.05 level of probability was utilized as the criterion of significance.

RESULTS

Both in nonpregnant and pregnant animals, short-term PAF infusion (15 min/dose) resulted in dose-dependent increases in MAP as is shown in Fig. 1. MAP tended to increase to a greater degree in nonpregnant compared with pregnant sheep. At the highest dose of PAF (100 ng·kg⁻¹·min⁻¹) the magnitude of this response became significantly different between the two groups, with MAP increasing 41 ± 9% from baseline (from 82 ± 5 to 115 ± 7 mmHg) in nonpregnant sheep compared with only 16 ± 8% from baseline (from 75 ± 2 to 87 ± 6 mmHg) in pregnant sheep.

PAF also caused significant vasoconstriction within the renal vasculature both in nonpregnant and pregnant sheep (Fig. 2). As illustrated, RVR increased in a dose-dependent manner in both groups. In nonpregnant sheep, RVR was significantly elevated from baseline for the two highest doses of PAF, whereas in pregnant sheep a significant effect was observed only at the highest dose. Although at the lower two doses of PAF, RVR tended to increase more in nonpregnant relative to pregnant animals, this difference was not statistically significant (P = 0.09 and 0.08, respectively). By the highest dose of PAF the magnitude of the response was nearly identical between the two groups. The dramatic increase in renal resistance observed at this dose (100 ng·kg⁻¹·min⁻¹) corresponded to only modest decreases in RBF (from 448 ± 5 to 403 ± 46 ml/min in nonpregnant ewes and from 466 ± 52 to 406 ± 75 ml/min in pregnant ewes). Thus, although the increase in vascular resistance in response to PAF indicates active vasoconstriction within the kidney, it would seem that the simultaneous increase in perfusion pressure afforded protection against a significant drop in RBF.

In the uterine vascular bed, an interesting dichotomy was observed with respect to the effects of PAF on vascular resistance and consequently on UBF. In nonpregnant animals, a marked dose-dependent decrease in UVR was observed in response to PAF infusion (Fig.
3), indicative of active vasodilation within the uterus. This was reflected by an increase in total UBF to ~3.5-fold over baseline by the highest PAF dose (from 21 ± 5 to 76 ± 15 ml/min; Fig. 4). In contrast to the response observed in nonpregnant ewes, pregnant sheep demonstrated a dose-dependent increase in UVR (and hence active constriction of the uterine vasculature) in response to PAF (Fig. 3). This corresponded to decreases in UBF by as much as 24 ± 7% below baseline by the highest dose of PAF (787 ± 65 to 637 ± 90 ml/min; Fig. 4). To address the possibility that this decrease in UBF was occurring secondary to contraction of the uterine smooth muscle, amniotic fluid pressure was measured in three animals as an index of intrauterine pressure. Amniotic fluid pressure remained quite stable throughout the PAF dose-response curve (range of 5 ± 1 to 8 ± 2 mmHg), thus indicating that the changes in UVR and UBF were not merely the result of uterine contraction.

Other hemodynamic effects of PAF included increases in microvascular permeability both in pregnant and nonpregnant sheep. At the systemic level this was suggested by an increase in hematocrit, which presumably resulted from protein and water leakage out of the vascular space, a previously documented effect of PAF (17). In nonpregnant sheep, hematocrit was significantly increased from baseline at 30 and 100 ng·kg⁻¹·min⁻¹ PAF, whereas in pregnant sheep the increase became significant at 100 ng·kg⁻¹·min⁻¹ PAF; at this highest PAF dose, hematocrit was elevated to a similar extent in both groups (nonpregnant 27 ± 1 to 33 ± 1%; pregnant 28 ± 1 to 33 ± 1%; Fig. 5). According to a mathematical derivation described by Van Beaumont (37), the percent difference between the original and final hematocrit ratios can be used in conjunction with a proportionality factor to estimate the percent change in plasma volume. Thus, assuming that red blood cell volume remained constant throughout the experiment, the increases in hematocrit reflect a significant decrease in plasma volume, which was estimated to drop by 16 ± 2% in pregnant and 17 ± 2% in nonpregnant sheep by the end of the experiment.
Increased protein permeability was also evident within the kidney, as urinary protein excretion was elevated in nonpregnant sheep in response to the highest dose of PAF (Fig. 6). In pregnant sheep, baseline urinary protein excretion was somewhat higher than that of nonpregnant animals, as expected. In this group urinary protein excretion also tended to increase with the highest PAF dose, although this was not a significant elevation over baseline. The PAF-induced proteinuria, although somewhat variable between animals, is noteworthy in that it was observed after only 15 min of PAF infusion. It is possible that longer periods of PAF infusion may have elicited a more pronounced and consistent response.

DISCUSSION

The present studies were conducted to evaluate the effects of exogenously administered platelet-activating factor (PAF) on vascular tone and permeability in nonpregnant sheep and to determine if pregnancy alters these effects. In both nonpregnant and pregnant sheep, PAF was found to increase MAP in a dose-dependent fashion. This effect was somewhat surprising, because reports of PAF administration in other species are typically associated with hypotensive responses (19, 38). It is possible that the elevation in pressure observed in the present study was transient as each dose of PAF was maintained for only 15 min; longer term infusions may show changes in pressure secondary to the PAF-induced decrease in intravascular volume or may invoke more complex secondary vasoactive mechanisms. It is not clear from the present studies whether mediation of the observed hypertension is carried out by an endothelial (or perhaps platelet derived) vasoconstrictor or whether it is a direct effect of PAF.

Compelling evidence for the ability of pregnancy to alter vascular responsiveness to PAF was found in the sheep uterus. In nonpregnant animals, PAF administration produced a very clear dose-dependent uterine vasodilation, whereas, in pregnant sheep, quite the opposite response was observed. It is possible that the uterine vasculature may release a vasodilator in response to PAF in the nonpregnant but not in the pregnant state. However, preliminary experiments showed that in nonpregnant sheep the decrease in UVR was not altered by pretreatment with either nitro-L-arginine methyl ester or indomethacin (13), suggesting that the PAF-induced vasodilation is not mediated by either nitric oxide or prostacyclin. In pregnant sheep, amniotic fluid pressure remained quite stable throughout the PAF dose-response curve, thus indicating that the observed increase in UVR and decreased UBF were not merely the result of uterine constriction but rather are reflective of active constriction of the uterine vasculature. One explanation for this is that pregnancy may upregulate receptor-mediated signaling mechanisms that specifically produce vasoconstriction. It is also possible that during pregnancy, PAF may modify its own actions by secondary stimulation of an endothelial vasoconstrictor within the uterine vasculature. This is of interest as it can be speculated that decreased capacity for compensatory endothelial cell vasodilator synthesis, as has been theorized to occur with pre-eclampsia, may amplify the ability of locally produced PAF to increase vascular tone and decrease blood flow in this bed. This is significant in that any mechanism that may potentially serve to decrease UBF during pregnancy could, over time, compromise placental perfusion and thereby reduce delivery of oxygen and nutrients to the developing fetus.

In the renal circulation, vasoconstriction was observed in both groups, as evidenced by progressively increased RVR and decreased RBF with each dose of PAF. These findings are consistent with those of Baer and Cagen (2), who found that equivalent doses of PAF infused directly into the renal artery in the dog constricted this vascular bed by ~15–20%. In that study, PAF-induced renal vasoconstriction appeared to be a direct, primary effect as it was not altered by blockade of suspected secondary mediators such as serotonin, angiotensin II, or alpha-adrenergic receptors, and was in fact potentiated by meclofenamate. This suggests that in addition to a direct effect on the vascular smooth muscle in the kidney, PAF may also stimulate the release of vasodilator prostaglandins, presumably from the endothelium.

To assess the effects of PAF on microvascular protein permeability, the present studies measured urinary protein excretion as a direct index of renal permeability and hematocrit as an indirect index of systemic permeability. The latter measurement must be somewhat cautiously interpreted because it is unknown whether PAF stimulates splenic contraction in the sheep, an effect that would independently increase hematocrit. However, it has been reported using direct measurement in the conscious sheep that PAF increases vascular leakage by significantly decreasing the protein reflection coefficient of the microvasculature (3). In addition, studies in rats and guinea pigs have shown similar increases in hematocrit in response to PAF administration (19). This can only reflect a decrease in
plasma volume because these species do not have the ability to alter hematocrit via splenic contraction. Thus it is assumed that our observed rise in hematocrit, although possibly augmented by splenic release of red blood cells, was at least in part secondary to an increase in microvascular permeability.

Given this assumption it was found that, consistent with reports in other species, PAF significantly increased both systemic and renal glomerular microvascular permeability in the sheep, and this effect was not markedly different during pregnancy. Enhancement of microvascular permeability is an action of PAF that has been described by others (20, 28), and it appears that other agents that have also been observed to enhance vascular permeability, such as endothelin-1, vascular endothelial growth factor, and angiotensin II, may in fact share PAF as a common mediator of this effect (9, 29, 34). In support of this, previous studies from this laboratory that reported increases in systemic and renal vascular permeability induced by infusion of endothelin-1 in pregnant sheep (15) also observed that endothelin administration produced significant increases in plasma PAF (14). In general, it is thought that the PAF-stimulated increase in vascular protein permeability arises from a direct effect on venular endothelial cell contraction and formation of gap junctions leading to protein extravasation (39). A similar phenomenon is thought to occur in the kidney, where the mechanism of the proteinuric effect of PAF appears to involve rearrangement of cytoskeleton in endothelial and/or epithelial cells, resulting in an alteration of the size-selective properties of the glomerular barrier (30). It has been shown in the kidney as well as in other vascular beds that PAF-induced protein leakage is a specific, receptor-mediated event that is not inhibited by antagonists of cyclooxygenase or lipoxygenase or by pretreatment with antioxidants (9, 28, 34).

On the basis of the effects of PAF on vascular tone and permeability, it has been speculated that elevated production of PAF in certain disease states may play an important role in the progression of pathological conditions. In particular, PAF has been investigated as a potential mediator of various experimental models of progressive proteinuric renal disease (12, 27, 31) as well as gentamicin-induced nephrotoxicity (8). Additionally, blockade of PAF receptors has been shown to significantly delay the onset of proteinuria and prolong survival in lupus autoimmune mice (25). PAF has also been implicated in the pathophysiology of neonatal necrotizing enterocolitis (5) as well as various pulmonary diseases such as asthma, chronic obstructive pulmonary disease, lung fibrosis, and adult respiratory distress syndrome (21). Finally, although PAF is thought to be involved in a number of normal reproductive processes including ovulation, implantation, and parturition, it has been suggested that alterations in PAF production and/or metabolism may contribute to complications of pregnancy such as preeclampsia and preterm labor (23, 36). That PAF may participate importantly in normal pregnancy as well as in certain complications of pregnancy has been suggested by studies that show that the production and metabolism of PAF is significantly influenced by changes in estrogen, progestins, and cytokines, all of which are central to the progression of pregnancy (36). These observations also suggest, however, the possibility that the pharmacokinetics of PAF may be altered during pregnancy, which may have contributed to some of the quantitative differences between pregnant and nonpregnant sheep in the present study, particularly at the lower doses.

In summary, the results of the present study show that systemic administration of PAF in pregnant sheep produces increased maternal MAP, renal and uterine vascular resistances, and urinary protein excretion. We also observed marked increases in hematocrit, indicative of reduced blood volume secondary to increased systemic microvascular protein permeability. These responses were similar in nonpregnant sheep, with the exception that UVR was decreased in the nonpregnant sheep (thus UBF was increased), whereas UVR was increased in the pregnant sheep, resulting in decreased UBF. This suggests that pregnancy alters the mechanism of action of PAF within the uterine vasculature in a way that can reduce UBF and thereby potentially compromise placental perfusion.

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