Levels of prostaglandins and their precursors in EFA-deficient rabbits—a new concept of prostaglandin biosynthesis

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Pathological changes that accompany essential fatty acid (EFA) deficiency have been ascribed to deficiency in prostaglandin (PG) production (Van Dorp, 1971; Willis & Stone, 1976), and/or impaired membrane function (Sun & Sun, 1974). We have examined the relationship between tissue levels of prostaglandins and their precursor fatty acids in rabbits maintained on EFA deficient diets.

Groups of weanling Dutch male rabbits (Hyeline) were maintained for up to 8 weeks on either a normal diet (Diet RAF, The Christopher Hill Group Ltd., Poole, Dorset) or a completely fat free diet. Tissues removed at the time of killing were immediately frozen in liquid nitrogen and stored (−20°C) until powdered and extracted for PG determination (Denton, Marples & Willis, 1978). For fatty acid analysis (Hassam, Rivers & Crawford, 1977), the tissues were homogenized in an ice-cold mixture of chloroform/methanol (2:1), containing anti-oxidant. The lipids were subsequently separated into classes, transmethylated and the fatty acid methyl esters estimated by gas–liquid chromatography. Liver and red blood cell lipids are usually analyzed to indicate essential fatty acid status. The results (Table 1a) showed that in the EFA deficient animals there was a reduction in linoleic acid (18:2ω6) levels as expected, but surprisingly no change or even an increase in the PG precursors DHLA (20:3ω6) and arachidonic acid (20:4ω6) in the principle phosphoglyceride fractions. Similar results to those shown for the fat-free diet were

Table 1a

Fatty acid	*Liver ethanolamine phosphoglycerides* Control (n = 4)	Fat-free (n = 4)	*R.B.C. ethanolamine phosphoglycerides* Control (n = 4)	Fat-free (n = 4)
Linoleic acid (18:2ω6)	20.3 ± 3.6	12.4 ± 3.1	30.4 ± 1.9	16.5 ± 1.9
Dihomo-γ-linolenic acid (20:3ω6)	0.32 ± 0.11	0.27 ± 0.06	0.56 ± 0.22	0.65 ± 0.14
Arachidonic acid (20:4ω6)	8.7 ± 2.2	15.7 ± 1.6	11.3 ± 1.2	13.7 ± 1.3

Table 1b

Tissue	Diet	PGE_1	PGE_2	PGF_2
Lung	Control (n = 5)	61 ± 30	205 ± 88	187 ± 104
	Fat-free (n = 4)	15 ± 8	19 ± 5	27 ± 16
Skin	Control (n = 5)	139 ± 12	402 ± 103	111 ± 24
	Fat-free (n = 4)	43 ± 27	48 ± 6	40 ± 26
Eye	Control (n = 5)	136 ± 62	193 ± 48	96 ± 35
	Fat-free (n = 4)	8 ± 1	27 ± 4	13 ± 5

a: Mean (±s.e. mean) values for linoleate, dihomo-γ-linolenate and arachidonate content of cell membrane phospholipid. These representative figures are expressed at % total fatty acids in phosphatidylethanolamine (P.E.). Liver is the principal initial site of fatty acid metabolism, while red blood cells (RBC) provide an index of structural lipid status.

Similar fatty acid composition values were obtained for other phospholipids and other tissues.

b: Mean (±s.e. mean) values for PG content in some tissues. Results are expressed as ng PG/g of frozen tissue, allowing for recovery of [^{3}H] tracer.

obtained with an EFA-deficient diet, supplemented with hydrogenated coconut oil (6% of calories as saturated fat).

By contrast, however, levels of prostaglandins E_1, E_2 and $F_{2\alpha}$ were markedly decreased in all tissues examined (Table 1b).

The results suggest that in the rabbit basal turnover of these prostaglandins is more directly related to dietary status rather than phosphoglyceride stores. The question raised by these results is: are there different metabolic pools for basal and stimulated prostaglandin synthesis?

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Release of slow-reacting substance from guinea-pig and human lung by calcium ionophore A23187

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Slow-reacting substance of anaphylaxis (SRS-A) was first described in the effluent from guinea-pig sensitized lungs *in vitro* following antigenic challenge. Consequently, many subsequent studies have been directed towards the immunological mechanisms involved. However, it has been reported that a slow-reacting substance(s) (SRS) with the characteristics of SRS-A may be released by a non-immunological stimulus, calcium ionophore A23187 from human leucocytes (Conroy, Orange & Lichtenstein, 1976) and rat peritoneal cells (Bach & Brashler, 1974). The experiments described were performed to determine whether A23187 would release SRS from guinea-pig and human lung.

Unsensitized or actively sensitized male guinea-pigs (350–600 g) were killed, the lungs removed and perfused with Tyrode via the pulmonary artery until free of blood. The lung tissue was cut into pieces, approximately 2 mm^3, weighed into 1.0–1.7 g portions and incubated in 4.5 ml of A23187 (5 μg/ml) in Tyrode for 45 min at 37°C or challenged with antigen (15 min at 37°C). Macroscopically normal human lung, obtained from operative specimens resected for bronchogenic carcinoma was washed in Tyrode, cut into small pieces, divided into 450 mg replicates and incubated with A23187 as above. The SRS activity in the supernatant was assayed on smooth muscle stripped from guinea-pig ileum (Rang, 1964) and blocked with mepyramine and hyoscine. Histamine was assayed fluorimetrically.

During incubation with A23187 unsensitized guinea-pig lung released SRS (560 ± 183 mu ml^{-1}, $n = 7$, for units see Engineer, Niederhauser, Piper & Sirois, 1978). After pretreatment with indomethacin (1 μg/ml) for 1 h and subsequent incubation with A23187 and indomethacin (1 μg/ml), the release of SRS was significantly greater than with A23187 alone (930 ± 234 mu/ml, $P < 0.005$). When sensitized guinea-pigs were used, the chopped lung released SRS with A23187 and SRS-A following antigenic challenge. Both these substances were antagonized by FPL 55712 (1 μg/ml) (Augstein, Farmer, Lee, Sheard & Tattersall, 1973), destroyed by arylsulphatase and by low pH but were stable to base hydrolysis. In unsensitized and sensitized lung cysteine (10^{-3} M) caused an increase (mean 92%) in ionophore-induced SRS release. In experiments with human lung SRS was also released by A23187 (5 μg/ml) and the release potentiated by cysteine (10^{-3} M). Human SRS was found to be indistinguishable from guinea-pig SRS. A23187 also caused a dose-dependent release of histamine from human and guinea-pig lung.

These findings extend earlier observations (Bach & Brashler, 1974; Conroy *et al.*, 1976) and support the possibility that a SRS, which is similar or identical to SRS-A associated with IgE and IgG-mediated allergic reactions, may be involved in non-immunological inflammatory processes. Since indomethacin increased the ionophore-induced release of SRS, some product(s) of arachidonic acid metabolism by cyclooxygenase may modulate its release.