Hypoxia attenuates metabolism of platelet activating factor by fetal and newborn lamb lungs

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Abstract The metabolism of platelet activating factor (PAF) by lungs of near-term fetal and 5- to 9-day-old lambs was studied during normoxia and hypoxia at 37°C in 30 mM Tris buffer. PAF synthesis was studied in lung cytosol and membrane using 250 μM [^{3}H]acetyl CoA, 40 μM lyso-PAF, and 50 μg protein. PAF catabolism was studied in lung homogenate (LH) using 50 μM [^{3}H]alkyl-PAF. PAF was extracted and assayed by thin-layer chromatography (TLC) and liquid scintillation spectrometry. Levels of PAF synthesized (nmol/min per mg protein) by fetal lung membrane versus cytosol were 1.35 ± 0.07 versus 0.61 ± 0.08, which were greater than those by newborn which were 0.33 ± 0.07 versus 0.17 ± 0.03. Hypoxia did not alter PAF synthesis by the lungs. PAF catabolism (nmol lyso-PAF/min per mg protein) by fetal LH was 0.07 ± 0.01, which increased to 0.24 ± 0.02 during normoxia. In newborn LH, the rate was 0.24 ± 0.04 and increased to 0.33 ± 0.01 during normoxia. PAF catabolism was higher in newborn than in fetal LH. An increase in pO_2 augmented PAF catabolism, more in fetal than in newborn LH. Thus rate of PAF synthesis decreases from fetus to newborn, but PAF catabolism increases from fetus to newborn. The higher rate of PAF synthesis coupled with a low rate of PAF catabolism in the hypoxic environment of fetal lungs may predispose the fetus to a high PAF level, which may contribute to the high basal vasomotor tone in fetal lungs. A fall in PAF level with oxygenation, due to increased PAF catabolism, may facilitate the normal fall in pulmonary vascular resistance at birth.—**Salva, A. M. L., B. O. Ibe, E. Cliborn, G. Reyes, and J. U. Raj.** Hypoxia attenuates metabolism of platelet activating factor by fetal and newborn lamb lungs. *J. Lipid Res.* 1996. **37:** 783–789.

Supplementary key words perinatal • acetyltransferase • acetylhydrolase • normoxia • hypoxia • postnatal • pulmonary circulation

The pulmonary circulation is modulated by some endogenous mediators such as eicosanoids (1, 2) endothelin (3, 4), nitric oxide (5), and platelet activating factor (PAF) (6), among others. Although PAF is a potent inflammatory mediator, it also possesses other biological properties (7). PAF is synthesized by many types of cells including platelets, lung epithelial and endothelial cells. After a stimulus, 1-O-alkyl-phospholipids released from membrane phospholipids are acted upon by phospholipase A_2 (PLA_2) to form lyso-PAF (8–10). The lyso-PAF so formed may be acted upon by acetyltransferase to form PAF. PAF synthesized in vivo is catabolized by acetylhydrolase, the PAF catabolic enzyme, to form lyso-PAF. Synthesis and catabolism of PAF are probably controlled by some intracellular or local tissue events (11, 12).

We have previously shown that PAF may play some role in the maintenance of vasomotor tone in both the pulmonary and systemic circulations in the perinatal period (13). This suggests that apart from being an inflammatory mediator, PAF may have some physiological function, especially in the perinatal pulmonary circulation. Circulating PAF level in a system will depend on both rate of PAF synthesis and catabolism. Therefore, we studied the synthesis and catabolism of PAF in ovine lungs during the perinatal period. As the fetal environment is hypoxic, changing from a pO_2 <50 torr in utero, to pO_2 ~100 torr postnatally, we also determined the effect of oxygen tension on PAF synthesis and catabolism. Our hypothesis is that a high rate of PAF synthesis in conjunction with a low rate of PAF catabolism in the hypoxic environment of fetal lung may sustain a high PAF level in the fetus due to an attenuation in PAF catabolism. The reduction in PAF catabolism in the hypoxic environment of the fetus may contribute to the high basal vasomotor tone in the fetal lung.

Abbreviations: PAF, platelet activating factor; 1-O-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine; [^{3}H]acetyl-C_{16}-PAF, hexadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine, 1-O-[acetyl-^{3}H(N)]-; [^{3}H]lyso-C_{16}-PAF, hexadecyl-*sn*-glyceryl-3-phosphorylcholine, 1-O-[alkyl-1′-,2′-^{3}H]-; [^{3}H]alkyl-C_{16}-PAF, hexadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine, 1-O-[hexadecyl-1′-,2′-^{3}H(N)]-; PMSF, phenylmethylsulfonyl fluoride; BSA, bovine serum albumin; TLC, thin-layer chromatography; 4-BPB, 4-bromophenacylbromide; DTT, dithiothreitol; PLA_2, phospholipase A_2; LH, lung homogenate; GSH, reduced glutathione.

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MATERIALS AND METHODS

Materials

Pregnant ewes (142–148 d gestation, term being 150 d) and newborn lambs, 5- to 9-d of age, were purchased from Nebekar Farms, Santa Monica, CA. Authentic standards of PAF: hexadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine, (C_{16}-PAF); hexadecyl-*sn*-glyceryl-3-phosphorylcholine, (lyso-C_{16}-PAF); octadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine, (C_{18}-PAF), were purchased from Biomol Research Laboratories (Plymouth Meeting, PA). Radiolabeled PAF standards and substrates were purchased from DuPont, New England Nuclear (Boston, MA). They are: hexadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine, 1-O-[acetyl-^{3}H(N)]-, (^{3}H-acetyl-C_{16}-PAF), 10 Ci/mmol (370 GBq/mmol); hexadecyl-*sn*-glyceryl-3-phosphorylcholine, 1-O-[alkyl-1′-,2′-^{3}H]-, (^{3}H-lyso-C_{16}-PAF), 60 Ci/mmol (2.2 TBq/mmol); hexadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine, 1-O-[hexadecyl-1′-,2′-^{3}H(N)]-, (^{3}H-alkyl-C_{16}-PAF), 60 Ci/mmol (2.2 Tbq/mmol); acetyl coenzyme A, [acetyl-^{3}H]-, ([^{3}H]acetyl CoA), 2.78 Ci/mmol (103 GBq/mmol). Phenylmethysulfonyl fluoride (PMSF) and bovine serum albumin (BSA) were purchased from Sigma Chemical Company (St. Louis, MO). Stock solution of the lipids was made in ethanol and stored at -70°C. Dilutions for use in each experiment were made fresh each day in the experimental buffers. Glass silica gel TLC plates were purchased from J. T. Baker (Phillipsburg, NJ). Ecolite(+) liquid scintillation cocktail and 4-bromophenacylbromide (4-BPB) were purchased from ICN Biochemicals (Irvine, CA). All other solvents and reagents were purchased from Fisher Scientific (Santa Clara, CA).

Subcellular fractionation of perinatal ovine lungs

Lung subcellular fractions of the near term lamb fetuses and the neonatal lambs were prepared as previously described (14). Briefly, lungs isolated from freshly killed fetal and newborn lambs were cannulated and perfused at 80 ml/min through the pulmonary artery cannula, with phosphate-buffered saline, pH 7.4, to wash the lung vasculature of blood components. Perfusion was continued until the effluent was clear of blood cells, as verified by light microscopy, and the lung appeared bleached. Washed lungs were then placed in Krebs' bicarbonate buffer, pH 7.4, containing (mM) NaCl 119, KCl 4.7, $MgSO_4$ 1.17, NaH_2CO_3 22.6, KH_2PO_4 1.18, $CaCl_2$ 1.6, and glucose 5.5. The lungs were sliced in the buffer and homogenized in 3 ml/g lung weight of 46 mM KH_2PO_4, pH 7.4, containing 0.1 mM PMSF. A portion of the lung homogenate was saved for metabolic studies. Microsome (membrane) and cytosol were prepared by differential centrifugation of the remainder of the lung homogenate at 9,000 *g* for 20 min followed by 100,000 *g* for 60 min. Membrane pellet was washed and resuspended in 30 mM Tris buffer, pH 7.5. Protein concentration of the lung homogenate, cytosol, and the membrane suspension was determined by the method of Lowry et al. (15), using crystalline bovine serum albumin (BSA) as the standard. Then aliquots (2.0 ml for lung homogenate and 0.5 ml for cytosol and membrane suspension) were transferred to polypropylene vials, frozen in liquid nitrogen and stored at -70°C until used for metabolic studies. Storage at this temperature did not affect the metabolic profile of the subcellular fraction being examined. However, thawed protein sample was discarded after use. Also, the low concentration of PMSF used in enzyme preparation did not affect the synthesis or catabolism of PAF by the lung homogenate, cytosol, and membrane compared to homogenates prepared without 0.1 mM PMSF.

Experimental protocol

Lung homogenate, cytosol, and membrane from each lung was studied at pO_2 ~100 torr (normoxia), and at pO_2 <50 torr (hypoxia). Before the addition of substrates for PAF synthesis or catabolism, incubation buffer was equilibrated in polypropylene tubes with air for normoxia and a gas mixture of 2% O_2, 10% CO_2 and balance nitrogen for hypoxia. After the desired oxygen tension (pO_2) has been determined using a blood-gas instrument, (Radiometer BMS3MK2PHM73, Copenhagen), synthesis or catabolism of PAF was studied as follows.

Normoxia

Duplicate samples of fetal and neonatal lung homogenate, cytosol or membrane were incubated at pO_2 ~100 torr.

Hypoxia

Duplicate samples of fetal and neonatal lung homogenate, cytosol or membrane were incubated at pO_2 <50 torr. All incubations were terminated by the addition of 1 ml of methanol to denature the protein.

Specific protocol for synthesis of PAF by lung cytosol and membrane

PAF biosynthesis by the perinatal lungs was studied in cytosol and membrane following published methods (16, 17) by measuring the activity of lyso-PAF:acetyl-CoA acetyltransferase (EC 2.3.1.67). The incubation buffer was 30 mM Tris, pH 7.4, containing 10 μM $CaCl_2$, 1 mM dithiothreitol (DTT), and 0.025% BSA. Fifty μg of fetal cytosol, neonatal cytosol, and membrane proteins were incubated (0.5 ml total volume) in normoxia and hypoxia with 40 μM of lyso-PAF and [^{3}H]acetyl CoA, 250 μM (2.78 Ci/mmol), for 15 min at 37°C in a constant

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temperature shaker bath. The substrates were solubilized in the incubation buffer. Incubations were terminated by adding 1 ml methanol. Control incubation was done as above without any added cytosolic or membrane protein.

Specific protocol for PAF catabolism by perinatal lungs

PAF catabolism was studied in lung homogenate, which we used instead of cytosol or membrane because our aim in this protocol was not to localize PAF catabolism in a specific lung subcellular fraction or catabolism by any specific lung cell type.

We first incubated different amounts of lung homogenate: 0.25, 0.5, 1.0, 2.0, and 5.0 mg of lung homogenate protein, using 1.5 μM [^{3}H]acetyl-PAF (10 Ci/mmol) in 30 mM Tris buffer, pH 7.5, containing 0.01% BSA (total volume, 2.0 ml) for 0, 15, and 60 min. From the results of these preliminary studies, we chose to use 1 mg lung homogenate/ml of incubation for each experiment. Thereafter, each incubation was done in a total volume of 2.0 ml of 30 mM Tris buffer, pH 7.5, containing 0.01% BSA, in a constant temperature shaker bath at 37°C in polypropylene plastic tubes following the method reported by Maki, Hoffman, and Johnston (18). The [^{3}H]acetyl-PAF was suspended in this Tris buffer.

All incubations were done with three sets (normoxia, hypoxia, and control) of duplicate samples of lung homogenate at various time periods, viz; 0, 5, 15, 30, and 60 min. Each sample contained 1.5 μM [^{3}H]acetyl-C_{16}-PAF and 0.01% BSA and was incubated as described under general protocols for incubations. Control incubations were done with lung homogenate protein denatured by boiling for 3 min and incubated during normoxia. Each incubation was terminated by adding 1 ml of methanol.

To study the rate of PAF catabolism, lung homogenate was incubated for 15 min with [^{3}H]alkyl-C_{16}-PAF (50 μM, 60 Ci/mmol), during normoxia and hypoxia, in 30 mM Tris buffer containing 0.01% BSA (18). Each incubation was terminated by addition of 1 ml methanol. [^{3}H]alkyl-C_{16}-PAF was used in this step of the PAF catabolic study because it allowed the direct measurement of both PAF and lyso-PAF from the thin-layer chromatography (TLC) plate. The effect of 4-bromophenacylbromide (4-BPB), an inhibitor of PLA_2, on the rate of PAF catabolism was also determined. Aliquots of lung homogenate were preincubated for 15 min during normoxia with 2 mM 4-BPB (19), after which incubations were continued as described above.

Extraction of PAF

Prior to extraction, 25 μg each of authentic PAF and lyso-PAF standards were added to each tube to aid in establishing PAF and lyso-PAF bands on TLC. Then PAF and lyso-PAF were extracted from the incubation media by the method of Bligh and Dyer (20). After addition of 1 vol methanol and 2 vol chloroform, the mixture was vortexed and then centrifuged at 3000 rpm for 15 min to maximize phase separation. The chloroform layer was carefully removed with Pasteur pipettes into clean polypropylene test tubes and dried under a stream of nitrogen.

Thin-layer chromatography (TLC) of PAF

Dried residue was resuspended in 200 μl chloroform and then 10 μl of each sample was spotted on silica gel G-plates and developed in a solvent mixture containing chloroform–methanol–water 65:35:5 against authentic radiolabeled and nonradiolabeled PAF and lyso-PAF. TLC spots were visualized under iodine vapor. The TLC spot corresponding in R_f values with the standard PAF or lyso-PAF was scraped off into a scintillation vial, 4 ml of Ecolite(+) was added to the sample, and radioactivity measured by scintillation spectrometry (Tricarb, Nuclear Chicago).

Data analysis

Data are mean ± SEM. Data from PAF synthesis by lungs are presented as nmol PAF/min per mg protein. Data from time-course of PAF catabolism are presented as %PAF radioactivity, i.e., the percent of the total radioactivity used at each time point that co-migrated with authentic PAF standard on the TLC chromatogram. Data on acetylhydrolase activity are normalized to protein concentration and expressed as nmol lyso-PAF/min per mg protein. All data were subjected to statistical analysis using a two-tailed Student's *t*-test to discover differences between the groups. For example, to test for differences in PAF synthesis or PAF catabolism between normoxia and hypoxia, a *t*-test was used. To test the trend in PAF catabolism during normoxia and hypoxia a linear correlation analysis was used. For multiple comparisons to detect differences among more than two experimental groups, for example, data during normoxia, hypoxia, and control in fetus and newborn, an analysis of variance (ANOVA) with Tukey test was used. A P value < 0.05 was accepted as statistically significant difference.

RESULTS

Synthesis of PAF by cytosol and membrane

Figure 1 shows amount of PAF synthesized by fetal (panel A) and newborn (panel B) lamb lung cytosol and membrane during normoxia and hypoxia. During normoxia, synthesis of PAF by fetal lamb lung membrane

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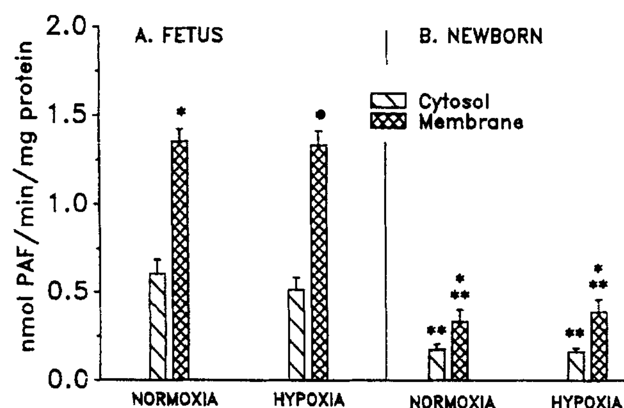

Fig. 1. PAF synthesized by lung cytosol and membrane of near term fetal lambs (panel A) and newborn lambs (panel B) during normoxia and hypoxia. Data are means ± SEM, n = 10 different lungs. Data were analyzed statistically using a Student's *t*-test. An ANOVA was used to compare sets of data (e.g., cytosol: normoxia and hypoxia) from the fetus with those of the newborn. Hypoxia did not alter the amount of PAF synthesized by cytosol or membrane of fetal or newborn lambs. However, newborn lamb lung cytosol and membrane synthesized less PAF than those of the fetuses. $^*P < 0.05$, different from cytosol; $^{**}P < 0.05$, different from synthesis by fetal lamb lung cytosol and membrane.

(1.35 ± 0.07) was significantly greater than the synthesis by cytosol (0.61 ± 0.08). Hypoxia did not change the amount of PAF synthesized by the membrane (1.33 ± 0.08) or cytosol (0.51 ± 0.07). However, PAF synthesis by fetal lung membrane was still higher than the synthesis by cytosol.

The amount of PAF synthesized by newborn lamb lung cytosol and membrane during normoxia and hypoxia is also shown in Fig. 1 (panel B). During normoxia, synthesis of PAF by the lung membrane (0.33 ± 0.07) was significantly greater than the synthesis by cytosol (0.17 ± 0.03). As was observed in the fetal lung, hypoxia did not change the amount of PAF synthesized by the newborn lamb lung membrane (0.39 ± 0.07) or cytosol (0.16 ± 0.02). However, during hypoxia, PAF synthesis by newborn lung membrane was still higher than the synthesis by cytosol. In addition, the amount of PAF synthesized by newborn lamb lung cytosol or membrane was significantly less than the amount synthesized by the cytosol or membrane of the fetal lamb lungs.

Catabolism of PAF by lung homogenate

Figure 2 shows the profile of catabolism of [^{3}H]acetyl-C_{16}-PAF by fetal lamb lung homogenate during normoxia and hypoxia. During normoxia, the catabolism of PAF increased with duration of incubation. Catabolism of PAF at 5 min was significantly different from that at 0 min. By 30 min of incubation, 0.086 ± 0.004 nmol (about 40%) of PAF had been metabolized into lyso-PAF which is significantly different from the amount at 60 min which was 0.238 ± 0.02 nmol (about 60%), thus indicating a time-dependent catabolism of PAF.

During hypoxia, catabolism of PAF into lyso-PAF by the lung homogenate was not significant until 30 min, at which point 0.012 ± 0.001 nmol (about 19%) of PAF was catabolized. At all time points, catabolism of PAF, by the lung homogenate, during hypoxia was significantly lower than that during normoxia. The slope of PAF catabolism during hypoxia was significantly different from that during normoxia. Denaturing the lung homogenate by boiling significantly decreased PAF catabolism to background radioactivity.

Figure 3 shows the profile of catabolism of [^{3}H]acetyl-C_{16}-PAF by lung homogenate of neonatal lamb during normoxia and hypoxia. During normoxia, catabolism of PAF increased with duration of incubation. Catabolism of PAF by newborn lamb lung homogenate was significantly different from that of fetal lung homogenate at 5 min and at all time points thereafter. By 30 min of incubation, 0.15 ± 0.02 nmol (50%) of PAF had been metabolized into lyso-PAF and by 60 min, 0.39 ± 0.06 nmol (about 65%) was metabolized. The catabolism of PAF by acetylhydrolase in neonatal lamb lung homogenate showed a time-dependent profile, but at a relatively accelerated rate as compared to that by fetal lung homogenate.

During hypoxia, there was also a time-dependent catabolism of PAF. Catabolism of PAF after 5 min incubation was significantly different from that at 0 min and after 30 min incubation, 0.12 ± 0.01 nmol (40%) of PAF was catabolized. This is significantly greater than the amount catabolized by fetal lung homogenate under

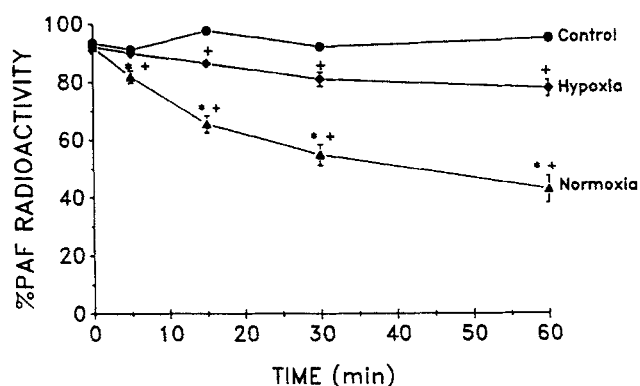

Fig. 2. Catabolism of [^{3}H]PAF by near term fetal lamb lung homogenate during normoxia and hypoxia. Data are means ± SEM, n = 6 different animal lungs for each time point. Data were analyzed statistically using a Student's *t*-test. An ANOVA was used to compare sets of data (e.g., normoxia and hypoxia) from the fetus with those of the newborn. The Y-axis represents the percent of the total PAF radioactivity, used in the study at each time point, that co-migrated with authentic nonradiolabeled PAF standard on the TLC chromatogram. Control experiments were done with lung homogenate denatured by boiling for 3 min. Catabolism of PAF was time-dependent during normoxia and hypoxia. Hypoxia attenuated PAF catabolism. $^+P < 0.05$, different from control and $^*P < 0.05$, different from hypoxia.

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the same conditions. At all time points, the catabolism of PAF during hypoxia was significantly lower than that during normoxia. Also, as in fetal lung homogenate, the slope of the profile during hypoxia was significantly different from that during normoxia. The catabolism of PAF by denatured neonatal lamb lung homogenate was significantly reduced to background radioactivity.

Figure 4 shows the rate of production of lyso-PAF from [^{3}H]alkyl-C_{16}-PAF by fetal and neonatal lamb lung homgenate during normoxia and hypoxia. In fetal lamb lung homogenate, the rate of production of lyso-PAF was increased by about 300% from 0.07 ± 0.01 nmol lyso-PAF/min per mg protein during hypoxia to 0.24 ± 0.02 nmol lyso-PAF/min per mg protein during normoxia. In neonatal lamb lung homogenate, the rate was 0.24 ± 0.04 nmol lyso-PAF/min per mg protein during hypoxia, which increased by 36% to 0.33 ± 0.01 nmol lyso-PAF/min per mg protein during normoxia. During hypoxia and normoxia, fetal lamb lung homogenate produced lyso-PAF at a slower rate than neonatal lamb lung homogenate during normoxia. The rates of catabolism of [^{3}H]alkyl-C_{16}-PAF by fetal and neonatal lamb lung homogenate were not altered by 4-BPB.

DISCUSSION

Among its wide ranging physiological as well as pathological effects in vivo, PAF is an important vasoactive mediator in the circulation. Due to its potent action on blood vessels, it has been implicated in some abnormalities of fetal and neonatal circulations, such as neonatal pulmonary hypertension (21, 22). However, its role in modulating normal fetal and neonatal circulations still needs further study. PAF may play a role in the maintenance of pulmonary and systemic vasomotor tone during the perinatal period (13). This suggests that apart from being a potent inflammatory mediator, or modulator of fetal lung development (17), PAF may perform some other beneficial functions in the circulation under physiologic conditions.

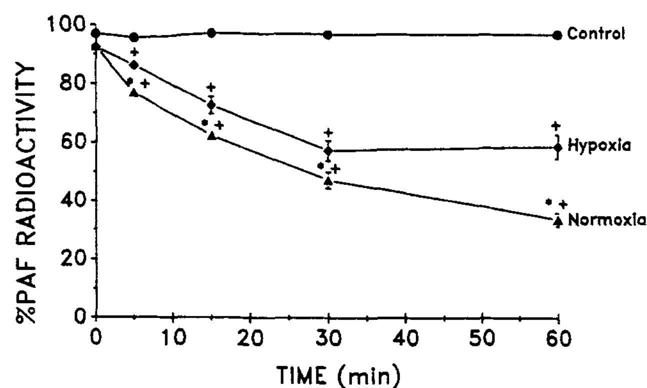

Fig. 3. Catabolism of [^{3}H]PAF by neonatal lamb lung homogenate during normoxia and hypoxia. Data are means ± SEM, n = 6 different animal lungs for each time point. Data were analyzed statistically using a Student's *t*-test. An ANOVA was used to compare sets of data (e.g., normoxia and hypoxia) from the fetus with those of the newborn. The Y-axis represents the percent of the total PAF radioactivity, used in the study at each time point, that co-migrated with authentic nonradiolabeled PAF standard on the TLC chromatogram. Control experiments were done with lung homogenate denatured by boiling for 3 min. Catabolism of PAF was time dependent during normoxia and hypoxia. Hypoxia attenuated PAF catabolism; $^+P < 0.05$, different from control; and $^*P < 0.05$, different from hypoxia.

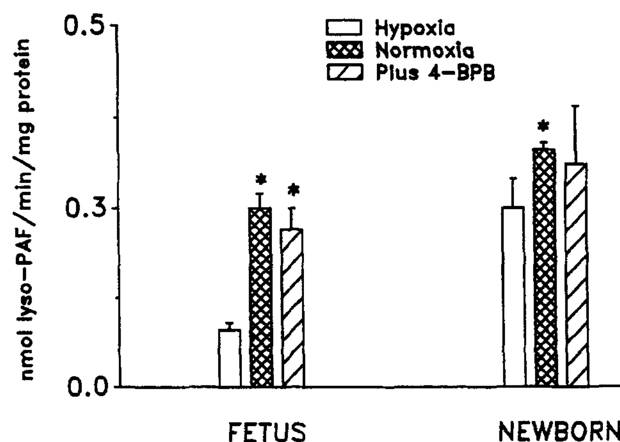

Fig. 4. Rate of [^{3}H]lyso-PAF production from [^{3}H]alkyl-C_{16}-PAF by fetal and neonatal lung homogenates during hypoxia and normoxia. Data are means ± SEM, n = 6 different animal lungs. Data were analyzed statistically using a Student's *t*-test. Lung homogenate was preincubated with 2 mM of 4-bromophenacylbromide (4-BPB) or the vehicle for 15 min, after which 50 µM of [^{3}H]alkyl-C_{16}-PAF was added to the system and incubation was continued for another 15 min. Media were extracted and assayed as described under Methods. Rate of [^{3}H]lyso-PAF production by fetal or neonatal lung homogenate was greatly increased during normoxia, although about ten-fold more in fetal than in neonatal lung homogenate. Addition of 2 mM 4-BPB did not alter the rate of PAF catabolism by fetal or newborn lamb lung homogenate; $^*P < 0.05$, different from hypoxia.

Circulating level of PAF in vivo will depend upon its biosynthesis by acetyltransferase and/or catabolism by acetylhydrolase. Thus, the level of PAF in a system will be high in situations where the synthesis of PAF is stimulated with a concomitant decrease in the catabolic process. In this study, we have investigated the synthesis and catabolism of PAF by perinatal ovine lungs. As the oxygen tension of the fetal environment is hypoxic and increases to normoxic conditions at birth, we have also studied the effect of hypoxia on the synthesis and catabolism of PAF by the lung.

PAF synthesis by perinatal lung cytosol and membrane

Fetal and neonatal lung cytosol and membrane synthesized PAF from [^{3}H]acetyl CoA. Lung membrane of each age synthesized more PAF than the cytosol suggesting that PAF acetyltransferase is more membrane bound

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than cytosolic. Our data also suggest that in ovine lungs, PAF acetyltransferase activity decreases from the fetus to the neonate, and the activity is insensitive to changes in oxygen tension. In rabbit lungs, acetyltransferase activity increases with the gestational age (17), but the activity during postnatal life was not reported. It is possible that in ovine lungs, acetyltransferase activity may increase with gestation, with peak activity at term, but decreases after birth. Our observation in this report that acetyltransferase activity is more membrane bound than cytosolic is similar to the observation in fetal rabbit lungs (16) and human fetal membranes (17) in which acetyltranferase activity was found to be more membrane bound than cytosolic.

PAF catabolism by perinatal lung homogenate

Both fetal and neonatal lung homogenates catabolized PAF in a time-dependent manner. We found very striking differences in the catabolism of PAF by fetal and neonatal sheep lungs at $pO_2 \sim 100$ torr and $pO_2 < 50$ torr. Hypoxia attenuated the kinetics of lyso-PAF production in fetal lung homogenate by 336%, but only by 36% in neonatal lung homogenate. As the fetal environment is hypoxic, it would seem that with a greatly retarded rate of PAF catabolism by acetylhydrolase, the level of PAF in fetal pulmonary circulation will be high. Comparison of slopes of PAF catabolism by fetal and neonatal lung homogenates showed a significant difference ($P < 0.05$) in the catabolic kinetics between hypoxia and normoxia. In the neonate, hypoxia will also lead to a higher PAF level in the pulmonary circulation, but to a lesser degree than in the case of the fetus.

In cultures of human fetal lung explants (17), acetylhydrolase activity was reported to be between 0.4 and 0.8 nmol lyso-PAF/min per mg protein. However, in fractionated human amniotic tissue, the values were 0.16–1.33, but 0.53–1.02 in the crude homogenate (16). Our values for acetylhydrolase activity in fetal lamb lung homogenate during hypoxia were significantly lower than these reported values (16). On the other hand, our values in fetal and neonatal lamb lung homogenate during normoxia are within these reported values. It seems that the difference in rate of PAF catabolism between normoxia and hypoxia in the two age groups may be related to the function of PAF in the respective systems. In the ovine fetal lung, a lower acetylhydrolase activity may be needed to maintain a high PAF level necessary to sustain the high pulmonary vascular resistance in fetal lungs whereas a faster catabolic rate after birth is necessary to sustain a low pulmonary vascular resistance.

Hypoxia decreases phospholipase A_2 (PLA_2) activity in rat ventricular myocytes (23), although in rat lung alveoli it was suggested that accumulation of lyso-PAF during hypoxia may be due to enhanced PLA_2 activity (24). The action of PLA_2 at the *sn*-2 position of phospholipids is preferential for long chain fatty acids, but some deacetylation by PLA_2 may also occur. In our study, when we tested for the possible contribution of PLA_2 to the observed PAF catabolism by treatment of the lung homogenates with 2 mM 4-BPB, a PLA_2 inhibitor, during incubation, there was no affect on the rate of lyso-PAF production. Therefore, it is unlikely that the catabolism of [^{3}H]PAF by lung homogenate is due to the action of PLA_2.

The lung is composed of numerous cell types (25) including endothelial cells, macrophages, and epithelial cells. In endothelial cells cultured from pulmonary arteries, oxygen tension did not alter the profile of PAF synthesis (26), and no simultaneous production of lyso-PAF was observed, suggesting that PAF is not being catabolized by acetylhydrolase or PLA_2 during synthesis. Indeed, further studies are needed to elucidate the effect of hypoxia on the PAF catabolic enzyme, acetylhydrolase. However, we can speculate that hypoxia may act to inhibit lung PAF acetylhydrolase by causing some structural modification of the enzyme via hydrophobic effects. One other reason could be that hypoxia may enhance the depletion of endogenous antioxidants which may be necessary in the activation of the enzyme active site by serving as proton donor and/or acceptor. Reduced glutathione (GSH) is one such endogenous antioxidant which may be depleted by hypoxia (27) by promoting the formation of the oxidized form, glutathione disulfide. The level of GSH in ovine fetal lung tissue has been shown to be significantly lower than the levels in newborn and adult lung tissues (14). Thus, if GSH were a necessary proton shuttle in acetylhydrolase activity on PAF, any fractional decrease in the tissue level caused by hypoxic condition may reduce the catabolic activity of PAF acetylhydrolase. We can also infer that the effect of oxygen tension in the regulation of PAF level in the circulation probably depends on a complex interplay among the substrate (PAF), the environment of the system, and PAF metabolizing enzymes. Therefore, the use of lung homogenate rather than any specific cell type or lung subfraction affords a system relatively closer to the lung, as the objective of this study of PAF catabolism by the lung was not to characterize PAF catabolism by any specific lung cell or localize PAF catabolic enzyme to a subcellular fraction.

In summary, we have shown that acetyltransferase activity in ovine lungs is greater in membrane, decreases from fetus to the newborn and is unaffected by hypoxia. We have also shown that hypoxia greatly attenuates the rate of catabolism of PAF by fetal lamb lung homogenate, a 10-fold greater decrease in catabolism than in neonatal lamb lung homogenate. Our data demonstrate

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that a low acetylhydrolase activity in the hypoxic environment of fetal lamb lung may help maintain a high PAF level in the fetal pulmonary circulation. In addition, an increase in pO_2 results in an increase in the catabolism of PAF by acetylhydrolase that will lead to a rapid fall in PAF level in pulmonary circulation. Our data would suggest that the regulation of PAF level by acetylhydrolase activity in the lung may be important for the normal transition of the pulmonary circulation at birth.

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