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Coronary vasoconstriction in the rat, isolated perfused heart induced by platelet-activating factor is mediated by leukotriene C_4

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1 Platelet-activating factor (Paf, 0.04–4.50 nmol) dose-dependently induced coronary vasoconstriction and decreased cardiac contractility in rat, isolated perfused hearts and concomitantly released leukotriene-like bioactivity into the cardiac effluent.

2 Platelet-activating factor (0.9 nmol) induced an increase in 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), $PGF_{2\alpha}$, PGE_2 and thromboxane B_2 (TXB_2) measured by radioimmunoassay (RIA) of cardiac effluents following partial purification using C_{18} Sep-Paks.

3 The leukotriene-like bioactivity released by Paf was identified as leukotriene C_4 (LTC_4) using a combination of isolation on reverse phase-h.p.l.c. (r.p.h.p.l.c.) and quantitation by RIA. In addition, LTB_4 was also identified by r.p.h.p.l.c. and the levels, determined by RIA, were within the range having biological activity.

4 The release of cyclo-oxygenase products by Paf was prevented by indomethacin (2.8 μM), markedly attenuated by diethylcarbamazine (7.7 mM) but unaffected by FPL 55712 (1.9 μM)-pretreatment. Furthermore, LTC_4 (50 pmol) did not increase the release of the cyclo-oxygenase products measured.

5 The release of LTB_4 and LTC_4 appeared to be unaffected by indomethacin pretreatment whereas diethylcarbamazine-pretreatment markedly inhibited release.

6 The coronary vasoconstriction induced by Paf (0.9 nmol) was attenuated by pretreatment with indomethacin or diethylcarbamazine, whereas FPL 55712 caused a marked inhibition of the response. In contrast, the decrease in cardiac contractility was prevented by indomethacin or diethylcarbamazine and unaffected by FPL 55712 pretreatment.

7 It is concluded that LTC_4 may be largely responsible for the coronary vasoconstriction induced by Paf with cyclo-oxygenase products having a possible modulatory role whereas the latter appear to be involved in the Paf-induced decrease in cardiac contractility.

Introduction

Platelet-activating factor (Paf), a putative mediator of inflammation, has recently been implicated in cardiac anaphylaxis (Levi *et al.*, 1984). Paf exerts both platelet-dependent (Heffner *et al.*, 1983; Lefer *et al.*, 1984) and platelet-independent actions (Voelkel *et al.*, 1982) in the cardiovascular system.

In rat isolated perfused lungs Paf induces pulmonary vasoconstriction (Voelkel *et al.*, 1982) which is accompanied by release of cysteinyl-containing leukotrienes and is inhibited by the putative lipoxygenase inhibitor, diethylcarbamazine. Furthermore, Paf elicits the formation of leukotrienes from cat chopped pulmonary and vascular tissues and from rat and guinea-pig chopped lung tissues (Lefer *et al.*, 1984). Platelet-activating factor induces coronary vasoconstriction in the guinea-pig, isolated perfused heart (Benveniste *et al.*, 1983; Levi *et al.*, 1984) in the dog *in vivo* (Kenzora *et al.*, 1984; Sybertz *et al.*, 1985) and in the pig *in vivo* (Feuerstein *et al.*, 1984). It has been suggested that the coronary vasoconstriction induced by Paf may be indirectly mediated via the release of leukotrienes (Feuerstein *et al.*, 1984; Kenzora *et al.*, 1984; Sybertz *et al.*, 1985) on the basis of the inhibitory actions of the combined cyclo-oxygenase/lipoxygenase inhibitor, BW755C and the leukotriene receptor antagonist, FPL 55712.

The present study was undertaken to define the role of arachidonic acid metabolites in coronary vasoconstrictor responses to Paf in rat, isolated perfused hearts. The use of rats to study the mechanism of action of Paf precludes the confounding influence of platelets since the actions of Paf in the rat appear to be platelet-independent (Terashita *et al.*, 1983).

A preliminary account of some of this work has been presented to the British Pharmacological Society (Piper & Stewart, 1985).

Methods

Isolated, perfused hearts

Hearts from male Wistar rats (300–400g) were perfused via the aorta using Krebs solution which was maintained at 37°C and gassed with 95% O_2 plus 5% CO_2. Rats were pretreated with heparin (3000 u kg^{-1}, i.p.), 15 min before obtaining the hearts, then killed by a blow to the head and exsanguinated. The heart was rapidly excised and placed in ice-cold Krebs solution for dissection. The aorta was cannulated retrogradely and suspended from the perfusion apparatus within two minutes. The composition of the Krebs solution was as follows (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgCl_2$ 0.5, NaH_2PO_4 1.0 and D-glucose 11.1. The Krebs solution was perfused through the hearts at a constant flow rate of 8 ml min^{-1} for 45 min before the commencement of the experiments. Each heart received only one dose of Paf which induced a complete tachyphylaxis. Measurements of cardiac contractility were made by attaching a cotton thread, hooked by a pin to the apex of the left ventricle, to a force displacement transducer (GRASS FT03). A resting tension of 4 g weight was applied and the developed isometric tension was displayed on a multi-channel recorder (Graphtec linearcorder Mark VII VR3101) following preamplification. Coronary perfusion pressure was measured by attaching a side-arm of the aortic cannula to a pressure transducer (Elcomatic EM50) and displayed as above. Heart rate was measured intermittently by increasing the trace speed to count the beats over a 2s period.

Drug pretreatments

When the heart was treated with synthesis inhibitors, these were added to the Krebs reservoir before commencement of heart perfusion (indomethacin, 2.8 μM; diethylcarbamazine, 7.7 mM). FPL 55712 (1.9 μM) was infused for 20 min before the administration of Paf. Drugs administered by bolus injection were given in a volume of 10–100 μl into the perfusion line 2 cm proximal to the aortic cannula. All drugs except indomethacin and FPL 55712 were dissolved in Krebs solution. Indomethacin was initially dissolved in 0.1 M Na_2CO_3 then added to Krebs (ml l^{-1}). FPL 55712 was initially dissolved in distilled H_2O then infused at a rate of 0.1 ml min^{-1} into the perfusion fluid.

Bioassay

Male Dunkin-Hartley guinea-pig (0.5–1.0 kg) were killed by a blow to the head and exsanguinated. Three strips of longitudinal smooth muscle from the ileum (GPISM) were prepared according to the method of Rang (1964) and superfused in series to detect the release of leukotriene-like material. The strips were placed under a load of 0.5 g and continuously superfused with the cardiac effluent to which a mixture of antagonists was added to increase the specificity of the bioassay (0.84 μM hyoscine; 0.35 μM mepyramine; 0.57 μM methysergide; 7.7 μM propranolol). In addition, the last strip was continuously superfused with the leukotriene receptor antagonist, FPL 55712 (1.9 μM) after confirming that this tissue was sensitive to leukotriene D_4 (LTD_4). Tissues were calibrated with LTD_4 (1–40 pmol) administered directly over the GPISM. Changes in length of the tissues were measured auxotonically using Havard Smooth Muscle Transducers and the outputs were displayed on a multi-channel recorder (Graphtec Linearcorder Mark VII 3101).

Preparation of samples for radioimmunoassay

In experiments in which the cardiac effluents were analysed by radioimmunoassay (RIA) for cyclo-oxygenase and lipoxygenase products, the cardiac effluent was collected on ice for a 10 min control period. A further 10 min collection was made following Paf (0.9 nmol) or LTC_4 (50 pmol) administration. The resulting cardiac effluent samples were divided into aliquots of equal volume (40 ml) and passed through Sep-Pak C_{18} cartridges (Waters Associates) at a flow rate of 5 ml min^{-1}. Before sample application, the Sep-Pak cartridges were washed with methanol (5 ml) then distilled H_2O (5 ml). Following sample application, Sep-Paks were washed with distilled H_2O (5 ml), the arachidonic acid metabolites were eluted with methanol (5 ml), evaporated to dryness under vacuum and stored under N_2 at −20°C until RIA (cyclo-oxygenase products) or further purification (lipoxygenase products) was carried out. The percentage recoveries of the cyclo-oxygenase products were determined by the addition of tritium-labelled 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$; 99 + 3), $PGF_{2\alpha}$ (90 + 1), PGE_2 (64 + 1) or thromboxane B_2 (TXB_2 82 + 2) to 40 ml of Krebs solution ($n = 3$ for each product) and passed separately through Sep-Paks in the manner previously described. Since the recoveries showed little

variability the amounts of cyclo-oxygenase products determined by RIA have not been corrected for recovery.

Samples for assay of leukotrienes were further purified on reverse-phase (r.p.-) h.p.l.c. (Waters Associates; mobile phase: 80% methanol, 20% H_2O; 0.01% acetic acid; adjusted to pH 5.4 with NH_4OH; flow rate 1.0 ml min^{-1}; Spherisorb 5u ODS stainless steel column (25 × 0.5 cm, Hichrom Ltd). Before passing the sample for leukotriene RIA through Sep-Paks, approximately 4000 d.p.m. each of [^{3}H]-LTB_4 and [^{3}H]-LTC_4 were added to enable estimates of recovery to be made. In addition, 100 µl aliquots of 500 µl fractions from the h.p.l.c. were counted (Packard Tri-Carb 4640, liquid scintillation counter) to determine retention times of [^{3}H]-LTB_4 and [^{3}H]-LTC_4. The percentage recoveries of LTB_4 (79 ± 3, n = 46) and, in particular LTC_4 (46 ± 3, n = 46) were lower and more variable than those of the cyclo-oxygenase products and have been used to correct the levels of these lipoxygenase products. u.v. absorbance at 229 and 280 nm was measured and used to determine the retention times of synthetic leukotrienes. Fractions corresponding to the retention times of ^{3}H-leukotrienes (LTB_4 7.6 ± 0.1 min; LTC_4 5.5 ± 0.1 min) were collected for subsequent RIA of LTB_4 (Salmon *et al.*, 1982) and LTC_4 (Hayes *et al.*, 1983). The fraction containing the radioactive peak of either [^{3}H]-LTC_4 or [^{3}H]-LTB_4 and the two preceding and two following fractions were analysed by RIA.

In addition, fractions corresponding to the retention time of synthetic LTD_4 (16–18 min) were analysed by RIA using the LTC_4 antiserum which shows a significant cross-reaction (limit of detection 0.2 pmol) with LTD_4 (Hayes *et al.*, 1983). Since the LTC_4 antiserum shows little cross-reactivity with LTE_4, the fractions corresponding to the retention time of synthetic LTE_4 were bioassayed on GPISM.

Radioimmunoassay

Radioimmunoassay for cyclo-oxygenase products was performed according to the method of Jose *et al.* (1976). The cross-reactivities for the antisera for 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 (TXB_2) have been previously reported (Watts *et al.*, 1982). The percentage cross-reactions of the PGE_2 antiserum at 50% binding with other cyclo-oxygenase products were PGE_1, 26.00; $PGF_{2\alpha}$, 1.40; TXB_2, 0.03; 6-keto-$PGF_{1\alpha}$, 0.03; arachidonic acid, 0.06. Those for $PGF_{2\alpha}$ antiserum at 50% binding were 6-keto-$PGF_{1\alpha}$, 8.80; PGE_2 0.20; TXB_2 3.60; arachidonic acid 0.01.

LTB_4 (Salmon *et al.*, 1982) and LTC_4 (Hayes *et al.*, 1983) were assayed using the antisera and methods described, with the exception of the separation of bound from free [^{3}H]-LTC_4. The latter was modified to shorten the assay by 24 h using ammonium sulphate precipitation rather than immunoprecipitation. The limit of detection (0.1–0.2 pmol LTC_4) was similar to that reported by Hayes *et al.* (1983).

Statistical analyses

The data were analysed by two-tailed unpaired Student's t test. A P value less than 0.05 was considered to be significant. Data are presented as the means and standard errors of the means (s.e.mean) of n observations.

Materials

All chemicals used were of analytical grade. The solvents used for h.p.l.c. were of h.p.l.c. grade. The compounds used were obtained from the following sources: A23187, Calbiochem; Arg-vasopressin, Parke-Davis; diethylcarbamazine, Sigma; FPL 55712, sodium 7-[3-(4-acetyl-3-hydroxy-2-propyl-phenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate), Fisons Pharmaceuticals; heparin, Evans; indomethacin, Merck, Sharp and Dohme; synthetic LTB_4, LTC_4, LTD_4 and LTE_4, LTC_4 antiserum, Merck Frosst Laboratories, Canada; LTB_4 antiserum, Wellcome Research Laboratories; 6–5, 8, 9, 11, 12, 14, 15, -[^{3}H]-keto $PGF_{1\alpha}$ 100 Ci $mmol^{-1}$, 14, 15, -[^{3}H]-LTB_4 32 Ci $mmol^{-1}$, 14, 15, -[^{3}H]-LTC_4 40 Ci $mmol^{-1}$, New England Nuclear; 5, 6, 8, 11, 12, 14, 15, -[^{3}H]-PGE_2 160 Ci $mmol^{-1}$, 5, 6, 8, 11, 12, 14, 15 [^{3}H]-$PGF_{2\alpha}$ 160 Ci $mmol^{-1}$ and 5, 6, 8, 9, 11, 12, 14, 15 -[^{3}H]-TXB_2 180 Ci $mmol^{-1}$, Amersham.

Results

Paf-induced cardiac effects and release of leukotriene-like material

Injection of Paf (0.04–4.50 nmol) into the fluid perfusing isolated hearts elicited an increase in coronary perfusion pressure and a decrease in cardiac contractility (Figures 1 and 2, Table 1) whereas, over this dose range, heart rate remained unaltered (data not shown). The increase in coronary perfusion pressure and the release of leukotriene-like material occurred between 15 and 30s after Paf administration. The time-course of the increase in coronary perfusion pressure (Figure 3) indicates that the peak effect occurs within 5 min and that the increase is maintained in excess of 10 min. The decrease in contractility was maintained over the 10 min observation period (Table 1). The superfused GPISM tissues relaxed to the pre-Paf tone within 20 min (Figure 1).

The release of leukotriene-like material by Paf was dose-dependent (0.04–4.50 nmol) whereas maximal

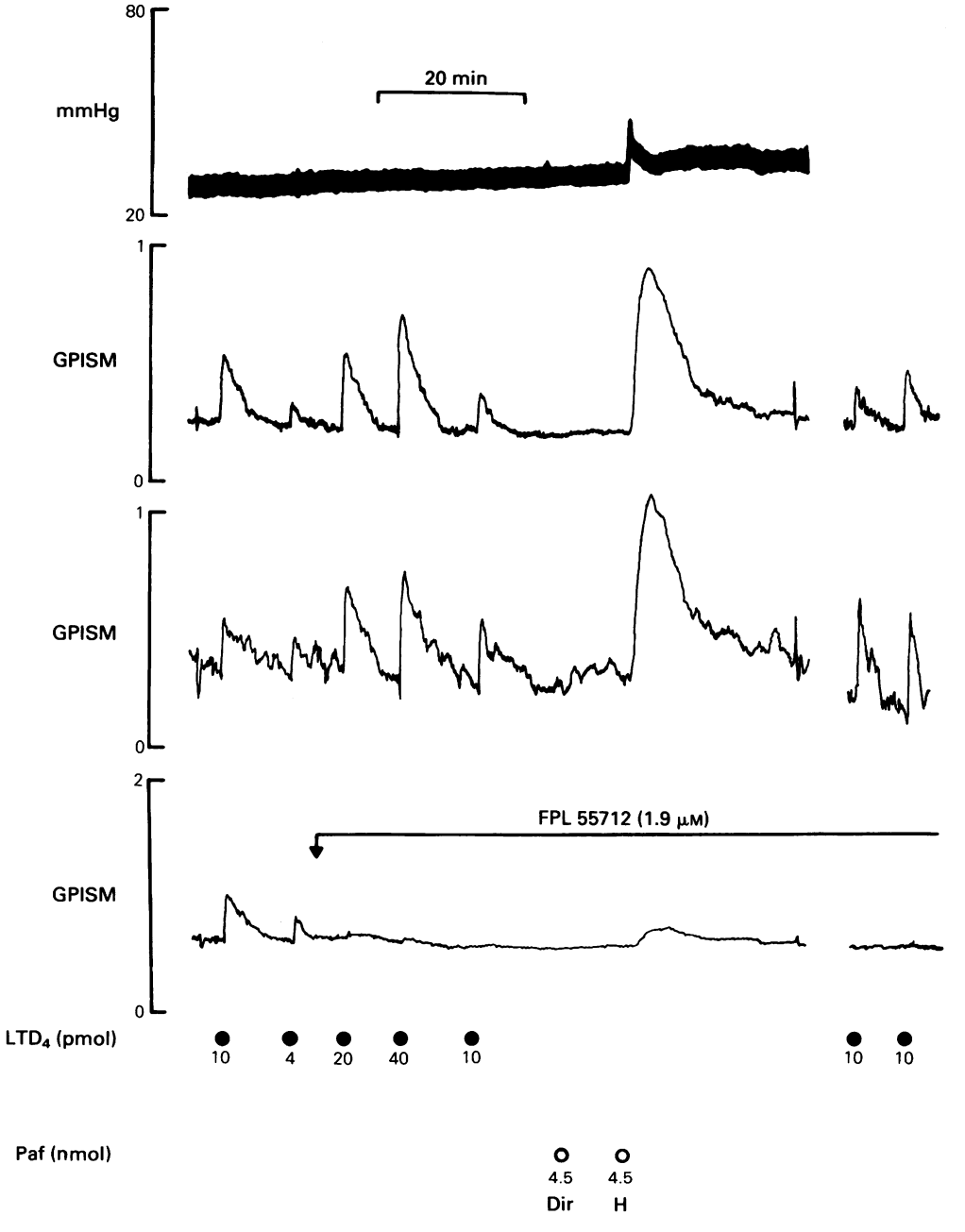

Figure 1 The effect of a bolus dose of platelet-activating factor (Paf) on coronary perfusion pressure (upper tracing, mmHg) and on superfused guinea-pig longitudinal smooth muscle from the ileum (GPISM; lower tracings, V). Leukotriene D_4 (LTD_4, 4–40 pmol) was administered directly over the GPISM tissues. The last tissue was continuously exposed to FPL 55712 following control responses to LTD_4. Paf administered directly (DIR) over the assay tissues did not affect the tone. In contrast, when Paf was administered to the heart (H) there was a substantial contraction of the upper 2 assay tissues which was markedly inhibited by FPL 55712 in the third tissue.

Table 1 The effect of platelet-activating factor (Paf) (0.90 nmol) on cardiac contractility in control, indomethacin, diethylcarbamazine and FPL 55712 pretreated hearts

Treatment	n	*Control cardiac contractility* (g)	*Paf-induced change in cardiac contractility (g)*[1] *2 min*	*5 min*	*10 min*
Control	17	1.35 ± 0.15	− 0.24 ± 0.08*	− 0.37 ± 0.11*	− 0.42 ± 0.12*
Indomethacin (2.8 μM)	12	1.12 ± 0.12	+ 0.02 ± 0.04	+ 0.03 ± 0.04	+ 0.02 ± 0.02
Diethylcarbamazine (7.7 mM)	11	1.20 ± 0.14	+ 0.06 ± 0.01	+ 0.20 ± 0.09	+ 0.10 ± 0.05
FPL 55712 (1.9 μM)	9	1.33 ± 0.21	− 0.21 ± 0.08*	− 0.29 ± 0.06*	− 0.26 ± 0.07*

*$P < 0.05$, compared to resting value of contractility, paired Student's t test. [1]Data are presented as mean ± s.e.mean of changes from control cardiac contractility at 2, 5 and 10 min after Paf administration.

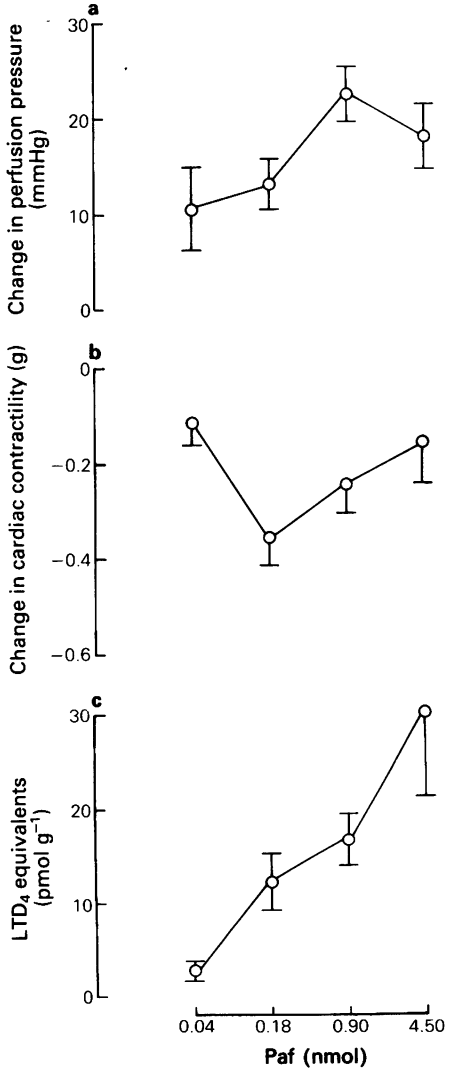

Figure 2 The effects of platelet-activating factor (Paf) (0.04–4.50 nmol) on (a) coronary perfusion pressure, (b) cardiac contractility, and (c) release of leukotriene-like material from rat isolated perfused hearts detected by superfusion bioassay on strips of GPISM and quantitated by comparison to synthetic leukotriene D_4 (LTD_4) ($n = 4–6$).

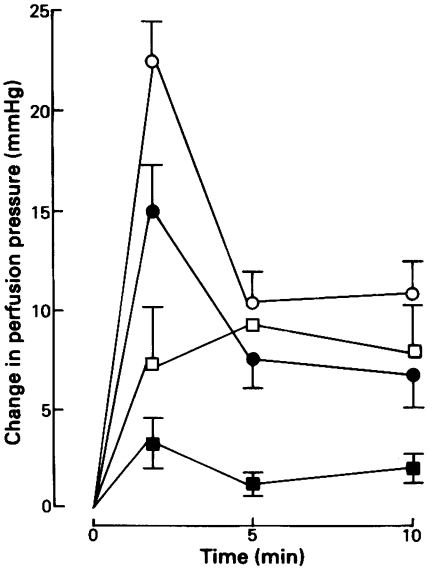

Figure 3 Platelet-activating factor (Paf, 0.9 nmol)-induced increases in coronary perfusion pressure in control hearts (○) and in those pretreated with indomethacin 2.8 μM (●), diethylcarbamazine 7.7 mM (□) or FPL 55712 1.9 μM (■), at 2, 5, and 10 min post-Paf ($n = 9–13$). Ordinate scale: change in coronary perfusion pressure (mmHg). Abscissa scale: time after Paf administration (min).

Table 2 The effects of pretreatments on the resting values of coronary perfusion pressure, cardiac contractility, heart rate and heart wet weight

Treatment	n	*Coronary perfusion pressure* (mmHg)	*Cardiac contractility* (g)	*Heart rate* (beats min^{-1})	*Heart wet weight*[1] (g)
Control	13	43.5 ± 2.7	1.39 ± 0.18	258 ± 9	1.35 ± 0.05
Indomethacin (2.8 μM)	12	40.6 ± 2.1	1.12 ± 0.12	248 ± 8	1.40 ± 0.05
Diethylcarbamazine (7.7 mM)	11	68.5 ± 5.0*	1.20 ± 0.14	150 ± 8*	1.55 ± 0.08
FPL 55712 (1.9 μM)	9	42.8 ± 2.9	1.37 ± 0.19	237 ± 16	1.33 ± 0.04

[1]Heart wet weight was measured at the end of the experiment.
*$P < 0.05$, compared to control (unpaired Student's t test).

increases in coronary perfusion pressure and decreases in cardiac contractility were achieved with 0.90 nmol and 0.18 nmol, respectively (Figure 2).

Effects of pretreatments modifying the actions and release of arachidonic acid metabolites

The resting values of the cardiac parameters (Table 2) were unchanged by the drug pretreatments used with the exception of diethylcarbamazine which increased coronary perfusion pressure and reduced heart rate.

Paf (0.9 nmol)-induced increases in coronary perfusion pressure were attentuated ($P < 0.05$, unpaired Student's t test) by indomethacin (2.8 μM) or diethylcarbamazine (7.7 mM) pretreatment at 2 min, but not at 5 or 10 min post-Paf. In contrast, pretreatment with FPL 55712 (1.9 μM) markedly inhibited the increase in coronary perfusion pressure at 2, 5 and 10 min post-Paf. Conversely, the decrease in contractility induced by Paf was prevented by pretreatment with either indomethacin or diethylcarbamazine (Table 1), whereas FPL 55712 had no significant effect ($P < 0.05$, unpaired Student's t test).

Paf-induced release of cyclo-oxygenase and lipoxygenase products

During the control period, there was a significant production and release of cyclo-oxygenase products (Table 3) whereas in none of the control, indomethacin- or diethylcarbamazine-pretreated hearts was there a significant detectable release of either LTB_4 or LTC_4. Indomethacin reduced the resting production and release of cyclo-oxygenase products to a greater extent than diethylcarbamazine which only significantly reduced ($P < 0.05$, unpaired Student's t test) the release of $PGE_{2\alpha}$.

PAF (0.9 nmol)-induced increases in the production and release of each of PGE_2, $PGF_{2\alpha}$ 6-keto-$PGF_{1\alpha}$ and TXB_2 were prevented by indomethacin and markedly inhibited by diethylcarbamazine-pretreatment (Figure 4).

Table 3 The resting production and release of arachidonic acid metabolites by control, indomethacin- and diethylcarbamazine-treated perfused hearts ($n = 6-8$)

Arachidonic acid metabolite	*Resting production and release* (pmol min^{-1}) *Control*	*Indomethacin* (2.8 μM)	*Diethylcarbamazine* (7.7 mM)
PGE_2	1.67 ± 0.50	2.40 ± 0.17	4.04 ± 1.13
$PGF_{2\alpha}$	3.00 ± 0.80	0.30 ± 0.05*	0.56 ± 0.13*
6-keto-$PGF_{1\alpha}$	4.99 ± 1.54	1.53 ± 0.16	4.99 ± 0.58
TXB_2	0.54 ± 0.12	0.24 ± 0.05*	0.36 ± 0.08
LTB_4	0.01 ± 0.01	ND	0.05 ± 0.05
LTC_4	0.13 ± 0.09	0.19 ± 0.19	ND

*$P < 0.05$, compared to control.
ND = not detectable.

The release of PGE_2 from Paf-challenged, diethylcarbamazine- pretreated hearts was highly variable and did not differ ($P>0.05$, paired Student's t test) from that before Paf administration.

Significant amounts ($P<0.05$, paired Student's t test) of LTB_4 and LTC_4 were released into the cardiac effluents of hearts challenged with 0.9 nmol Paf (Figure 4). This release of leukotrienes was not altered ($P>0.05$ unpaired Student's t test) by indomethacin-pretreatment. However, diethylcarbamazine-pretreatment resulted in an inhibition ($P<0.05$, unpaired Student's t test) of the release of both LTB_4 and LTC_4. The release of LTC_4 was not significantly ($0.1>P>0.05$ paired Student's t test) greater than that of LTB_4.

LTD_4 release was detected in the cardiac effluents of one control and two indomethacin-treated hearts (1.7, 0.5 and 0.6 pmol min^{-1}, respectively) following isolation on r.p.-h.p.l.c. and quantitation by RIA. The corresponding values of LTC_4 release were 4.6, 4.8 and 3.4 pmol min^{-1}, respectively. There was no detectable leukotriene-like material in fractions corresponding to the retention time of synthetic LTE_4 11–13 min.

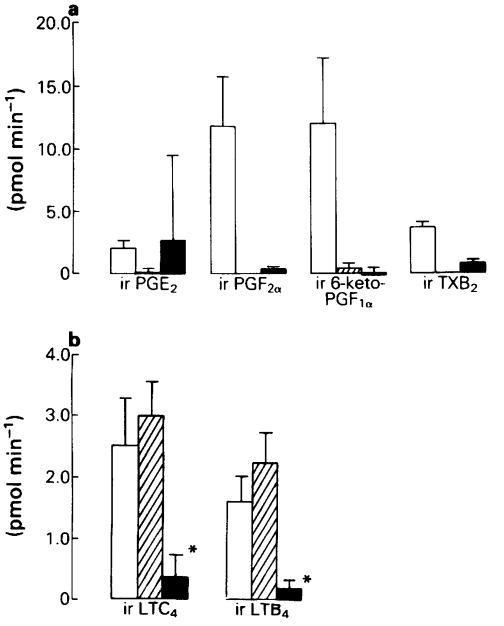

Figure 4 Platelet-activating factor (Paf, 0.9 nmol)-induced increase in the production and release of immunoreactive (ir) cyclo-oxygenase and lipoxygenase products from control (open columns), indomethacin (2.8 μM; hatched columns)- and diethylcarbamazine (7.7 mM; solid columns)-pretreated hearts. Ordinate scales: (a) increase in release of cyclo-oxygenase products ($n=9-13$) in pmol min^{-1}, (b) increase in release of lipoxygenase products ($n=5-7$) in pmol min^{-1}.

The effects of LTC_4 and Paf on coronary perfusion pressure and the release of cyclo-oxygenase products

Administration of a bolus dose of LTC_4 (50 pmol) elicited an increase in coronary perfusion pressure (Table 4) similar in magnitude to that of Paf whereas LTC_4 did not significantly alter $P>0.05$, paired Student's t test) either cardiac contractility (resting value, 1.15 ± 0.12 g; post-LTC_4 change, -0.025 ± 0.025) or heart rate (resting value, 270 ± 12; post-LTC_4 change, $+8+14$). The increases in coronary perfusion pressure induced by LTC_4 (50 pmol) and Paf (0.9 nmol) were respectively prevented and markedly inhibited in FPL 55712-pretreated hearts.

In contrast to the action of Paf on the release of cyclo-oxygenase products, LTC_4 increased the release only of PGE_2 and this increase was significantly less ($P<0.05$, unpaired Student's t test) than that induced by Paf (Table 5). The Paf-induced increase in the release of cyclo-oxygenase products was not prevented ($P>0.05$, paired Student's t test) by pretreatment with FPL 55712 even though the release of $PGF_{2\alpha}$ was significantly reduced ($P<0.05$, unpaired Student's t test).

Effects of Paf, A23187, Arg-vasopressin and lyso-Paf on coronary perfusion pressure and the release of leukotriene-like material

In doses eliciting similar increases in coronary perfusion pressure, Paf (0.9 nmol, $+22.8\pm2.9$ mmHg) or A23178 (4.0 nmol, $+20.9\pm3.7$ mmHg) but not Arg-vasopressin (1.2 nmol, $+28.6\pm7.5$ mmHg) released significant ($P<0.05$, paired Student's t test) amounts of leukotriene-like material (21.0 ± 3.6 pmol, 11.6 ± 2.0 pmol, 1.2 ± 0.8 pmol, respectively). The precursor/metabolite of Paf, lyso-Paf (0.9 nmol) had no effect on either coronary perfusion pressure or the release of leukotriene-like bioactivity. In addition, lyso-Paf did not desensitize hearts to the coronary vasoconstrictor actions of Paf (0.9 nmol).

Discussion

The findings of the present study provide direct experimental evidence for previous suggestions that leukotrienes may contribute to Paf-induced cardiac dysfunction (Feuerstein *et al.*, 1984; Kenzora *et al.*, 1984, Sybertz *et al.*, 1984).

Paf-induced dose-dependent increases in coronary perfusion pressure were accompanied by the release of leukotriene-like material detected by on-line super-

Table 4 A comparison of the effects of leukotriene C_4 (LTC_4) and platelet-activating factor (Paf) on coronary perfusion pressure in control and FPL 55712-pretreated hearts

Treatment	n	*Resting coronary perfusion pressure* (mm Hg)	*Increase in coronary perfusion pressure* (mm Hg)
50 pmol LTC_4	4	46.3 ± 1.4	+ 16.3 ± 3.1
50 pmol LTC_4 + 1.9 μM FPL 55712	4	46.8 ± 2.2	+ 0.8 ± 1.1*
0.90 nmol Paf	17	43.5 ± 2.7	+ 19.4 ± 2.1
0.90 nmol Paf + 1.9 μM FPL 55712	9	42.8 ± 2.9	+ 2.1 ± 0.8*

*$P < 0.05$, unpaired Student's t test.

fusion bioassay on GPISM tissues. The leukotriene-like material was identified as LTC_4 on the basis of co-elution with [^{3}H]-LTC_4 on r.p. h.p.l.c. and detection by LTC_4 antiserum (Hayes *et al.*, 1983). Furthermore, the putative lipoxygenase inhibitor, diethylcarbamazine, reduced the Paf-induced release of both leukotriene-like material determined by bioassay and immunoreactive (ir) LTC_4 determined by RIA. Diethylcarbamazine has previously been shown to reduce the antigen-induced release of SRS-A from guinea-pig chopped lung (Piper & Temple, 1981). Diethylcarbamazine, at concentrations similar to those used in the present study, has been reported to inhibit the release of leukotrienes rat perfused lungs induced by Paf, arachidonic acid or hypoxia (Voelkel *et al.*, 1982; 1984; Morganroth *et al.*, 1984).

LTB_4 release from Paf-challenged hearts was identified by co-elution of ir LTB_4 with [^{3}H]-LTB_4 added to the cardiac effluents before purification and isolation on r.p.-h.p.l.c. The release of ir LTB_4 was markedly inhibited by diethylcarbamazine pretreatment, providing evidence consistent with the suggestion that this compound is an inhibitor of 5-lipoxygenase (Engineer *et al.*, 1978). It seems unlikely that LTB_4 contributes to the acute cardiac responses to Paf in these experiments since it has previously been shown to be devoid of any acute effects in rat, isolated perfused hearts (Letts & Piper, 1983). Nevertheless, this potent inducer of chemotaxis (Ford-Hutchinson *et al.*, 1980) has the potential, at the concentrations determined in the cardiac effluents, to induce an inflammatory response similar to that observed in an experimental model of myocardial infarction (Mullane *et al.*, 1984).

Prostaglandin $F_{2\alpha}$ has been found to increase the production and release of both 6-keto-$PGF_{1\alpha}$ and PGE_2 concomitantly with the induction of an increase in coronary flow rate in rat, perfused hearts (de Deckere & Ten Hoor, 1980). Thus, the $PGF_{2\alpha}$, PGE_2 and PGI_2 released by Paf may reduce the Paf-induced coronary vasoconstriction. There is considerable evidence that the release of TXA_2 in the coronary circulation leads to vasoconstriction (Anhut *et al.*, 1978, Allan & Levi, 1981; Aeringhaus *et al.*, 1984). The

Table 5 Change in production and release of cyclo-oxygenase products induced by leukotriene C_4 (LTC_4) or platelet-activating factor (Paf) in control and FPL 55712-pretreated hearts

Treatment	n	*Cyclo-oxygenase product* (pmol min^{-1}) *6-keto-$PGF_{1\alpha}$*	*TXB_2*	*$PGF_{2\alpha}$*	*PGE_2*
50 pmol LTC_4	4	+ 0.58 ± 0.41	+ 0.25 ± 0.21	+ 0.38 ± 0.34	+ 0.76 ± 0.26*
50 pmol LTC_4 + 1.9 μM FPL 55712	4	− 0.33 ± 0.24	+ 0.03 ± 0.04	+ 0.08 ± 0.50	+ 0.63 ± 1.13
0.9 nmol Paf	12	+ 9.82 ± 2.65*	+ 3.11 ± 0.28*	+ 9.89 ± 2.83*	+ 1.96 ± 0.44*
0.9 nmol Paf + 1.9 μM FPL 55712	4	+ 7.52 ± 2.24*	+ 2.77 ± 0.25*	+ 4.83 ± 0.83*	+ 3.01 ± 0.91*

*$P < 0.05$, compared to release during pre-Paf/LTC_4 control period (paired Student's t test).

small inhibitory effect of indomethacin on the Paf-induced coronary vasoconstriction suggests that the predominant influence of cyclo-oxygenase metabolites is vasoconstrictor. In addition, indomethacin pretreatment failed to increase the release of either LTB_4 or LTC_4 in contrast to observations in guinea-pig cardiac (Aeringhaus *et al.*, 1984) and pulmonary (Engineer *et al.*, 1978) anaphylaxis. However, the release of leukotrienes from chopped lung induced by Paf (Beaubien *et al.*, 1984) and that released from human lung parenchyma by Ca^{2+} ionophore (Sautebin *et al.*, 1985) has been found not to be enhanced by indomethacin pretreatment, suggesting that the enhancing effect of indomethacin may be stimulus-specific.

It seems likely that TXA_2 is responsible for the indomethacin-sensitive vasoconstriction. Although a contribution of the vasoconstrictor PGD_2 (Anhut *et al.*, 1978) cannot be excluded, it has been shown that PGD_2 is only a minor product of arachidonic acid from rat heart compared to the release of 6-keto-$PGF_{1\alpha}$ (de Deckere *et al.*, 1977). The release of cyclo-oxygenase products appears to be a direct result of the action of Paf; equiactive vasoconstrictor doses of LTC_4 failed to release cyclo-oxygenase products. Furthermore, FPL 55712, at a concentration which prevented LTC_4-induced vasoconstriction and markedly attentuated Paf-induced vasconstriction, did not modify the release of cyclo-oxygenase metabolites. Pretreatment with either diethylcarbamazine or indomethacin markedly inhibited the release of cyclo-oxygenase metabolites and prevented the decrease in cardiac contractility induced by Paf. Exogenous LTC_4 had no effect on the cardiac contractility, in agreement with earlier findings in the rat (Letts & Piper, 1983). It appears that the Paf-induced decrease in cardiac contractility is not a result of the actions of leukotrienes but may be related to the release of cyclo-oxygenase products.

The administration of a dose of LTC_4 of the same magnitude as that released by Paf elicited a vasoconstriction similar to that induced by Paf. The release of leukotriene-like material did not appear to be a non-specific result of coronary vasoconstriction since Arg-vasopressin, at equiactive vasoconstrictor doses, did not elicit leukotriene release. In addition, the Ca^{2+} ionophore, A23187, at a dose which elicited a similar coronary vasoconstriction, released similar amounts of leukotriene-like material into the cardiac effluents. The putative lipoxygenase inhibitor, diethylcarbamazine, or the leukotriene receptor antagonist, FPL 55712, inhibited Paf-induced vasoconstriction. It therefore appears that Paf-induced release of LTC_4 is causally related to the vasoconstrictor response. The failure to detect consistently LTD_4 or LTE_4 suggests that neither of these active metabolites of LTC_4 makes a significant contribution to the Paf-induced coronary vasoconstriction.

The source of the leukotrienes released by Paf in the present experiments has not been identified. However, the appearance of leukotriene-like material within 15s of Paf administration may suggest that the coronary blood vessels are a potential source. Previous studies from this laboratory indicate that porcine coronary blood vessels release leukotrienes on stimulation with the Ca^{2+} ionophore, A23187 (Piper *et al.*, 1983). In addition, cat coronary arteries have been found to release leukotrienes in response to Paf (Lefer *et al.*, 1984). There is no evidence to suggest that either endothelial or smooth muscle cells or the myocardium have the capacity to generate leukotrienes. The finding that the adventitia of pulmonary blood vessels makes the largest contribution to the release of leukotrienes (Piper *et al.*, 1983) is consistent with the speculation that perivascular mast cells may have contributed to the leukotriene release in the present experiments.

The release of Paf during cardiac (Levi *et al.*, 1984), pulmonary (Fitzgerald *et al.*, 1985) and systemic anaphylaxis (McManus *et al.*, 1979) and the inhibition of endotoxin shock by CV-3988, a Paf receptor antagonist (Terashita *et al.*, 1985) indicate the potential of Paf as a contributor to inflammatory diseases. Furthermore, cultured endothelial cells have recently been found to synthesize Paf in response to a diverse range of stimuli including histamine, bradykinin and A23187 (Camussi *et al.*, 1985). The possible interrelationships between the release of Paf and of the leukotrienes in the models mentioned above warrant further investigation.

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