DECREASED PULMONARY VASCULAR RESPONSIVENESS IN RATS RAISED ON AN ESSENTIAL FATTY ACID DEFICIENT DIET

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ABSTRACT

Leukotriene C_4 is produced during hypoxic pulmonary vasoconstriction and leukotriene inhibitors preferentially inhibit the hypoxic pressor response in rats. If lipoxygenase products are important in hypoxic vasoconstriction, then an animal deficient in arachidonic acid should have a blunted hypoxic pressor response. We investigated if vascular responsiveness was decreased in vascular rings and isolated perfused lungs from rats raised on an essential fatty acid deficient diet (EFAD) compared to rats raised on a normal diet. Rats raised on the EFAD diet had decreased esterified plasma arachidonic acid and increased 5-, 8-, 11- eicosatrienoic acid compared to rats raised on the normal diet (control). Compared to the time matched responses in control isolated perfused lungs the pressor responses to angiotensin II and alveolar hypoxia were blunted in lungs from the arachidonate deficient rats. This decreased pulmonary vascular responsiveness was not affected by the addition of indomethacin or arachidonic acid to the lung perfusate. Similarly, the pulmonary artery rings from

arachidonate deficient rats demonstrated decreased reactivity to norepinephrine compared to rings from control rats. In contrast, the tension increases to norepinephrine were greater in aortic rings from the arachidonate deficient rats compared to control. Stimulated lung tissue from the arachidonate deficient animals produced less slow reacting substance and platelet activating factor like material but the same amount of 6-keto-$PGF_{1\alpha}$ and TXB_2 compared to control lungs. Thus there is an association between altered vascular responsiveness and impairment of stimulated production of slow reacting substance and platelet activating factor like material in rats raised on an EFAD diet.

INTRODUCTION

Hypoxia causes vasoconstriction in the lung, but the mechanism by which it occurs remains unknown. One proposed mechanism is that alveolar hypoxia may elicit the release of a mediator substance from some lung cell. In the rat, leukotriene C_4 is produced during hypoxic vasoconstriction (1) and in rats, sheep, and newborn lambs, but not in piglets, leukotriene inhibitors preferentially inhibit the hypoxic pressor response (2,3,4,5). We hypothesized that if lipoxygenase products are important in hypoxic vasoconstriction, then an animal deficient in their substrate arachidonic acid should have a blunted hypoxic pressor response.

Our approach was to examine whether vascular responsiveness was depressed in isolated perfused lungs and vascular rings from rats raised on an arachidonate deficient diet compared to rats raised on a normal diet. In addition, we examined whether the alteration in vascular responsiveness correlated with the lung production of cyclooxygenase metabolites (TXB_2, 6-keto-$PGF_{1\alpha}$), lipoxygenase metabolites (slow-reacting substance-like material) and platelet activating factor-like material. The isolated perfused rat lung was used in part because in this system we could test whether the integrated pulmonary vascular response to alveolar hypoxia was preferentially inhibited in lungs from rats with a dietary deficiency of arachidonic acid. Vascular rings were used because in this system we could further assess if there was an alteration in pulmonary and/or systemic vascular reactivity secondary to the dietary deficiency of arachidonic acid.

METHODS

Lungs or vessel rings were isolated from male Sprague-Dawley rats fed ablibitum either on a normal diet (Rat Chow) or on a vitamin supplemented essential fatty acid deficient diet (EFAD) (ICN Biochemical, Cleveland). Rats were started on this diet 3 weeks after birth and continued for 4-8 months. Rats were anesthetized with pentobarbitol (10 mg/kg ip).

Preparation of vascular rings: The main pulmonary artery and thoracic aorta were removed (6) from rats raised on the normal and EFAD diet following the intracardiac injection of 500 units of heparin. After the vessels were cleaned of adherent fat and connective tissue, a ring 5 mm in width was separated from the remainder of the vessel. The vessel rings were connected to a Grass force transducer suspended in physiologic salt solution bubbled with 21% O_2 and 5% CO_2 (pH 7.35). Temperature was maintained at 37-40°C. The vascular rings were equilibrated for 1 hour at a resting tension of 2 g (aorta) or 1.5 g (pulmonary artery) prior to the initiation of any intervention. Following the equilibration period, the bath concentration of KCL was increased to 80 mM and the resultant vessel tension development was recorded until a stable plateau occurred. The chamber was then flushed with fresh solution until tension returned to baseline. This process was repeated at pretensions varying from 1 to 3 grams to determine the optimal pretension as judged by determining the maximal increase in tension to KCL. For a given ring all subsequent interventions were performed at this optimal pretension. In all rings, a dose-response to norepinephrine was established by increasing the bath concentration of norepinephrine (5×10^{-9} M to 5×10^{-6}M) and measuring the resultant increases in tension development.

Preparation of isolated lungs: Lungs were isolated and perfused as previously described (2). Lungs were perfused at constant flow (.03 ml/g rat body weight/min) with physiologic salt solution osmotically stabilized with 4 g/100 ml of Ficoll (7). Mean pulmonary artery inflow pressure was measured with a Statham transducer and recorded on a Grass recorder. Perfusion pressure was proportional to pulmonary vascular resistance since the flow for a given lung was held constant. The lungs were ventilated via a tracheal cannula at a rate of 60 strokes/minute by a pressure-cycled Harvard animal ventilator with a posi-

tive end-expiratory pressure of 1 cm H_2O. Lungs were ventilated with a normoxic (FIO_2 0.21) gas mixture containing 5% CO_2. The lungs and perfusate reservoir were kept at a temperature of 37-40°C. The lungs were perfused for 30 minutes to reach a stable perfusion pressure and temperature prior to initiation of the experimental protocols.

Three sequential series of pressor responses to intra-arterial angiotensin II followed by alveolar hypoxia were elicted. Five minutes were allowed for recovery or return of perfusion pressure to baseline between angiotensin II and hypoxic pressor responses. Pressor responses to angiotensin II were elicited by close arterial bolus injection of 0.05 ug dissolved in 50 ul of normal saline. Alveolar hypoxia was induced by ventilating the lung with a gas mixture of 0% O_2, 5% CO_2 and 95% N_2 for 4 minutes. The resultant lung effluent PO_2 was 46 ± 5 mmHg. The plateau of the pressor response to alveolar hypoxia occurred within the period of the hypoxic exposure. In a separate series of lungs from rats raised on the EFAD diet, indomethacin (5 ug/ml of perfusate), was added to the lung perfusate at the onset of lung perfusion to inhibit prostaglandin and thromboxane synthesis. Also in these lungs, twenty minutes prior to the third angiotensin II pressor response, arachidonic acid (1 ug, Nuchek) was also added to the lung perfusate as a bolus injection.

Measurement of arachidonate metabolites and platelet activating factor-like activity: To assess cyclooxygenase metabolite production in rats raised on the normal and EFAD diet, 2 ml of lung perfusate was collected from each isolated lung preparation before and after the first angiotensin II pressor response. The samples were placed in tubes containing 20 ug of indomethacin to prevent the formation of cyclooxygenase metabolites following sample collection. Samples were frozen until they were subsequently analyzed by radioimmunoassay (8) for 6-keto-$PGF_{1\alpha}$ and TXB_2.

Slow-reacting substance (SRS)-like activity and platelet activating factor (PAF) -like activity were assessed in lung tissue obtained from the isolated lung preparations used above. Following the last hypoxic pressor response, the lungs were weighed following separation from the heart and other attached structures. The lung tissue was chopped and then incubated in physiologic salt solution containing

calcium ionophore (10 uM) for 30 minutes. An ethanol precipitated supernatant was dried in a rotary evaporator, subjected to XAD-7 separation, and then resuspended in 2 ml of 30% methanol in water. Aliquots of this product were then used to bioassay for SRS-like myotropic activity and PAF-like activity.

Bioassay for SRS-like myotropic activity was performed using an isolated guinea pig ileum suspended in a 10 ml organ bath in the presence of atropine (10^{-6} M) and pyrilamine (10^{-6} M) as previously described (1,9). Contractions were considered to be indicative of SRS-like myotropic activity if they met the following criteria: slow onset, prolonged duration, dose-dependence, and reversible by the leukotriene receptor antagonist FPL 55712 (9). In each case the assay organ was responsive to synthetic leukotriene C_4 (1 ng) before and after sample testing.

Bioassay for PAF-like activity was carried out after passing the sample through a silicic acid column. Briefly, following the last step of a Bligh-Dyer extraction (10), the sample was loaded onto a silicic acid column in a 95:5 chloroform methanol mixture. The column was then sequentially washed with various mixtures of solvents. The effluent from washes with 95:5 and 80:20 chloroform: methanol mixtures and 100% methanol were discarded. The effluents from subsequent washes with 70:30 methanol:water were collected and subjected to a second Bligh-Dyer lipid extraction. The final sample was resuspended in Krebs-Ringer phosphate dextrose buffer with 0.25% nonpyrogenic human serum albumin (Miles Scientific). Aliquots of this final sample were used for the PAF assay.

Bioassay for PAF was performed by measuring the release of H3-serotonin from rabbit platelets as previously described (11). Briefly, blood was collected from the central ear artery of New Zealand white rabbits into a 1:7 volume of acid citrate dextrose and centrifuged at 300 x g for 20 minutes. A pool of the resultant platelet-rich plasma was then incubated for 30 minutes with 1 uCi of H3-serotonin binoxalate (New England Nuclear, Boston, MA) at 37°C. The platelets were sedimented at 2500 x g for 15 minutes and washed in Tyrode's gelatin without calcium in the presence of 0.1 mM ethyleneglycoltetraacetic acid (EGTA). The platelets were again pelleted and washed in Tyrode's gelatin without calcium (no EGTA). After a final centrifugation, the platelets were resuspended in

Tyrode's gelatin without calcium to a concentration of 1.25×10^9 (determined by absorbance at 530 nm). Each reaction tube contained various dilutions of the sample to be assayed in 450 ul volume of Tyrode's gelatin with calcium to which 50 ul of the platelet preparation was added. After 90 seconds of incubation at room temperature, the reaction was stopped by the addition of 20 ul of 9% formaldehyde. Following centrifugation at 2500 x g for 15 minutes, 100 ul of the supernatant was removed into 3 ml of scintillant (Scintiverse II, Fisher Scientific). The samples were counted for radioactivity in a liquid scintillation counter (model 8000, Bechman Instruments, Fullerton, CA). The reported cpms were converted to dpms using the H number and a quench curve generated specifically for the scintillant, vials and buffer employed in this assay. The platelets in one reaction tube containing only buffer and platelets were lysed by Triton X-100 and were counted to determine the maximal radioactivity in this batch of platelets. The bioactivity in each sample was expressed as a percentage of this maximal radioactivity. The bioactivity in each sample was reported in ng-equivalent of PAF after comparison with a standard curve of serotonin-release elicited by known amounts of synthetic hexadecyl PAF (Bachem Chemicals, Bubendorf, Switzerland). In four samples, addition of a known amount of synthetic PAF prior to the purification process resulted in recovery rates ranging from 85 to 95%. Thus, values for PAF are here reported without correction for recovery.

Polyenoic fatty acid analysis: Ten ul of rat plasma was transesterified to the methyl esters by heating in methanol (8% in HCl) at 60°C for one hour. The methyl esters were then analyzed by reverse-phase high performance liquid chromatography utilizing an IBM Instruments Company C-18 column, with a mobile phase of 10% methanol in water. The flow rate was 1 ml/min and the eluate was monitored for absorption at 214 nm. Arachidonic (20:4), and 5-,8-, 11- eicosatrienoic (20:3, Mead Acid) eluted at 18.3 and 27.1 minutes respectively. Arachidonic acid and Mead acid were quantitated externally by comparison to standard curves (12). Arachidonic acid and Mead acid were expressed as ng/ul of plasma. The analyses were in complete agreement with gas chromatographic analysis.

Statistics Values are expressed as mean ± SEM. Paired t-tests were used when responses in the same lung were compared. One-way analysis of variance was

used when multiple groups were compared. Multiple comparisons were made by the Student-Newman-Keul test. Dose response curves to norepinephrine were compared by two factor fixed effect two way analysis of variance concluding with contrasts. A $p < 0.05$ was accepted as significant.

RESULTS

Effect of EFAD diet on rat body weight and plasma polyenoic fatty acid composition: Rats raised on the EFAD diet (n = 16) tended to have lower body weights (384 ± 25 g) compared to rats raised on the normal diet (453 ± 33 g, n = 8, p = 0.11). Plasma from rats raised on the EFAD diet (n = 14) had 16 ± 3% (54 ± 12 ng/ul) of the mean amount of arachidonic acid found in the plasma of rats raised on the normal diet (336 ± 55 ng/ul; n = 7). In contrast, 5-, 8-, 11-eicosatrienoate was present in the plasma (660 ± 80 ng/ul) of the rats raised on the EFAD diet but was below (< 1 ng/ul) the level of detection in plasma from rats raised on the normal diet.

Effect of EFAD diet on pulmonary vascular responsiveness in isolated perfused lungs: Baseline mean pulmonary artery perfusion (PA) pressure was not different (p = NS) in lungs from rats raised on the EFAD diet (6.5 ± 0.6, n=12) compared to lungs from rats raised on a normal diet (5.5 ± 0.6, n=4). The second hypoxic challenge caused PA pressure to increase 7.9 ± 0.6 mmHg in lungs from rats raised on the normal diet. The magnitude of the time-matched pressor response to alveolar hypoxia was less ($p < 0.05$) in lungs from rats raised on the EFAD diet (Table 1). This inhibition of the hypoxic pressor response in lungs from rats raised on the EFAD diet was not reversed by adding either indomethacin (5 ug/ml of perfusate) or indomethacin plus arachidonic acid (1 ug) to the lung perfusate approximately 45 minutes prior to the hypoxic challenge (Table 1).

Compared to the time-matched response in control lungs, the magnitude of the PA pressor response to angiotensin II (0.05 ug) was less ($p < 0.05$) in lungs from rats raised on the EFAD diet (Table 1). As with the hypoxic pressor response, the inhibition of the angiotensin II pressor response in lungs from rats raised on the EFAD diet was not reversed by adding either indomethacin or indomethacin plus arachidonic acid to the lung perfusate approximately 40 minutes

prior to eliciting a pressor response to angiotensin II (Table 1).

TABLE 1

$\overline{PA}$ PRESSOR RESPONSES IN ISOLATED PERFUSED LUNGS FROM CONTROL AND ARACHIDONATE-DEFICIENT RATS

Experiment	n[a]	$\overline{PA}$ Pressor Responses (mmHg) Alveolar Hypoxia	Angiotensin II
Control (Normal Diet)	4	7.9 ± 0.6	8.8 ± 0.8
Arachidonate Deficient	8	4.3 ± 0.7*[b]	4.0 ± 0.9*
Arachidonate Deficient + Indomethacin	4	5.0 ± 0.6*	3.4± 1.2*
Arachidonate Deficient + Indomethacin + Arachidonic Acid	4	5.5 ± 0.5*	3.3 ± 0.9*

a n = number of animals
b * = $p < 0.05$ compared to control

Effect of an EFAD diet on pulmonary and systemic vascular reactivity in vessel rings: The maximum increase in tension to 80 mM KCl tended to be less ($p = 0.08$) in the pulmonary artery rings from rats raised on an EFAD diet (0.9 ± 0.2 g, n=4) compared to controls (1.6 ± 0.2 g, n=4). A dose-response curve to norepinephrine showed decreased reactivity ($p < 0.05$) in the pulmonary artery rings from the rats raised on the EFAD diet (Figures 1 and 2). In contrast, reactivity to norepinephrine (Figure 2) was increased ($p < 0.05$) in aortic rings from rats raised on the EFAD diet compared to controls.

Effects of EFAD diet on lipid mediator production: Typical SRS-like myotropic activity was present in calcium ionophore stimulated lung homogenate in all of the lungs tested (n = 4) from rats raised on the normal diet. A typical response is shown in Figure 3. For each lung homogenate from rats raised on the

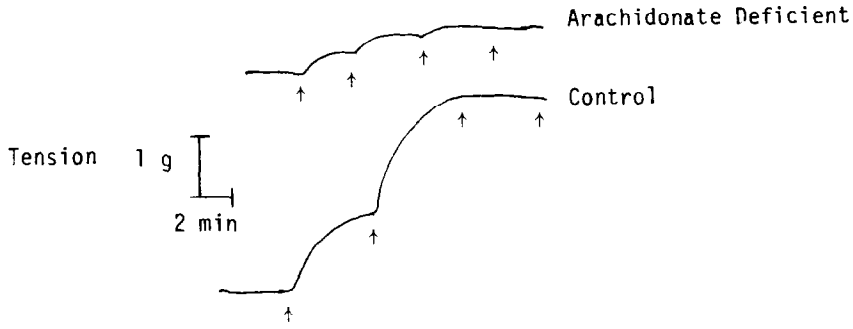

Figure 1: Typical tracing showing increases in tension development by pulmonary artery rings from rats raised on the essential fatty acid deficient (top tracing) and normal (bottom tracing) diet in response to norepinephrine. Upward arrows indicate an increase in bath concentration of norepinephrine to 5×10^{-9}, 5×10^{-8}, 5×10^{-7} and 10^{-6} M, respectively.

normal diet, 25 ul of the lung homogenate (total sample 2 ml) produced SRS-like contractions on the guinea pig ileum (Figure 4). In contrast, in none of the rats raised on the EFAD diet did 25 ul of the lung homogenate (total sample 2 ml) produce SRS-like contractions on the guinea pig ileum (Figure 4). However, larger aliquots from lung homogenates from rats raised on the EFAD diet did demonstrate SRS-like myotropic activity in six of the eight lung homogenates tested (Figure 4).

Platelet activating factor-like bioactivity was also decreased ($p < 0.05$) in calcium ionophore stimulated lung homogenate from rats raised on the EFAD diet (28 ± 6 ng/g lung, $n = 8$) compared to lung homogenate from rats raised on the normal diet (65 ± 9 ng/g lung, $n = 4$).

Baseline levels of 6-keto-$PGF_{1\alpha}$ and TXB_2, as well as the respective levels following an angiotensin II pressor response were not different ($p = NS$) in lung

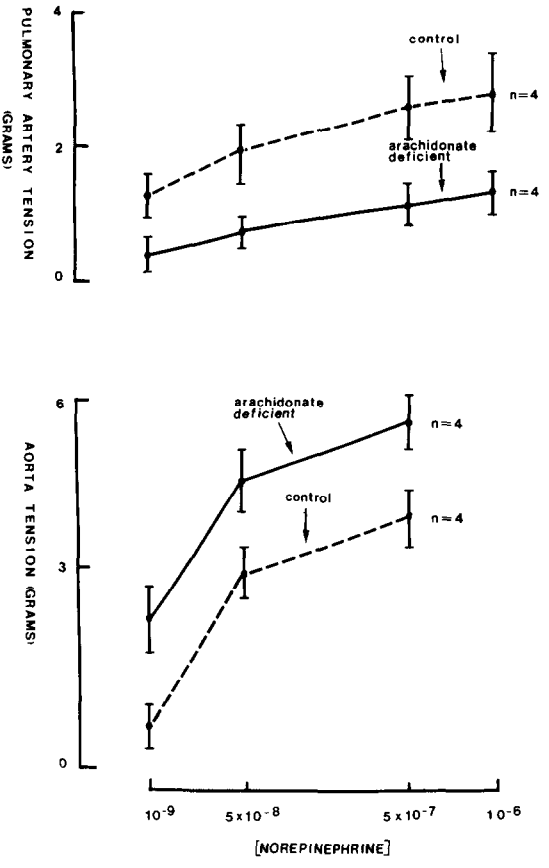

Figure 2: Tension development in response to norepinephrine in vascular rings from rats raised on the normal (control) and essential fatty acid-deficient diet.

effluent from lungs isolated from rats raised on the normal or EFAD diet (Table 2). Addition of indomethacin to the lung perfusate of lungs from rats raised on the EFAD diet did inhibit ($p < 0.05$) the production of 6-keto-$PGF_{1\alpha}$ and TXB_2 (Table 2). In these same lungs, and as expected, the addition of arachidonic acid to the lung perfusate did not reverse the inhibition of the production of 6-keto-$PGF_{1\alpha}$ and TXB_2 caused by indomethacin.

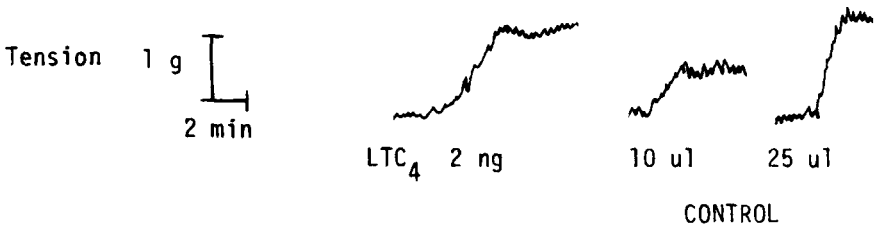

Figure 3: Contractile responses of the guinea pig ileum to exogenous LTC_4 and to material extracted from whole lung homogenate stimulated by calcium ionophore from a rat raised on a normal diet. Time scale and magnitude of contraction are shown.

TABLE 2

CYCLOOXYGENASE METABOLITE PRODUCTION IN EFFLUENT FROM ISOLATED LUNGS IN RESPONSE TO ANGIOTENSIN II

DIET	INTERVENTION	n[a]	6-Keto-$PGF_{1\alpha}$ (ng/ml)	TXB_2 (pg/ml)
CONTROL[b]	-------	4	4.7 ± 1.7[d]	194 ± 82[e]
EFAD[c]	NONE	8	7.2 ± 2.4[d]	122 ± 33[e]
EFAD	INDOMETHACIN	4	0.2 ± 0.07*[f]	10 ± 3*
EFAD	INDOMETHACIN + ARACHIDONIC ACID	4	0.1 ± 0.01*	13 ± 3*

a n = number of rats.
b = Control refers to rats raised on a normal diet.
c = EFAD refers to rats raised on the essential fatty acid deficient diet.
d - Baseline levels for the control and EFAD groups were 5.4 ± 2.0 ng/ml and 8.2 ± 2.8 ng/ml respectively.
e - Baseline levels for the control and EFAD groups were 77 ± 26 and 92 ± 24 respectively.
f * = $p < 0.05$ compared to control

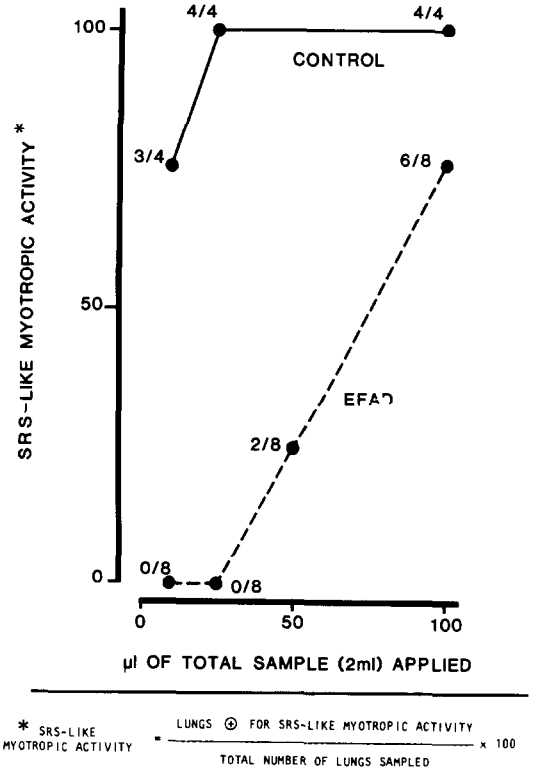

Figure 4: Amount of lung homogenate extract from rats raised on the normal (control) and essential fatty acid-deficient (EFAD) diet required to produce SRS-like myotropic activity on the guinea pig ileum. The homogenates were stimulated with calcium ionophore. Numbers above data points indicate number of lung homogenates demonstrating SRS-like myotropic activity (number above diagonal line) and total number of lung homogenates tested (number below diagonal line).

DISCUSSION

In this study we demonstrated in plasma from rats raised on the EFAD diet that there was a decreased level of arachidonic acid, the precursor for the pro-

duction of prostaglandins, thromboxane, and leukotrienes. As expected, in essential fatty acid deficiency (13) an increased level of 5-, 8-, 11- eicosatrienoic acid and a tendency for decreased weight gain were observed.

The main finding of this study was that both hypoxic pulmonary vasoconstriction and calcium ionophore stimulated lung production of SRS-like material were inhibited in lungs isolated from rats with decreased plasma levels of arachidonic acid. This inhibition of SRS production and hypoxic vasoconstriction is consistent with the hypothesis that lipoxygenase products of arachidonic acid may have a role in the mediation of hypoxic pulmonary vasoconstriction in isolated rat lungs. Although we did not demonstrate in the current study that there was an alteration in hypoxia-induced SRS production, we have previously demonstrated in isolated perfused rat lungs that leukotriene C_4 is produced in response to acute alveolar hypoxia and that both leukotriene C_4 production and hypoxic pulmonary vasoconstriction were inhibited by leukotriene synthesis blockers (1,2). Similarly, previous work has demonstrated that chronic alveolar hypoxia caused pulmonary hypertension and production of SRS-like material in the intact rat (14). The pulmonary hypertension and SRS production were inhibited by pretreatment with a leukotriene synthesis blocker (14). If leukotrienes mediate hypoxic pulmonary vasoconstriction, then inhibition of their synthesis should inhibit vasoconstriction secondary to alveolar hypoxia but not vasoconstriction to other agonists. Previous studies (2,3,4) have demonstrated that inhibitors of leukotriene synthesis or action inhibit hypoxic pulmonary vasoconstriction without causing a nonspecific decrease in pulmonary vascular responsiveness. However, in the current study there was not a preferential inhibition of hypoxic pulmonary vasoconstriction, since in lungs isolated from rats raised on the EFAD diet both the pressor responses to hypoxia and angiotensin II were similarly inhibited. This nonspecific decrease in pulmonary vascular responsiveness was confirmed in isolated pulmonary artery rings from rats raised on the EFAD diet where there was decreased reactivity to norepinephrine induced vasoconstriction.

This nonselective decrease in pulmonary vascular responsiveness could have been due to decreased production of SRS-like material in lung parenchyma or

vascular tissue from rats raised on the EFAD diet. Despite the potential of lungs from the arachidonate-deficient rats to produce LTC_3 and LTC_5, which possess SRS-like myotropic activity (15,16), the EFAD diet must have resulted in the production of less leukotrienes of the 3, 4, and 5 series since less SRS-like myotropic activity was detected by bioassay. Because of the decrease in plasma arachidonic acid one might expect decreased production of LTC_4, D_4, and E_4, however the mechanism responsible for inhibition of SRS production in our experiments is unclear. Stenson (13) has shown that a metabolite of 5-, 8-, 11- eicosatrienoic acid may inhibit LTA_4 hydrolase and LTB_4 production. Thus, metabolites produced in response to the EFAD diet could possibly inhibit SRS production. The inability of exogenous arachidonic acid to restore pulmonary vascular responsiveness to control levels would be consistent with the presence of an inhibitory substance. However, the failure of exogenous arachidonic acid to restore pulmonary vascular responsiveness could also be due to other factors. Time allowed for arachidonic acid incorporation, the amount supplied, or pools with different affinities for exogenous arachidonic acid all could explain this finding.

Cyclooxygenase metabolite production as assessed by production of 6-keto-$PGF_{1\alpha}$ and TXB_2 did not appear to be involved in the decreased pulmonary vascular responsiveness. A surprising finding was the lack of inhibition of prostacyclin and thromboxane production in lungs from rats raised on the EFAD diet. High baseline values for these metabolites in all lung effluents likely represents activation of the cyclooxygenase system due to the artificial circulation and ventilation. However, continued prostacyclin production did not explain the decrease in pulmonary vascular responsiveness since cylooxygenase inhibition by indomethacin did not reverse the decrease in pulmonary vascular responsiveness. The reason for the lack of inhibition of 6-keto-$PGF_{1\alpha}$ and TXB_2 production in lungs from rats raised on the EFAD diet is unclear, but this finding indicates a more complex relationship between substrate and arachidonic acid products. If there were different arachidonic acid pools conceivably dietary manipulation could affect these pools differently.

Since in our study the EFAD diet led to decreased production of SRS and PAF-like material after calcium ionophore stimulation but did not affect 6-keto-$PGF_{1\alpha}$

or TXB_2 production in the isolated lung, one might speculate that the EFAD diet affected the availability of phospholipids released from a pool from which both PAF and lipoxygenase products originate but not the pool from which prostaglandins are derived. That leukotrienes and PAF share a common precursor phospholipid (possibly 1-0-alkyl-2-arachidonyl-sn-glycero-3-phosphocholine) has also been suggested in studies of neutrophils derived from EFAD rats. In these studies, neutrophils depleted of arachidonic acid did not synthesize PAF or leukotrienes (17). Alternatively, inhibition of PAF synthesis in the EFAD lungs could be merely a consequence of inhibition of leukotriene synthesis since LTC_4 is known to stimulate PAF production (7).

Although there is an association between altered vascular reactivity and impairment of stimulated production of SRS and PAF like material in the EFAD rat lungs, it has not been established that these two findings are causally related. One alternative possibility is that the EFAD diet may have altered membrane permeability to calcium (18) or membrane fluidity by altering the lipid composition of vascular membranes. Finally, the tendency for decreased weight gain in rats raised on the EFAD diet does not seem to account for the decreased pulmonary vascular reactivity, because the difference in weight was small and less than that reported to inhibit pulmonary changes due to alveolar hypoxia (14,19). Furthermore, the finding of increased systemic vascular responsiveness in aortic rings from rats raised on the EFAD diet makes a general depression of vasoreactivity due to impaired weight gain unlikely.

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