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Effect of exogenous 5, 8, 11, 14, 17-eicosapentaenoic acid on cardiac anaphylaxis

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1 The effects of infusions of eicosapentaenoic acid (EPA) (6×10^{-8} mol min^{-1} and 15×10^{-8} mol min^{-1}) on the coronary constriction and the release of immunoreactive sulphidopeptide-leukotrienes (SP-LT), thromboxane B_2(TXB_2) and 6-keto-prostaglandin $F_{1\alpha}$($PGF_{1\alpha}$) from perfused anaphylactic guinea-pig hearts were investigated.

2 EPA dose-dependently inhibited the profound early coronary flow reduction after antigen injection. The less pronounced late phase of anaphylactic coronary flow reduction was, however, not significantly affected. EPA (15×10^{-8} mol min^{-1}) significantly shortened the average duration of antigen-induced arrhythmias.

3 EPA dose-dependently decreased release of immunoreactive TXB_2 and 6-keto-$PGF_{1\alpha}$ from anaphylactic guinea-pig hearts.

4 Release of immunoreactive SP-LT was dose-dependently increased after antigen challenge in the presence of EPA. Inhibition of the release of SP-LT by the lipoxygenase inhibitor esculetin (1×10^{-7} mol min^{-1}) was accompanied by a significant attenuation of flow reduction during the late phase of anaphylactic vasoconstriction.

5 Reversed phase h.p.l.c. of perfusates from anaphylactic guinea-pig hearts revealed immunoreactivity comigrating with authentic leukotriene C_4 (LTC_4), LTD_4 and LTE_4. In perfusates from hearts treated with EPA infusions, additional immunoreactivity was detected comigrating with LTC_5, LTD_5 and LTE_5. In addition to immunoreactivity migrating with LTB_4, as observed in control heart perfusates, in perfusates from EPA-treated hearts, a second peak was observed, which coincides with the retention time described for LTB_5.

6 Exogenous LTC_5 (1×10^{-12} mol min^{-1} and 20×10^{-12} mol min^{-1}) induced dose-dependent reductions of coronary flow and was found to be a slightly weaker constrictor than LTC_4, but no significant differences were observed. Coronary vasoconstriction elicited by infusion of exogenous LTC_4 (20×10^{-12} mol min^{-1}) was dose-dependently inhibited by infusions of EPA. However, the negative inotropic effect of LTC_4 remained unaffected.

7 Thus, in the isolated anaphylactic heart of the guinea-pig exogenous EPA was effectively metabolized via the 5-lipoxygenase pathway whereas the cyclo-oxygenase pathway of polyunsaturated fatty acid metabolism was found to be inhibited. The results are in agreement with the suggestion that cyclo-oxygenase products are mediators of the early phase of the anaphylactic coronary constriction, while vasoconstrictor SP-LT are involved in the later phase. However, in spite of enhanced release of SP-LT, EPA infusion did not result in increased coronary constriction. Considering the fact that EPA antagonizes LTC_4-induced coronary constriction, it seems possible, that EPA might act as a functional antagonist of vasoconstrictor eicosanoids including EPA-derived SP-LT.

Introduction

In man, anaphylactic reactions to drugs or foodstuffs have been accompanied by electrocardiographic changes, including all types of cardiac arrhythmias and even acute myocardial infarction. Anaphylactic cardiac arrest has been reported in patients after intravenous injection of propanidid or alcuronium. Cases of coronary spasm, associated with urticaria, have been seen within minutes of a bee sting and signs of acute

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myocardial infarction were also observed (for review see Simmet & Peskar, 1986).

The heart has now been recognized as a primary target organ in anaphylactic reactions and a substantial body of clinical evidence suggests that arrhythmia and cardiac failure may occur without preceding respiratory distress, which otherwise may be the cause of secondary cardiac symptoms (Austen, 1978; Simmet & Peskar, 1986).

Guinea-pig isolated sensitized hearts react very strongly to antigen, with a long-lasting reduction in coronary flow coupled with marked impairment of cardiac function (Feigen & Prager, 1969; Liebig *et al.*, 1975; Levi *et al.*, 1976). In addition, electrocardiographic changes have been found that are similar to those observed in man during anaphylaxis (Capurro & Levi, 1975). Furthermore, upon immunological challenge, various mediators, which could be responsible for the observed changes in cardiac function, like histamine (Brocklehurst, 1960; Feigen & Prager, 1969) slow-reacting substance of anaphylaxis (SRS-A) (Brocklehurst, 1960; Liebig *et al.*, 1975) or sulphidopeptide-leukotrienes (SP-LT) (Aehringhaus *et al.*, 1983), platelet-activating factor (Paf-acether) (Levi *et al.*, 1984), prostaglandins (Liebig *et al.*, 1975; Levi *et al.*, 1976; Anhut *et al.*, 1978; Peskar *et al.*, 1979; Allan & Levi, 1981), and thromboxane B_2 (TXB_2) (Anhut *et al.*, 1977; Allan & Levi, 1981), the stable degradation product of the biologically active TXA_2, have been detected in perfusates of anaphylactic guinea-pig hearts.

The ω-3 polyunsaturated fatty acid 5, 8, 11, 14, 17-eicosapentaenoic acid (EPA) is a major constituent of fish oil. Eskimos, whose diet is rich in ω-3 polyunsaturated fatty acids, have a prolonged bleeding time, and a very low incidence of cardiovascular death, which has been related to the fact that EPA is the main fatty acid for prostaglandin biosynthesis in Eskimos (Dyerberg *et al.*, 1978). The report, demonstrating the lack of effect of EPA-derived TXA_3 on platelet aggregation whereas the corresponding PGI_3 metabolite was found to be as effective as PGI_2 (Needleman *et al.*, 1979), further stimulated interest in dietary manipulation of prostanoid biosynthesis. The extent of EPA metabolism via the cyclo-oxygenase pathway giving rise to trienoic prostanoids (Dyerberg *et al.*, 1978; 1981; Needleman *et al.*, 1979; Fischer & Weber, 1983; 1984) and the effect of the substance on the cyclo-oxygenase pathway of polyunsaturated fatty acid metabolism seem to vary considerably with the experimental conditions. Thus, several authors suggested the compound was only a poor substrate for metabolism via cyclo-oxygenase (Culp *et al.*, 1979; Needleman *et al.*, 1979; Hornstra *et al.*, 1981; Juan & Sametz, 1983; Juan *et al.*, 1985) and in numerous preparations an inhibitory effect of EPA on the enzymatic conversion of the normal substrate, arachidonic acid, by the cyclo-oxygenase pathway has been described (Culp *et al.*, 1979; Needleman *et al.*, 1979; Hornstra *et al.*, 1981; Spector *et al.*, 1983). On the other hand, EPA seems to be readily metabolized via the 5-lipoxygenase pathway giving rise to the EPA-derived 5-series leukotrienes (Hammarström, 1980; Jakschik *et al.*, 1980; Lee *et al.*, 1984; Terano *et al.*, 1984; Strasser *et al.*, 1985).

Thus, during anaphylactic reactions, it seems possible that EPA might favourably affect the release of mediators derived from the cyclo-oxygenase pathway of polyunsaturated fatty acid metabolism. Furthermore, we recently obtained evidence that EPA might antagonize vasoconstriction induced by various vasoactive drugs. Therefore, this study was initiated to investigate the effects of exogenous EPA on the coronary vasoconstriction and eicosanoid release in guinea-pig isolated anaphylactic hearts.

Methods

Isolated hearts of ovalbumin-sensitized male guinea-pigs (500–850 g body wt.) were perfused under constant pressure and challenged by injection into the inflow cannula of 1 mg of ovalbumin dissolved in 0.1 ml of Tyrode solution (Liebig *et al.*, 1975). A thread hooked to the apex of the left ventricle was attached to a force displacement transducer (Hugo Sachs, D-7801 March-Hugstetten, F.R.G., model K 30). A basal tension of 4 g weight was applied and the spontaneous rate and force of contractions were recorded on a Watanabe multichannel recorder. Duration of arrhythmias was determined from the recordings of the heart rate. Coronary flow was measured by direct determination of the perfusate volume per collection period. Changes in coronary flow after challenge were compared with basal flow immediately before antigen challenge. After an equilibration period of 25–30 min, heart perfusates were collected before and, for a total of 23 min, after antigen injection. The sodium salt of EPA dissolved in saline was infused into the inflow cannula at a constant rate of 6×10^{-8} mol min^{-1} or 15×10^{-8} mol min^{-1} resulting in final perfusate concentrations between 5.0×10^{-6} M and 12.8×10^{-6} M, and 12.4×10^{-6} M and 33.5×10^{-6} M, respectively. Infusions started 30 min before ovalbumin challenge as did infusions of esculetin. Esculetin was first dissolved in saline containing NaOH (0.1 M) and was afterwards neutralized with Tris/HCl buffer 0.5 M, pH 7.4. After appropriate dilution in saline, esculetin was infused at a constant rate of 1×10^{-7} mol min^{-1} resulting in final concentrations between 0.8×10^{-5} M and 1.5×10^{-5} M. Control hearts always received the appropriate solvent infusions.

Exogenous LTC_4 and LTC_5 were infused for 5 min

into hearts of non-sensitized guinea-pigs (370–480 g body wt.) which were prepared and treated like the hearts of sensitized animals. Leukotrienes were infused at a constant rate of 1×10^{-12} mol min^{-1} and 20×10^{-12} mol min^{-1} resulting in final perfusate concentrations between 0.9×10^{-10} M and 1.7×10^{-10} M, and 1.8×10^{-9} M and 7.7×10^{-9} M, respectively.

Reversed phase h.p.l.c. of SP-LT and LTB_4 was performed as previously described (Peskar *et al.*, 1986). Briefly, perfusate samples were purified using C_{18}-SEP-PAK cartridges. LTB and SP-LT were eluted with methylformate and methanol, respectively. After solvent evaporation leukotrienes were redissolved in methanol and injected onto a C_{18}-Nucleosil column (250 × 4 mm, particle size 5 μm, Macherey Nagel, Düren, F.R.G.), using the solvent system methanol : water : acetic acid (68:32:0.01, v/v/v, pH 5.5). Finally, eluted fractions were tested for inhibition of binding of [^{3}H]-LTC_4 to an anti-LTC_4 antibody that exhibits 40% relative cross-reaction with both LTD_4 and LTE_4. Fractions from the methylformate extracts were likewise tested for inhibition of binding of [^{3}H]-LTB_4 to the anti-LTB_4 antibody.

Standard LTC_5 was converted to LTD_5 by a partially purified γ-glutamyl transpeptidase as described elsewhere (Bernström *et al.*, 1982). Since this commercially available enzyme preparation is contaminated with dipeptidase (Bernström *et al.*, 1982), LTD_5 was further degraded into LTE_5 resulting in a mixture of LTC_5, LTD_5 and LTE_5, which was separated by reversed phase h.p.l.c.

Radioimmunological determination of SP-LT in heart perfusates has been performed as described previously (Aehringhaus *et al.*, 1982). In fact the validity of the method in this particular system has been repeatedly checked by correlation to bioassay data (Aehringhaus *et al.*, 1983). Relative cross-reaction of LTC_5 with the anti SP-LT antiserum was 99%. Relative cross-reaction of LTB_5 with the LTB_4 antiserum has been reported to be 17.8% (Terano *et al.*, 1984). TXB_2 and 6-keto-$PGF_{1\alpha}$ in the perfusates were also determined radioimmunologically, as described previously (Anhut *et al.*, 1977; Peskar *et al.*, 1979; Aehringhaus *et al.*, 1983). Radioimmunoassays were always controlled for non-specific interference with EPA and were adequately corrected if necessary.

Means ± s.e.mean were calculated. Statistical analysis was performed using Student's *t* test.

Materials

Ovalbumin, esculetin, γ-glutamyl transpeptidase and the sodium salt of 5, 8, 11, 14, 17-eicosapentaenoic acid were purchased from Sigma Chemical Co., St Louis, Mo., U.S.A. Tritium labelled eicosanoids were obtained from New England Nuclear, Dreieich, F.R.G., and the anti-LTB_4 antiserum was from Wellcome Co., Beckenham, England.

Results

Cardiac function

Infusion of EPA 6×10^{-8} mol min^{-1} and 15×10^{-8} mol min^{-1} dose-dependently inhibited the profound coronary constriction observed early after antigen-challenge (Figure 1). Thus, coronary flow reduction after ovalbumin injection into control hearts was 63.5 ± 5.3% as compared to 37.7 ± 3.0% ($P < 0.01$) and 27.4 ± 3.5% ($P < 0.001$) in hearts which had been infused with EPA at 6×10^{-8} mol min^{-1} and 15×10^{-8} mol min^{-1}, respectively. On the other hand, during the less pronounced longer-lasting late phase of anaphylactic constriction, no significant differences were observed.

The later phase of anaphylactic coronary flow reduction was, however, also inhibited, when EPA (15×10^{-8} mol min^{-1}) was infused in the presence of esculetin (1×10^{-7} mol min^{-1}) (Figure 2). Thus, shortly after the initial anaphylactic coronary constriction, which was similar to that of EPA-treated control hearts, coronary flow increased again. It was found to be significantly enhanced during the last three collection periods (i.e. 11–23 min after challenge) by 23.6 ± 8.3%, 18.7 ± 6.4% and 18.0 ± 5.9% as compared to hearts receiving EPA (15×10^{-8} mol min^{-1}) alone. Similarly, when esculetin (1×10^{-7} mol min^{-1}) was infused in the absence of EPA the initial coronary

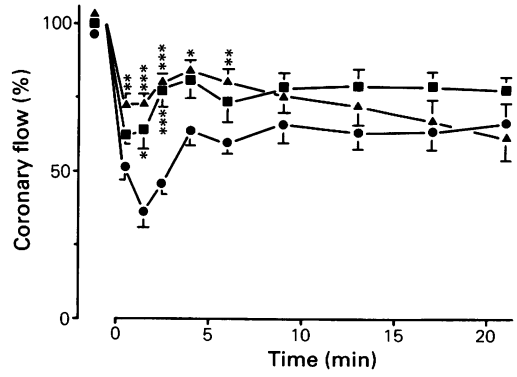

Figure 1 Coronary flow before and after antigen challenge of sensitized guinea-pig hearts under control conditions (●, $n = 8$) and during infusion of eicosapentaenoic acid (EPA) 6×10^{-8} mol min^{-1} (■, $n = 8$) and 15×10^{-8} mol min^{-1} (▲, $n = 8$). The EPA infusion was started 30 min before challenge. The coronary flow before antigen injection was 11.2 ± 0.9 ml min^{-1} in the control group and 12.5 ± 0.6 ml min^{-1} and 14.4 ± 0.7 ml min^{-1} in the presence of the lower and the higher dose of EPA, respectively, and was taken as 100% for each group. Antigen was injected at time 0. ***$P < 0.001$; **$P < 0.005$; *$P < 0.01$ as compared to coronary flow under control conditions.

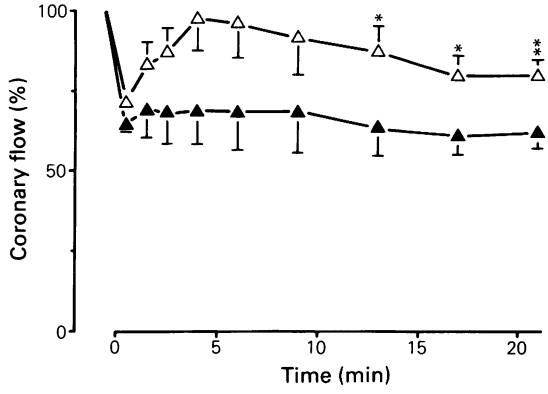

Figure 2 Coronary flow before and after antigen challenge of sensitized guinea-pig hearts during infusion of eicosapentaenoic acid (EPA) 15×10^{-8} mol min^{-1} (▲, $n = 5$) and during infusion of EPA 15×10^{-8} mol min^{-1} plus esculetin 1×10^{-7} mol min^{-1} (△, $n = 5$). All infusions were started 30 min before challenge. Coronary flow before antigen injection was 13.3 ± 1.2 ml min^{-1} in the presence of EPA alone and 11.5 ± 0.8 mol min^{-1} in hearts treated with EPA plus esculetin and was taken as 100% for each group. Antigen was injected at time 0. $^{**}P < 0.02$; $^{*}P < 0.05$ as compared to coronary flow during infusion of EPA 15×10^{-8} mol min^{-1} alone.

spasm remained unaffected (data not shown), whereas the late phase reaction was significantly attenuated. Again coronary flow was significantly enhanced during the last three collection periods by $17.1 \pm 3.6\%$, $16.1 \pm 4.1\%$ and $16.9 \pm 4.1\%$ ($P < 0.02$, $n = 5$) as compared to control hearts. There was no significant difference in coronary flow between both groups before antigen challenge.

The average duration of arrhythmia after antigen challenge was 434.9 ± 102.1 s ($n = 8$), which was significantly shortened during the infusion of EPA (15×10^{-8} mol min^{-1}) to 193.5 ± 35.7 s ($n = 8$, $P < 0.05$). When EPA (15×10^{-8} mol min^{-1}) was infused in the presence of esculetin (1×10^{-7} mol min^{-1}) the duration of arrhythmias observed was 210.3 ± 63.4 s ($n = 5$) suggesting that esculetin does not antagonize the antiarrhythmic effect of the higher dose of EPA. Infusion of esculetin (1×10^{-7} mol min^{-1}) alone did not significantly affect the duration of arrhythmias as compared to controls ($n = 5$ each). The lower infusion rate of EPA (6×10^{-8} mol min^{-1}), on the other hand, was without significant effect (628.7 ± 149.7 s, $n = 8$).

Neither infusion of EPA 6×10^{-8} mol min^{-1} or 15×10^{-8} mol min^{-1} nor infusion of esculetin 1×10^{-7} mol min^{-1} or its combination with EPA for 30 min before ovalbumin injection significantly affected heart rate or myocardial contractility compared to controls.

Mediator release

Levels of immunoreactive TXB_2 and 6-keto-$PGF_{1\alpha}$ in basal heart perfusates were either below or close to the detection limits of the radioimmunoassays (7.2×10^{-11} M and 6.1×10^{-11} M, respectively). However, large amounts of these cyclo-oxygenase products were detected in heart perfusates after antigen injection. As shown in Table 1, (upper part) EPA accelerated the initial release of TXB_2 after antigen challenge, an effect which was significant ($P < 0.05$) at the higher infusion rate of EPA (15×10^{-8} mol min^{-1}). In contrast, starting from the third minute after antigen challenge, significant and dose-dependent inhibition of TXB_2 release was observed in the presence of EPA (6×10^{-8} mol min^{-1} and 15×10^{-8} mol min^{-1}) (Table 1). Total release of TXB_2 during the 23 min observation period decreased to 149.4 ± 11.7 ng ($n = 8$, $P < 0.02$) and 119.1 ± 8.4 ng ($n = 8$, $P < 0.01$) in the presence of EPA 6×10^{-8} mol min^{-1} and 15×10^{-8} mol min^{-1}, respectively. Control hearts released 189.4 ± 8.0 ng TXB_2 ($n = 8$).

Whereas there was no significant inhibition of the release of 6-keto-$PGF_{1\alpha}$ during any of the single perfusate collection periods, total release of 6-keto-$PGF_{1\alpha}$ during the 23 min observation period was significantly decreased to 34.0 ± 2.6 ng ($n = 8$, $P < 0.025$) and 33.0 ± 2.8 ng ($n = 8$, $P < 0.02$) in the presence of EPA 6×10^{-8} mol min^{-1} and 15×10^{-8} mol min^{-1}, respectively. Control hearts released 48.3 ± 4.9 ng 6-keto-$PGF_{1\alpha}$ ($n = 8$).

Before antigen-challenge, no SP-LT-like material could be detected in heart perfusates. After ovalbumin injection considerable amounts of immunoreactive SP-LT could be found in the perfusates (Table 1, lower part). EPA infusions dose-dependently increased release of SP-LT-like immunoreactivity after antigen injection. In fact, during the second minute after antigen challenge the release of SP-LT-like material was increased by $117.1 \pm 49.0\%$ and $215.5 \pm 63.2\%$ during EPA infusions of 6×10^{-8} mol min^{-1} and 15×10^{-8} mol min^{-1}, respectively (Table 1, lower part). Total release of SP-LT during the 23 min observation period increased from 40.3 ± 4.5 ng under control conditions to 64.9 ± 11.5 ng (NS) and 70.9 ± 11.2 ng ($P < 0.025$) during EPA infusions of 6×10^{-8} mol min^{-1} and 15×10^{-8} mol min^{-1}.

In further experiments the release of SP-LT after antigen challenge during infusion of EPA (15×10^{-8} mol min^{-1}) was compared to that during infusion of EPA (15×10^{-8} mol min^{-1}) plus esculetin (1×10^{-7} mol min^{-1}). The lipoxygenase inhibitor esculetin significantly inhibited the total release of SP-LT from 95.1 ± 23.5 ng to 31.2 ± 10.0 ng ($n = 5$ each, $P < 0.05$) during the 23 min observation period. Infusion of esculetin (1×10^{-7} mol min^{-1}) alone reduced total

Table 1 Release of immunoreactive thromboxane B_2 (TXB_2) and sulphidopeptide-leukotrienes (SP-LT) after antigen challenge in the absence and presence of eicosapentaenoic acid (EPA)

Time (min)	0–1	1–2	2–3	3–5	5–7	7–11	11–15	15–19	19–23
TXB_2 (pg min^{-1})	7670	30898	33367	21273	11248	5780	3299	2387	1636
Control	±	±	±	±	±	±	±	±	±
($n = 8$)	2699	3980	2063	2657	1041	627	276	243	84
EPA	11657	36309	22399	11120	6287	4797	2690	1944	1631
6×10^{-8} mol min^{-1}	±	±	±	±	±	±	±	±	±
($n = 8$)	2263	4323	1874†	1039†	744†	345	165	210	202
EPA	17486	27921	17409	8697	4776	3805	1938	1218	1002
15×10^{-8} mol min^{-1}	±	±	±	±	±	±	±	±	±
($n = 8$)	3454*	4982	1359††	964††	802††	582*	380**	212†	199**
SP-LT (pg min^{-1})	536	5288	5529	4623	2545	1651	969	679	547
Control	±	±	±	±	±	±	±	±	±
($n = 8$)	211	915	1017	585	241	238	120	62	51
EPA	2046	11479	11105	7136	4138	2473	1085	521	357
6×10^{-8} mol min^{-1}	±	±	±	±	±	±	±	±	±
($n = 8$)	623*	2593*	2544	1323	720	506	227	86	127
EPA	3793	16682	12878	7428	4280	1647	919	572	420
15×10^{-8} mol min^{-1}	±	±	±	±	±	±	±	±	±
($n = 8$)	702††	3341***	1991***	1133*	1063	584	388	143	88

Values are mean ± s.e.mean. *$P < 0.05$, **$P < 0.02$, ***$P < 0.01$, †$P < 0.005$, ††$P < 0.001$, as compared to controls.

release of SP-LT to 23.2 ± 4.7 ng, which is a significant decrease of 45.6 ± 10.9% ($n = 5$, $P < 0.05$) as compared to controls. The compound also tended to decrease the release of TXB_2 and 6-keto-$PGF_{1\alpha}$, an effect which did not reach significance (data not shown).

Perfusates from six hearts (3 control hearts and 3 hearts treated with EPA 15×10^{-8} mol min^{-1}) were each subjected to reversed phase h.p.l.c. Typical profiles of SP-LT- and LTB_4-like immunoreactivity are shown in Figures 3 and 4. Analysis of the fractions obtained after reversed phase h.p.l.c. of anaphylactic guinea-pig heart perfusates, revealed SP-LT-like immunoreactivity comigrating with standard LTC_4, LTD_4 and LTE_4 (Figure 3, upper panel). When antigen challenge had been performed in the presence of EPA (15×10^{-8} mol min^{-1}) additional SP-LT-like immunoreactivity was found comigrating with LTC_5, LTD_5 and LTE_5, indicating metabolism of EPA via 5-lipoxygenase into the appropriate SP-LT (Figure 3, lower panel).

As shown in Figure 4, upper panel, the anaphylactic guinea-pig heart also releases immunoreactive material comigrating with LTB_4 on reversed phase h.p.l.c. When perfusates of EPA (15×10^{-8} mol min^{-1})-infused anaphylactic guinea-pig hearts were used, a second peak of LTB_4-like immunoreactivity was detected upon reversed phase h.p.l.c. profiling (Figure 4, lower panel). Even though this peak could not be identified by LTB_5 standard, which was not available to us, its relative retention time seems to be identical to that reported for LTB_5 (Lee *et al.*, 1984; Terano *et al.*, 1984; Strasser *et al.*, 1985).

Exogenous sulphidopeptide-leukotrienes

The effect of exogenous LTC_4 (1.0×10^{-12} mol min^{-1} and 20.0×10^{-12} mol min^{-1}) on coronary flow of non-sensitized guinea-pig hearts is shown in Figure 5. LTC_4 at the concentrations used, dose-dependently reduced coronary flow as did infusions of exogenous LTC_5 (1.0×10^{-12} mol min^{-1} and 20.0×10^{-12} mol min^{-1}, Figure 5). Although LTC_5 appeared to be a slightly weaker vasoconstrictor than LTC_4, no significant differences were found between flow reductions induced by 4- and 5-series LTC. Both LTC_4 and LTC_5 exerted dose-dependent negative inotropic effects but no significant differences were observed with respect to the efficacy of either compound (data not shown).

In other experiments the effect of EPA (6.0×10^{-8} mol min^{-1} and 15×10^{-8} mol min^{-1}) on LTC_4-induced coronary flow reduction was investigated. Figure 6 demonstrates that EPA at both concentra-

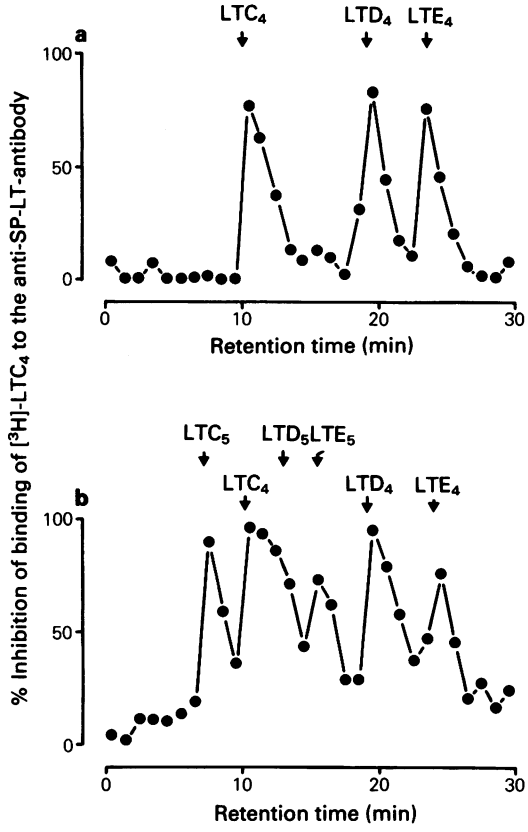

Figure 3 (a) Reversed phase h.p.l.c. profile of sulphidopeptide-leukotriene (SP-LT)-like material released from anaphylactic guinea-pig hearts as detected by SP-LT radioimmunoassay. (b) Effect of eicosapentaenoic acid (EPA) 15×10^{-8} mol min^{-1} infusion into the anaphylactic guinea-pig heart on the reversed phase h.p.l.c. elution profile of SP-LT-like material as detected by SP-LT radioimmunoassay. Arrows indicate the retention time of appropriate comigrating standards. A C_{18}-Nucleosil column was used. The solvent system consisted of methanol : water : acetic acid, 68:32:0.01, v/v/v.

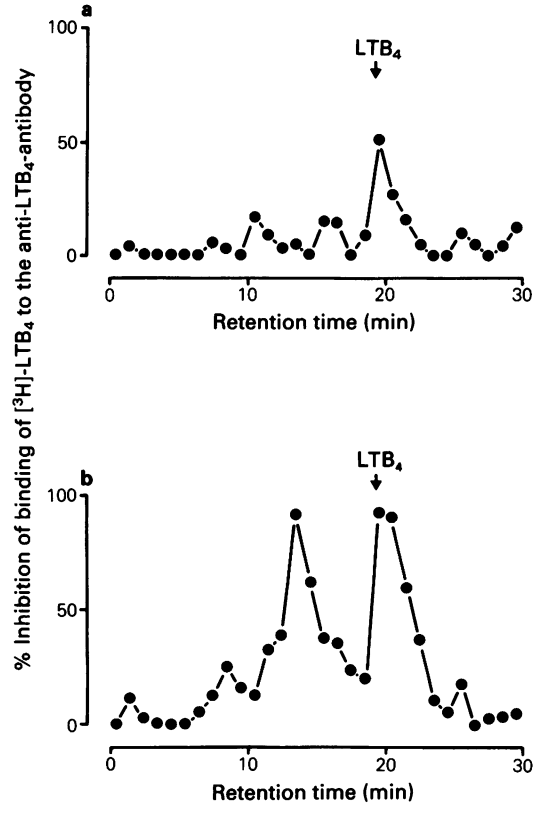

Figure 4 (a) Reversed phase h.p.l.c. profile of leukotriene B_4 (LTB_4)-like immunoreactivity released from anaphylactic guinea-pig hearts as detected by LTB_4 radioimmunoassay. (b) Effect of eicosapentaenoic acid (EPA) 15×10^{-8} mol min^{-1} infusion into the anaphylactic guinea-pig heart on the reversed phase h.p.l.c. elution profile of LTB_4-like immunoreactivity as detected by LTB_4 radioimmunoassay. Arrows indicate the retention time of appropriate comigrating standards. A C_{18}-Nucleosil column was used. The solvent system consisted of methanol : water : acetic acid, 68:32:0.01, v/v/v.

tions used, dose-dependently and significantly inhibited coronary flow reductions during infusion of LTC_4 (20×10^{-12} mol min^{-1}) for 5 min. Maximum decrease of coronary flow was $33.1 \pm 3.4\%$ ($n = 4$, $P < 0.02$) and $14.5 \pm 3.4\%$ ($n = 4$, $P < 0.001$) in the presence of EPA 6×10^{-8} mol min^{-1} and 15×10^{-8} mol min^{-1}, respectively, whereas in control hearts coronary flow decreased by $58.7 \pm 6.4\%$. Despite the marked effects of EPA infusions on the coronary flow reductions, EPA did not attenuate the negative inotropic effect of LTC_4. At the end of the 5 min LTC_4 infusion period, myocardial contractility was $79.1 \pm 2.3\%$ and $78.7 \pm 1.6\%$ in the presence of EPA 6×10^{-8} mol min^{-1} and 15×10^{-8} mol min^{-1}, respectively, and $75.6 \pm 5.0\%$ in hearts receiving LTC_4 20×10^{-12} mol min^{-1} alone, as compared to basal values ($n = 4$). The reduction in myocardial contractility was significant in all three cases ($P < 0.02$) compared to preinfusion values.

Discussion

In isolated perfused sensitized hearts of guinea-pigs the anaphylactic reaction is characterized by the occurrence of typical symptoms such as coronary

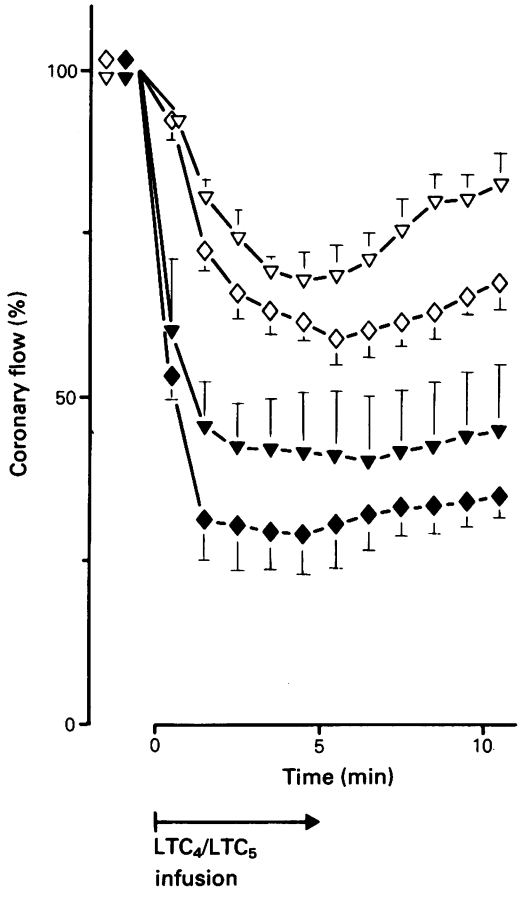

Figure 5 Effect of infusion of leukotriene C_4 (LTC_4) (◇, $n = 3$) and LTC_5 (▽, $n = 3$) at 1.0×10^{-12} mol min^{-1}, and LTC_4 (◆, $n = 3$) and LTC_5 (▼, $n = 3$) at 20.0×10^{-12} mol min^{-1} for 5 min on coronary flow in non-sensitized guinea-pig hearts. Coronary flow before leukotriene infusion was 10.1 ± 0.3 ml min^{-1} and was taken as 100%.

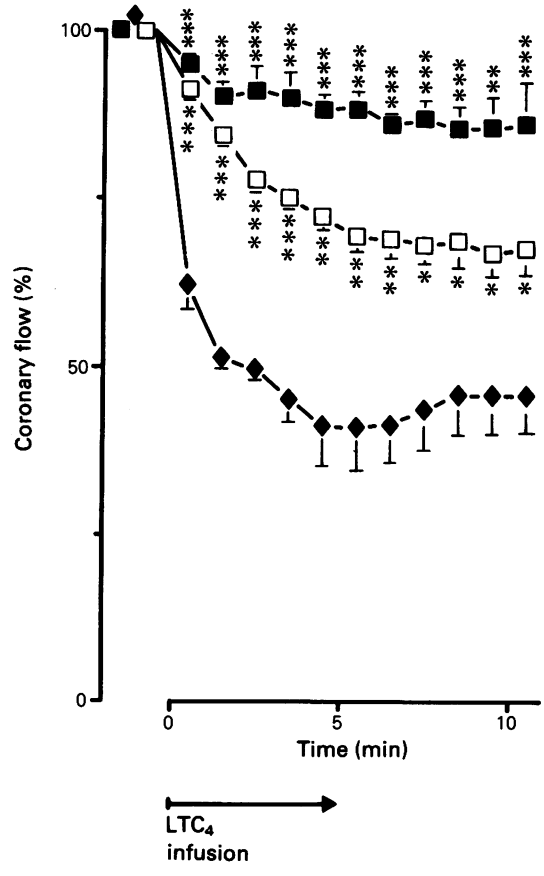

Figure 6 Effect of eicosapentaenoic acid (EPA) 6×10^{-8} mol min^{-1} (□, $n = 4$) and 15×10^{-8} mol min^{-1} (■, $n = 4$) on leukotriene C_4 (LTC_4) (20×10^{-12} mol min^{-1})-induced coronary flow reduction as compared to LTC_4 (20×10^{-12} mol min^{-1}, for 5 min) infusion alone (◆, $n = 4$). Coronary flow before LTC_4 infusion was 10.0 ± 0.6 ml min^{-1} and was taken as 100%. ***$P < 0.001$; **$P < 0.005$; *$P < 0.02$.

constriction, decrease in myocardial contractility and arrhythmias (Feigen & Prager, 1969; Capurro & Levi, 1975; Liebig *et al.*, 1975; Levi *et al.*, 1976).

The present results demonstrate that the anaphylactic event can be successfully modulated by infusion of exogenous EPA. Thus, EPA caused dose-dependent inhibition of the early more pronounced phase of anaphylactic coronary vasoconstriction and at the higher concentration used, effectively shortened the duration of arrhythmias.

EPA, a major unsaturated fatty acid in fish oil, is known to affect eicosanoid biosynthesis. Its metabolism via the cyclo-oxygenase pathway of polyunsaturated fatty acid metabolism gives rise to trienoic prostaglandins. In fact, bovine aortic microsomes and aspirin-treated platelets have been shown to convert PGH_3 into PGI_3 and TXA_3, respectively, the former exhibiting similar activities to PGI_2, whereas TXA_3 supposedly lacks any platelet aggregating activity (Needleman *et al.*, 1979). It has been suggested, however, that EPA is a rather poor substrate for cyclo-oxygenase and it was generally believed that it is an inhibitor of cyclo-oxygenase under normal physiological conditions, with no appreciable conversion into trienoic prostanoids (Culp *et al.*, 1979; Needleman *et al.*, 1979). Indeed, several investigators

failed to detect vascular PGI_3 formation after feeding rats with EPA or after incubation of rat aortic tissue or perfusion of rat hind legs and rabbit ears with EPA (Hornstra *et al.*, 1981; Juan & Sametz, 1983; Juan *et al.*, 1985). However, release of Δ^{17}-6-keto-$PGF_{1\alpha}$, the degradation product of PGI_3, from human umbilical artery incubated with EPA has been reported (Dyerberg *et al.*, 1981), as well as excretion of a urinary PGI_3 metabolite and release of TXA_3 from human platelets, both from subjects who had ingested large quantities of marine oils (Fischer & Weber, 1983; 1984).

As in the guinea-pig lung, thromboxane release predominates during anaphylaxis in guinea-pig hearts (Dawson *et al.*, 1976; Anhut *et al.*, 1977; Allan & Levi, 1981; Aehringhaus *et al.*, 1983), while under basal conditions lungs (Gryglewski *et al.*, 1978) and hearts (Schrör *et al.*, 1978) release mainly PGI_2. TXA_2 is known to induce coronary vasospasm without direct negative inotropic effects in guinea-pig isolated, working hearts (Terashita *et al.*, 1978). In the EPA-treated anaphylactic guinea-pig heart release of cyclo-oxygenase products such as immunoreactive TXB_2 and 6-keto-$PGF_{1\alpha}$ were significantly decreased. Even though infusion of EPA tended to increase release of TXB_2 during the first min after ovalbumin challenge, inhibition of TXB_2 release dominated during the later course of the anaphylactic reaction. Thus, for unknown reasons, the profile of TXB_2 release appeared to be somewhat different in the presence of EPA. The overall effect, however, was still a significant inhibition of the total release of TXB_2 during the 23 min observation period with both infusion rates of EPA. It is recognized that the cross-reaction of the corresponding EPA metabolites with the antisera are not known. However, it is highly likely that the 3-series prostanoids will cross-react to a considerable extent with the antisera employed. Thus, at least larger amounts of EPA metabolites should have been detected. Instead, significant inhibition of cyclo-oxygenase products was observed. Hornstra *et al.* (1981) reported inhibition of TXA_2 production by rat platelets and a decreased release of PGI_2-like material from rat aorta after dietary administration of EPA. Furthermore, decreased biosynthesis of PGI_2 has been observed when human endothelial cell cultures were enriched with EPA (Spector *et al.*, 1983). One could argue that inhibition of cyclo-oxygenase and thereby of the PGI_2 production of the coronary vascular bed might result in coronary vasoconstriction. However, this was not the case as shown in the results. This could be due to the fact that independent of its activity on eicosanoid biosynthesis, EPA seems to be able to antagonize smooth muscle contraction elicited by various agonists (Juan & Sametz, 1986; Simmet *et al.*, 1986).

The concept that EPA is an inhibitor of cyclo-oxygenase in the anaphylactic guinea-pig heart gains further support from the fact, that EPA antagonized the early more pronounced phase of anaphylactic coronary constriction and was accompanied by the inhibition of the antigen-induced immunoreactive TXB_2 release. Previously, it has been demonstrated, that addition of indomethacin to the perfusion medium abolished the anaphylactic release of immunoreactive TXB_2 and $PGF_{2\alpha}$, and simultaneously inhibited the early phase of anaphylactic coronary constriction, while leaving unaffected the later phase of the anaphylactic vascular reaction (Anhut *et al.*, 1977). Others described almost complete inhibition of anaphylactic coronary flow reduction by a rather high concentration of indomethacin (Levi *et al.*, 1976). However, these authors measured total coronary flow for a period of 10 min after challenge only, and might therefore, have not been able to discriminate between the indomethacin-sensitive and indomethacin-resistant phase of coronary flow reduction.

Arrhythmias of the anaphylactic guinea-pig heart are known to be due to conduction disturbances as well as to increased ventricular ectopic activity (Simmet & Peskar, 1986). In the present experiments it remains unclear whether the improvement of antigen-induced arrhythmias in the presence of the higher EPA dose is due to a direct effect of EPA or an unknown EPA-derived metabolite on the electrophysiological events or whether it merely reflects the restoration of coronary perfusion.

SP-LT have previously been shown to be potent coronary vasoconstrictors in various species (Letts & Piper, 1981; Terashita *et al.*, 1981; Burke *et al.*, 1982; Letts & Piper, 1982; 1983; Letts *et al.*, 1983; Roth *et al.*, 1985) and to be released from anaphylactic guinea-pig hearts (Aehringhaus *et al.*, 1983). Determination of SP-LT-like material in the perfusates revealed an up to 3 fold increased release during the early phase of anaphylaxis when anaphylactic guinea-pig hearts were perfused with EPA. This increased release of SP-LT-like immunoreactivity could be inhibited by the lipoxygenase inhibitor esculetin (Neichi *et al.*, 1983). Fatty acids containing a $\Delta^{5,8,11}$ unsaturation such as EPA, are readily converted by 5-lipoxygenase (Jakschik *et al.*, 1980). Therefore, it was anticipated, that the increased amount of immunoreactive SP-LT might include EPA-derived SP-LT. In fact, reversed phase h.p.l.c. revealed immunoreactivity comigrating with 4- and EPA-derived 5-series LTC, LTD and LTE, even though the solvent system used, did not clearly separate LTC_4 from LTD_5. However, due to the metabolic pathway of leukotrienes the presence of single peaks comigrating with LTC_5 and LTE_5 implicate the existence of LTD_5 as well (Samuelsson, 1983).

In the presence of EPA an increased immunological release of SRS-A from chopped guinea-pig lung tissue has previously been reported (Morris *et al.*, 1979; Piper *et al.*, 1980). On the basis of the present results, it

is believed that this increased generation of SRS-A is analagous to the increased release of SP-LT from EPA perfused anaphylactic guinea-pig hearts. Furthermore, these authors (Morris *et al.*, 1979) demonstrated release of LTB_4 upon antigen challenge from sensitized guinea-pig lung tissue. Immunologically-induced generation of SP-LT and LTB of the 4- and 5-series has recently been described in response to an IgG-mediated immune complex reaction in the peritoneal cavities of menhaden oil-fed rats (Leitch *et al.*, 1984). Using reversed phase h.p.l.c. these authors identified LTB_5 by comparison with a synthetic LTB_5 standard which was not available for the present study. The retention time of the unidentified peak migrating in front of LTB_4 in this study appeared to be identical to that of synthetic LTB_5 in the former study and in others (Lee *et al.*, 1984; Terano *et al.*, 1984; Strasser *et al.*, 1985). Since LTB_4 has been shown to have no effect on coronary flow, heart rate and contractility in isolated hearts of guinea-pig and rat (Letts & Piper, 1983), the question of its pathophysiological significance during cardiac anaphylaxis awaits further elucidation.

Inhibition of the lipoxygenase pathway of arachidonic acid metabolism in anaphylactic guinea-pig hearts by nordihydroguaiaretic acid (NDGA) abolished the late phase anaphylactic coronary constriction as did the SRS-antagonist FPL 55712, suggesting that SP-LT may be the relatively more important mediators in the late phase of anaphylactic coronary constriction (Aehringhaus *et al.*, 1983). Surprisingly, in the present study significantly increased release of SP-LT was not accompanied by significant changes during late phase anaphylactic coronary flow reduction even though it was demonstrated that vasoconstrictor activity of LTC_5 was similar to that of LTC_4, as had been described for other smooth muscle preparations, such as the guinea-pig ileum and lung parenchymal strip (Hammarström, 1980; Leitch *et al.*, 1984). Nevertheless, inhibition of the increased release of SP-LT in the presence of EPA by the lipoxygenase inhibitor esculetin (Neichi *et al.*, 1983) resulted in significantly increased coronary flow during the late phase of cardiac anaphylaxis. Like the lipoxygenase inhibitor NDGA, esculetin also inhibits cyclo-oxygenase activity, although the IC_{50} for platelet cyclo-oxygenase seems to be three orders of magnitude higher than that for platelet lipoxygenase (Sekiya *et al.*, 1982).

The assumption of a direct or indirect effect of EPA on the SP-LT-induced coronary flow reduction was verified by the experiments demonstrating a dose-dependent inhibition of LTC_4-induced coronary constriction by EPA. Although EPA is metabolized into 5-series SP-LT of comparable vasoconstrictor activity to 4-series SP-LT, the data suggest that EPA is able to antagonize the coronary constrictor activity of SP-LT, thus acting as a functional antagonist.

Interestingly, exogenous SP-LT which are known to have negative inotropic effects (Letts & Piper, 1981; 1982; 1983; Burke *et al.*, 1982; Roth *et al.*, 1985) and which dose-dependently decreased myocardial contractility of non-sensitized guinea-pig hearts continued to exhibit negative inotropic effects in the presence of EPA, which did, however, dose-dependently restore coronary flow. Several authors did not find any negative inotropic effect of exogenous SP-LT on spontaneously beating atria or electrically driven papillary muscles from cat, rat or guinea-pig (Letts & Piper, 1981; 1982; 1983; Roth *et al.*, 1985) and consequently suggested that the negative inotropism should be secondary to vasoconstriction. In contrast, however, Burke *et al.* (1982) have shown that an angiotensin II-induced coronary flow reduction, comparable to that induced by leukotrienes, was accompanied by only a slight negative inotropic effect. In potassium-depolarized guinea-pig myocardium, LTC_4 and LTD_4 have been reported to inhibit calcium-dependent contractile responses and it was suggested, that the negative inotropic effect of SP-LT might result from a reduction in calcium ion influx through the sarcolemma (Hattori & Levi, 1984). The present data suggest that SP-LT might have a direct negative inotropic effect, because the negative inotropic effect remained unchanged despite almost complete restoration of the coronary flow in the presence of the higher dose of EPA. Similarly, in sheep the effect of exogenous LTD_4 on coronary flow was abolished by FPL 55712, but the antagonist only partially inhibited the negative inotropic effect of LTD_4 (Michelassi *et al.*, 1982).

In conclusion the data suggest that EPA is able to modulate the anaphylactic reaction in guinea-pig isolated hearts with respect to coronary flow reduction and duration of antigen-induced arrhythmias. EPA was effectively metabolized via the 5-lipoxygenase pathway of polyunsaturated fatty acid metabolism, but inhibited the cyclo-oxygenase pathway. By an unknown mechanism, EPA seems to inhibit vasoconstrictor activity of exogenous SP-LT, thus acting as a functional antagonist. Furthermore, the data are in agreement with the suggestion that cyclo-oxygenase products are mediators of the early phase of anaphylactic coronary vasoconstriction, while vasoconstrictor SP-LT are involved in the later phase.

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References

AEHRINGHAUS, U., WÖLBLING, R.H., KÖNIG, W., PATRONO, C., PESKAR, B.M. & PESKAR, B.A. (1982). Release of leukotriene C_4 from human polymorphonuclear leucocytes as determined by radioimmunoassay. *FEBS Letters*, **146**, 111–114.

AEHRINGHAUS, U., PESKAR, B.A., WITTENBERG, H.R. & WÖLBLING, R.H. (1983). Effect of inhibition of synthesis and receptor antagonism of SRS-A in cardiac anaphylaxis. *Br. J. Pharmac.*, **80**, 73–80.

ALLAN, G. & LEVI, R. (1981). Thromboxane and prostacyclin release during cardiac immediate hypersensitivity reactions in vitro. *J. Pharmac. exp. Ther.*, **217**, 157–161.

ANHUT, H., BERNAUER, W. & PESKAR, B.A. (1977). Radioimmunological determination of thromboxane release in cardiac anaphylaxis. *Eur. J. Pharmac.*, **44**, 85–88.

ANHUT, H., BERNAUER, W. & PESKAR, B.A. (1978). Pharmacological modification of thromboxane and prostaglandin release in cardiac anaphylaxis. *Prostaglandins*, **15**, 889–900.

AUSTEN, K. (1978). The anaphylactic syndrome. In *Immunological Diseases*. ed. Samter, M. pp. 885–899. Boston: Little Brown.

BERNSTRÖM, K., ÖRNING, L. & HAMMARSTRÖM, S. (1982). γ-Glutamyl transpeptidase, a leukotriene metabolizing enzyme. *Methods Enzymol.*, **86**, 38–45.

BROCKLEHURST, W.E. (1960). The release of histamine and formation of slow-reacting substance (SRS-A) during anaphylactic shock. *J. Physiol.*, **151**, 416–435.

BURKE, J.A., LEVI, R., GUO, Z.G. & COREY, E.J. (1982). Leukotrienes C_4, D_4 and E_4: effects on human and guinea-pig cardiac preparations in vitro. *J. Pharmac. exp. Ther.* **221**, 235–241.

CAPURRO, N. & LEVI, R. (1975). The heart as a target organ in systemic allergic reactions. Comparison of cardiac anaphylaxis in vivo and in vitro. *Circulation Res.*, **36**, 520–528.

CULP, B.R., TITUS, B.G. & LANDS, W.E.M. (1979). Inhibition of prostaglandin biosynthesis by eicosapentaenoic acid. *Prostaglandins Med.*, **3**, 269–278.

DAWSON, W., BOOT, J.R., COCKERILL, A.F., MALLEN, D.N.B. & OSBORNE, D.J. (1976). Release of novel prostaglandins and thromboxane after immunological challenge of guinea-pig lung. *Nature*, **262**, 699–702.

DYERBERG, J., BANG, H.O., STOFFERSEN, E., MONCADA, S. & VANE, J.R. (1978). Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. *Lancet*, **ii**, 117–119.

DYERBERG, J., JØRGENSEN, K.A. & ARNFRED, T. (1981). Human umbilical blood vessel converts all cis-5, 8, 11, 14, 17-eicosapentaenoic acid to prostaglandin I_3. *Prostaglandins*, **22**, 857–862.

FEIGEN, G.A. & PRAGER, D.J. (1969). Experimental cardiac anaphylaxis. Physiologic, pharmacologic and biochemical aspects of immune reactions in the isolated heart. *Am. J. Cardiol.*, **24**, 474–491.

FISCHER, S. & WEBER, P.C. (1983). Thromboxane A_3 (TXA_3) is formed in human platelets after dietary eicosapentaenoic acid (C2o:5ω3). *Biochem. biophys. Res. Commun.*, **116**, 1091–1099.

FISCHER, S. & WEBER, P.C. (1984). Prostaglandin I_3 is formed in vivo in man after dietary eicosapentaenoic acid. *Nature*, **307**, 165–168.

GRYGLEWSKI, R.J., KORBUT, R. & OCETKIEWICZ, A. (1978). Generation of prostacyclin by lungs in vivo and its release into the arterial circulation. *Nature*, **273**, 765–767.

HAMMARSTRÖM, S. (1980). Leukotriene C_5: a slow reacting substance derived from eicosapentaenoic acid. *J. biol. Chem.*, **255**, 7093–7094.

HATTORI, Y. & LEVI, R. (1984). Negative inotropic effect of leukotrienes: leukotrienes C_4 and D_4 inhibit calcium-dependent contractile responses in potassium-depolarized guinea-pig myocardium. *J. Pharmac. exp. Ther.*, **230**, 646–651.

HORNSTRA, G., CHRIST-HAZELHOF, E., HADDEMANN, E., TEN HOOR, R. & NUGTEREN, D.H. (1981). Fishoil feeding lowers thromboxane- and prostacyclin production by rat platelets and aorta and does not result in the formation of prostaglandin I_3. *Prostaglandins*, **21**, 727–738.

JAKSCHIK, B.A., SAMS, A.R., SPRECHER, H. & NEEDLEMAN, P. (1980). Fatty acid structural requirements for leukotriene biosynthesis. *Prostaglandins*, **20**, 401–410.

JUAN, H. & SAMETZ, W. (1983). Uptake, stimulated release and metabolism of (1 − ^{14}C)-eicosapentaenoic acid in a perfused organ of the rabbit. *Naunyn-Schmiedebergs Arch. Pharmac.*, **324**, 207–211.

JUAN, H. & SAMETZ, W. (1986). Vasoconstriction induced by noradrenaline and angiotensin II is antagonized by eicosapentaenoic acid independent of formation of trienoic eicosanoids. *Naunyn-Schmiedebergs Arch. Pharmac.*, **332**, 288–292.

JUAN, H., WINDISCH, I. & SAMETZ, W. (1985). Metabolism of ^{14}C-timnodonic acid in rat tissue. In *Prostaglandins and Other Eicosanoids in the Cardiovascular System*. ed. Schrör, K. pp. 84–88. Basel: Karger.

LEE, T.H., MENCIA-HUERTA, J.M., SHIH, C., COREY, E.J., LEWIS, R.A. & AUSTEN, K.F. (1984). Characterization and biologic properties of 5, 12-dihydroxy derivatives of eicosapentaenoic acid, including leukotriene B_5 and the double lipoxygenase product. *J. biol. Chem.*, **259**, 2383–2389.

LEITCH, A.G., LEE, T.H., RINGEL, E.W., PRICKETT, J.D., ROBINSON, D.R., PYNE, S.G., COREY, E.J., DRAZEN, J.M., AUSTEN, K.F. & LEWIS, R.A. (1984). Immunologically induced generation of tetraene and pentaene leukotrienes in the peritoneal cavities of menhaden-fed rats. *J. Immunol.*, **132**, 2559–2565.

LETTS, L.G. & PIPER, P.J. (1981). The effects of leukotrienes (LT) D_4 and C_4 on the guinea-pig isolated heart. *J. Physiol.*, **317**, 94P–95P.

LETTS, L.G. & PIPER, P.J. (1982). The actions of leukotrienes C_4 and D_4 on guinea-pig isolated hearts. *Br. J. Pharmac.*, **76**, 169–176.

LETTS, L.G. & PIPER, P.J. (1983). Cardiac actions of leukotrienes B_4, C_4, D_4 and E_4 in guinea-pig and rat in vitro. *Adv. Prostaglandin Thromboxane Leukotriene Res.*, **11**, 391–395.

LETTS, L.G., NEWMAN, D.L., GREENWALD, S.E. & PIPER, P.J. (1983). Effects of intra-coronary administration of leukotriene D_4 in the anesthetized dog. *Prostaglandins*, **26**, 563–572.

LEVI, R., ALLAN, G. & ZAVECZ, J.H. (1976). Prostaglandins and cardiac anaphylaxis. *Life Sci.*, **18**, 1255–1264.

LEVI, R., BURKE, J.A., GUO, Z.G., HATTORI, Y., HOPPENS, C.M., MCMANUS, L.M., HANAHAN, D.J. & PINCKARD, R.N. (1984). Acetyl glyceryl ether phosphorylcholine (AGEPC). A putative mediator of cardiac anaphylaxis in the guinea-pig. *Circulation Res.*, **54,** 117–124.

LIEBIG, R., BERNAUER, W. & PESKAR, B.A. (1975). Prostaglandin, slow-reacting substance, and histamine release from anaphylactic guinea-pig hearts, and its pharmacological modification. *Naunyn-Schmiedebergs Arch. Pharmac.*, **289,** 65–76.

MICHELASSI, F., LANDA, L., HILL, R.D., LOWENSTEIN, E., WATKINS, W.D., PETKAU, A.J. & ZAPOL, W.M. (1982). Leukotriene D_4: a potent coronary artery vasoconstrictor associated with impaired ventricular contraction. *Science*, **217,** 841–843.

MORRIS, H.R., TAYLOR, G.W., PIPER, P.J. & TIPPINS, J.R. (1979). Slow-reacting substance of anaphylaxis: studies on purification and characterisation. *Agents & Actions*, Suppl. **6,** 27–36.

NEEDLEMAN, P., RAZ, A., MINKES, M.S., FERENDELLI, J.A. & SPRECHER, H. (1979). Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. *Proc. natn. Acad. Sci., U.S.A.*, **76,** 944–948.

NEICHI, T., KOSHIHARA, Y. & MUROTA, S.I. (1983). Inhibitory effect of esculetin on 5-lipoxygenase and leukotriene biosynthesis. *Biochem. biophys. Acta*, **753,** 130–132.

PESKAR, B.A., STEFFENS, Ch. & PESKAR, B.M. (1979). Radioimmunoassay of 6-keto-prostaglandin $F_{1\alpha}$ in biological material. In *Radioimmunoassay of Drugs and Hormones in Cardiovascular Medicine*. ed. Albertini, A., DaPrada, M. & Peskar, B.A. pp. 239–250. Amsterdam: Elsevier/North-Holland Biomedical Press.

PESKAR, B.M., KOZUSCHEK, W., MORGENROTH, K., HOPPE, U., DREYLING, K.W. & PESKAR, B.A. (1986). Leukotriensythese durch gastrointestinales Gewebe und ihre pharmakologische Beeinflussung. *Wien. Klin. Wochenschr.*, **98,** 98–104.

PIPER, P.J., TIPPINS, J.R., MORRIS, H.R. & TAYLOR, G.W. (1980). Potentiation of SRS-A release from guinea pig chopped lung by subtrates for arachidonate lipoxygenase. *Adv. Prostaglandin Thromboxane Res.*, **6,** 121–124.

ROTH, D.M., LEFER, D.J., HOCK, C.E. & LEFER, A.M. (1985). Effects of peptide leukotrienes on cardiac dynamics in rat, cat and guinea pig hearts. *Am. J. Physiol.*, **249,** H477–484.

SAMUELSSON, B. (1983). Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science*, **220,** 568–575.

SCHRÖR, K., MONCADA, S., UBATUBA, F.B. & VANE, J.R. (1978). Transformation of arachidonic acid and prostaglandin endoperoxides by the guinea pig heart. Formation of RCS and prostacyclin. *Eur. J. Pharmac.*, **47,** 103–114.

SEKIYA, K., OKUDA, H., & ARICHI, S. (1982). Selective inhibition of platelet lipoxygenase by esculetin. *Biochim. biophys. Acta*, **713,** 68–72.

SIMMET, Th. & PESKAR, B.A. (1986). Eicosanoids and the coronary circulation. *Rev. Physiol. Biochem. Pharmac.*, **104,** 1–64.

SIMMET, Th., AISSA, J., SUTTER, D. & JUAN, H. (1986). Effect of exogenous eicosapentaenoic acid (EPA) on the mechanical response and eicosanoid release in guinea-pig lung parenchymal strips. *Naunyn-Schmiedebergs Arch. Pharmac.*, **332** (Suppl), 148.

SPECTOR, A.A., KADUCE, T.L., FIGARD, P.H., NORTON, K.C., HOAK, J.C. & CZERVIONKE, R.L. (1983). Eicosapentaenoic acid and prostacyclin production by cultured human endothelial cells. *J. Lipid. Res.*, **24,** 1595–1604.

STRASSER, T., FISCHER, S. & WEBER, P.C. (1985). Leukotriene B_5 is formed in human neutrophils after dietary supplementation with icosapentaenoic acid. *Proc. natn. Acad. Sci., U.S.A.*, **82,** 1540–1543.

TERANO, T., SALMON, J.A. & MONCADA, S. (1984). Biosynthesis and biological activity of leukotriene B_5. *Prostaglandins*, **27,** 217–232.

TERASHITA, Z.I., FUKUI, H., NISHIKAWA, K., HIRATA, M., & KIKUCHI, S. (1978). Coronary vasospastic action of thomboxane A_2 in isolated, working guinea pig hearts. *Eur. J. Pharmac.*, **53,** 49–56.

TERASHITA, Z.I., FUKUI, H., HIRATA, M., TERAO, S., OHKAWA, S., NISHIKAWA, K. & KIKUCHI, S. (1981). Coronary vasoconstriction and PGI_2 release by leukotrienes in isolated guinea pig hearts. *Eur. J. Pharmac.*, **73,** 357–361.

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