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Research Article

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Release of Leukotriene C_4 by Isolated, Perfused Rat Small Intestine in Response to Platelet-activating Factor

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Abstract

We reported a rat model of necrotizing enterocolitis by injecting platelet-activating factor (PAF) into the mesenteric vascular bed, and suggested that leukotrienes (LT) are secondary mediators. The present study, using isolated, buffer-perfused rat small intestine, shows: (*a*) Isolated, perfused small intestine synthesizes LTs in response to PAF. (*b*) Leukotriene C_4 (LTC_4) was the predominant LT released. (*c*) The initial vasoconstriction after PAF injection was due to a transient release of LTC_4 since FPL 55712 pretreatment abolished the vasoconstriction. (*d*) The sustained rise in perfusion pressure was also blocked by FPL 55712, which suggests that other vasoconstrictors released are regulated by LTs. (*e*) The vasoconstrictor(s) responsible for sustained rise in perfusion pressure is unknown, but is not thromboxane. (*f*) Most of the LT was released from intestinal tissue rather than mesenteric arteries. (*g*) Vasodilating prostaglandins (PGs) were also released, probably secondary to LTs. The complex interaction of these lipid mediators (PAF, LTs, and PGs) and their subtle balance may affect the course of the disease.

Introduction

We have previously reported a model of necrotizing enterocolitis in the rat (1) by injecting synthetic platelet-activating factor (PAF,[1] also named AGEPC or PAF-acether [2, 3]) into the mesenteric vascular bed. This model may have some clinical relevance because PAF is a naturally occurring substance elaborated by many cells and tissues (4–6), and the lesions resulting from PAF injection resemble morphologically those seen in human patients with certain clinical conditions (1). The mechanism of PAF action and the pathogenesis of bowel necrosis is unclear, but our previous study (7) using in vivo administration of various drugs and inhibitors suggested that arachidonic acid metabolites of the lipoxygenase pathway, especially leukotrienes (LT), probably played an important role in its pathogenesis. However, the evidence from in vivo studies was largely indirect and circumstantial. Furthermore, the source of LTs was not known. Most studies regarding sulfidopeptide leukotriene (or slow reacting substance of anaphylaxis; SRSA) production in isolated organs have been done in the lung (8, 9), and release of LTs in response to PAF has only been reported in this organ (10, 11). Although PAF, the inducing agent, was injected into the aorta near the mesenteric artery in our model, it is possible that LTs may have been synthesized by the lung tissue and transported to the mesenteric vasculature or intestinal tissue. In the present study we used isolated perfused intestine and thereby confirmed that leukotriene C_4 (LTC_4) was indeed released by the intestinal tissue in response to PAF. This release probably accounts for at least the initial severe vasoconstriction that occurred in PAF-induced ischemic bowel necrosis.

Methods

Materials. PAF (1-*O*-alkyl-2-*O*-acetyl-sn-glycero-3-phosphocholine) was purchased from CalBiochem-Behring Corp., La Jolla, CA. Stock solution in ethanol was kept at −70°C. Working solution (in 2.5 mg/ml bovine serum albumin-saline solution) was prepared fresh daily. LTC_4 and leukotriene D_4 (LTD_4) standards were generously provided by Dr. J. Rokach at Merck Frosst Canada, Inc., Quebec, Canada. FPL 55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxy propoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate) (12) was provided by Sandoz, Inc., East Hanover, NJ and Fisons Corp., Bedford, MA. OKY-046 (Sodium(E)-3-[4-(1-imidazolylmethyl)phenyl]-2-propanoate) was a generous gift from ONO Pharmaceutical Co., Osaka, Japan.

Isolated intestine preparation. Male Sprague-Dawley rats with a body weight of 280±30 g were used in all experiments. The rats were anesthetized with Nembutal. The abdomen was incised along the midline and the superior mesenteric artery was cannulated via the abdominal aorta with polyethelene tubing (PE-50 or PE-10). The entire small intestine was removed from the body, the intestinal contents were gently washed out with 30–50 ml buffer, and the small intestine was perfused at a constant rate (10 ml/min) with Krebs-Hensleit buffer[2] (37°C, 95% O_2, 5% CO_2). Thus, changes in perfusion pressure (measured with a Harvard pressure transducer [Harvard Apparatus Co., Inc., S. Natick, MA] connected to a recorder) reflect changes in vascular resistance.

Bioassay. Two methods were used for bioassay. In most of the experiments, we used a semiquantitative, modified superfusion bioassay method of Vane (13, 14, 15) and Ferreira (16). The venous effluent of the perfused intestine was continuously monitored for bioassayable sulfidopeptide leukotrienes and prostaglandin (PG) E-like substance throughout the experimental period (1–2 h). The assay tissues included a guinea pig ileum segment and a rat stomach strip (16). A mixture of antagonists was added directly to the superfusion fluid (venous effluent) before it reached the assay organs (15). This mixture included: indomethacin (150 μg/min), methysergide (20 μg/min), pyralamine (80 μg/min), scopolamine (10 μg/min), propanolol (200 μg/min), and phenoxybenzamine (10 μg/min). This would abolish the response of assay tissue to catecholamines, acetylcholine, serotonin, and histamine, and inhibit the endogenous PG production of the assay organs. Rat stomach strips were also pretreated with FPL 55712 (0.5 μg/ml), a selective inhibitor of SRSA (12), for 15 min. PAF (2 μg) was injected directly into the cannulated mesenteric artery. The assay organs were calibrated with standards of LTC_4, LTD_4, and PGE_2 at the beginning and conclusion of each experiment. The contraction tension of the assay tissues was measured isotonically with myograph transducers (Harvard Apparatus

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1. *Abbreviations used in this paper:* HPLC, high performance liquid chromatography; LT, leukotriene; PAF, platelet-activating factor; PG, prostaglandin; SRSA, slow reacting substance of anaphylaxis.

2. Krebs-Hensleit buffer: 0.12 M NaCl, 0.0047 M KCl, 0.0012 M $MgSO_4$, 0.0025 M $CaCl_2$, 0.025 M $NaHCO_3$, 0.0012 M KH_2PO_4, 0.01 M dextrose.

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Co., Inc.). The release of LTs and PGs was estimated by comparing the contraction of the guinea pig ileum and rat stomach strip to contractions caused by standard LTC_4, LTD_4, and PGE_2. In some experiments, FPL 55712 (0.5 μg/ml) was used to pretreat the guinea pig ileum for 15 min to block the contracting effect of LTs on the assay organ.

A more quantitative organ bath method was later used to measure the release of peptidoleukotrienes and PGE by perfused intestine. Contraction of guinea pig ileum or rat stomach strip was measured in a Harvard jacketed glass organ bath (Harvard Apparatus Co., Inc.). The intestinal venous effluent was collected in 10-min aliquots, mixed with the antagonist mixture as mentioned above, and added to the organ bath in various dilutions. When the contraction response reached a plateau, the organ bath was drained, and the tissue was washed and allowed to return to the baseline. The contraction responses were compared with those caused by LTC_4 or PGE_2 standard. In some experiments, the perfused intestine was pretreated with FPL 55712 (0.5–1 μg/min), indomethacin (100 μg/min), or OKY-046 (0.3–50 μg/min), a selective inhibitor of thromboxane A_2 synthetase (17, 18), for 20–25 min before the injection of PAF.

Extraction of LTs and PGs from intestinal effluent and their separation by HPLC. To extract the effluent for LT quantitation, the animal was fasted for 24 h before being killed. The intestine was catheterized and drained to avoid the contamination of the venous effluent by intestinal contents. 100 ml of venous effluent was collected immediately after PAF injection and mixed with equal volume of cold ethanol containing 200 ng PGB_1 as internal standard and was extracted as previously described (19). The ethanol buffer mixture was spun down to remove any precipitated protein. The sample was then acidified to pH 3 with 85% formic acid, and extracted twice by one volume of chloroform. The pH was then adjusted to 7–8 by adding 10 N NH_4OH and the sample was dried by N_2. The residue was dissolved in 70% methanol and analyzed by high performance liquid chromatography (HPLC). All glassware was silanized and the final drying was done in teflon tubes. The extraction efficiency for standard LTC_4 (in buffer) was over 60%.

The HPLC was equipped with a Beckman Altex column (4.5 × 25 mm; Beckman Instruments, Inc., Fullerton, CA) packed with Ultrasphere ODS 5 μm particles. The flow rate was 1 ml/min. The solvent system used was 67% methanol containing 0.08% acetic acid brought to pH 6.15–6.2 by ammonium hydroxide and the effluent was monitored at 280 nm. The fractions with the same elution time as standard LTC_4, LTD_4, and LTE_4 were collected, dried, dissolved in saline, and assayed for activity to contract guinea pig ileum.

For separation and quantitation of PGs in the intestinal effluent, 100 ml of venous effluent was collected after PAF administration. The sample was acidified to pH 3.5 and loaded on a Sep-Pak C18 cartridge (Waters Associates, Millipore Corp., Milford, MA). After we washed the column with water, PGs were eluted with methanol, dried, and applied to HPLC. The solvent system used was 72% water (with 0.05% acetic acid), 28% acetonitrile from 0 to 30 min, 28–30% acetonitrile from 30 to 40 min, then stayed at 30% acetonitrile until 50 min. The flow rate was 1.5 ml/min and the effluent was monitored at 194 nm. The peak that co-migrated with standard PGE_2 was collected, dried, dissolved in saline, and assayed for activity to contract rat stomach.

In some experiments, both thin layer chromatography and HPLC were used to purify and confirm the production of PGs. The venous effluent was spiked with 50,000–100,000 cpm of [^{3}H]PGE_2 (165 Ci/mmol, New England Nuclear, Boston, MA) for determination of extraction efficiency. An equal volume of cold acetone was added to precipitate the protein. After discarding the protein precipitate, acetone was evaporated and the samples were acidified to pH 3.5 and extracted twice with an equal volume of ethyl acetate. A few drops of ammonium hydroxide were added to the ethyl acetate extracts to neutralize any remaining acid, and the extracts were dried, dissolved in methanol/chloroform (1:49, vol/vol), and applied to mini-columns containing 1 g of washed silicic acid. Methanol/chloroform (1:49, vol/vol) was used to wash the neutral lipids and fatty acids off the column, and methanol/chloroform (1:9, vol/vol) was used to elute the PGs. The elutes were applied to thin layer plates coated with silica gel G and developed in solvent system consisting of the organic phase of ethyl acetate/water/iso-octane/acetic acid, 110:100:50:20. (In this solvent system, PGE_2 is separated from $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$, but co-migrates with TXB_2). The zones co-migrating with PG standards were scraped, and extracted with chloroform/methanol (1:1, vol/vol). An aliquot of the extracted PGE_2 was counted to determine the final extraction efficiency. Each extracted PG fraction was analyzed separately by HPLC.

Isolated mesenteric vessel perfusion. The isolated perfused mesenteric vessels were prepared in the same way as the isolated small intestine, except that the intestine was separated from the blood vessels at the level where the mesenteric vessels enter intestinal serosa. The intestinal tissue was discarded and the mesenteric artery was perfused as described above.

Results

As shown in Fig. 1, the venous effluent from isolated perfused rat intestine caused little or no contraction of the guinea pig

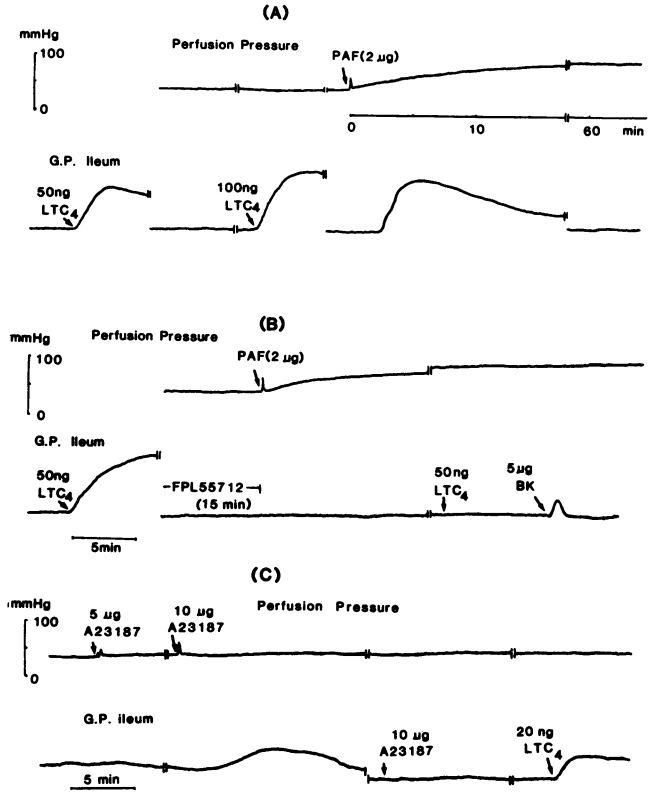

Figure 1. Release of peptide leukotriene by isolated, perfused rat small intestine after PAF injection. The isolated small intestine was perfused with oxygenated Krebs-Hensleit buffer and PAF was injected into the mesenteric artery. The venous effluent continuously dripped on the bioassay organ. $n = 7$, a typical experiment is shown. (*A*) Contractions of guinea pig ileum correspond to LT-like substance released into the venous effluent and are compared with a standard of LTC_4 injected as a bolus over the assay organ. LTD_4 was also used as standard, causing similar contraction of the guinea pig ileum (not shown). Direct application of 2 μg of PAF to the guinea pig ileum did not cause any contraction (data not presented). (*B*) After pretreatment of the guinea pig ileum with FPL 55712 (0.5 μg/ml) for 15 min, the contractile response of the assay organ to venous effluent after PAF injection was completely abolished. However, guinea pig ileum still contracted in response to bradykinin (BK). (*C*) Effect of calcium ionophore A23187 on release of peptide leukotrienes. ($n = 4$, a typical experiment is shown).

ileum. The perfusion pressure varied from preparation to preparation, depending largely on the amount of intraintestinal contents remaining and the degree of intestinal distension. The perfusion pressure remained unchanged or increased minimally (<20 mmHg) if no stimuli were applied. When PAF (2 μg) was injected into the mesenteric artery, there was an immediate rise of perfusion pressure, which gradually increased and was sustained for more than 1 h. The total increment varied from 30 to 200 mmHg (81±16 mmHg, n = 12), affected by the degree of intestinal distension. Concomitantly, there was a marked, sustained contraction of the guinea pig ileum (Fig. 1 *A*). The amount of bioactive substance released varied, but in most instances the magnitude and duration of contraction of guinea pig ileum were similar to those caused by 50–100 ng standard LTC_4 or LTD_4 (Fig. 1 *A*). This quantity represents an underestimate, since the contractile response of guinea pig ileum is only linear at a low dose range. By using organ bath and diluted venous effluent, we could determine the released quantities of LTs more accurately. After a bolus injection of 2 μg of PAF the perfused intestine released 4.2±1.6 μg (n = 3, mean±SEM) of LTC_4-like substance in the first 30 min. This release decreased markedly after 30 min and became almost undetectable after 40 min. The amount of LT release roughly correlated to the amount of PAF injected. 1 μg of PAF elicited variable responses ranging from <50 ng to 1.25 μg in 30 min (n = 3), while 4 μg of PAF caused a release (in 30 min) equivalent to 4.6±1.7 μg of standard LTC_4 (n = 3). The observed variability at a low dose (1 μg) of PAF is in keeping with our previous in vivo observation that bowel necrosis is inconstantly produced when only 1 μg of PAF is administered (1). Pretreatment of the ileum strip with FPL 55712 (0.5 μg/ml) for 15 min completely abolished the response of ileum to direct application of LTC_4, D_4, and to injection of PAF to the isolated perfused intestine (Fig. 1 *B*). Extraction of the intestinal effluent showed multiple peaks at 280 nm as detected by HPLC (Fig. 2 *B*), and the fraction (8–8.6 min) with the same elution time as authentic LTC_4 (8.3 min) showed >90% of the activity of contracting guinea pig ileum (Fig. 2 *C*), indicating that it was mainly LTC_4 that was released from the perfused intestine in response to PAF. The quantity of LTC_4 fraction (from HPLC) judged by the guinea pig ileum contraction varied from 20 to 200 ng in five experiments. This low quantity is probably a result of: the poor extraction efficiency of LTC_4 (<30%) from the venous effluent (due to its high protein content); degradation of LTC_4 during extraction, HPLC purification, and drying; and loss of LTC_4 during transfer from one container to another.

LTB_4 was not detected by HPLC. This is probably not due to a rapid metabolic degradation of LTB_4. To prove this point, we injected [^{3}H]LTB_4 (3×10^5 cpm, New England Nuclear) into the perfused intestine, collected venous effluent, and extracted and analyzed it by HPLC. We found that the majority (70%) of the [^{3}H]LTB_4 injected could be recovered as LTB_4, while only 16% of the radioactivity co-migrated with 20-hydroxy LTB_4 standard. (20-OH LTB_4 co-migrates with 20 carboxy LTB_4 in our solvent system).

The increase in perfusion pressure after PAF injection lasted much longer than the contraction of guinea pig ileum (Fig. 1). This is only partly due to tachyphylaxis of the contracting response of guinea pig ileum, because repeated injection of LTC_4 or LTD_4 directly to the ileum or a second injection of PAF to the perfused intestine still resulted in further contraction of the

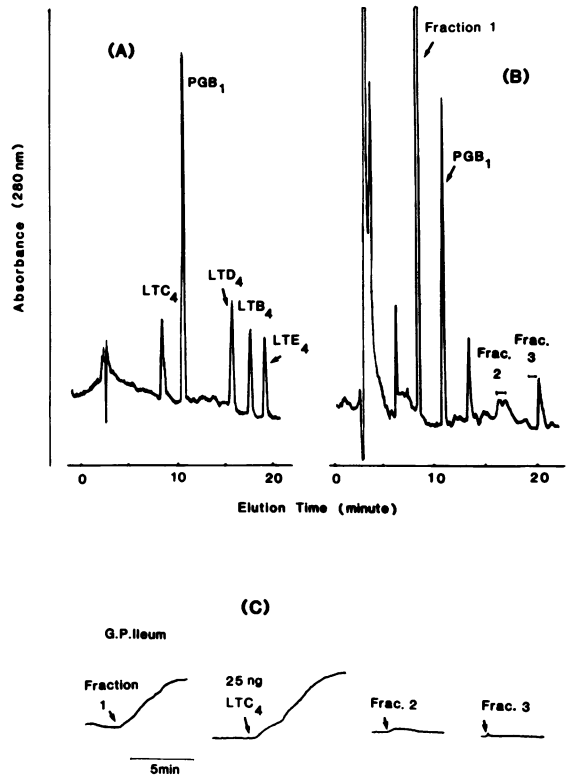

Figure 2. HPLC profile of LTs in intestinal venous effluent after PAF injection and contractile response of guinea pig ileum to various fractions collected from HPLC. (*A*) Standards. (*B*) After injection of PAF to the isolated perfused intestine, 100 ml of venous effluent was collected. 200 ng of PGB_1 was added as internal standard and the sample was extracted and injected into HPLC. Various fractions were collected and dried. Fraction 1 has the same elution time as LTC_4 (8.3 min), fraction 2 corresponds with LTD_4 (16.2 min), and fraction 3 corresponds with LTE_4 (18.4 min). (*C*) The dried fractions were dissolved in 300 μl of saline and 100 μl was applied directly to the guinea pig ileum. Only fraction 1 showed marked contractile effect on guinea pig ileum. Fraction 2 had minimal effect. Fraction 3 and other fractions in between showed no effect. (n = 4, a typical experiment is shown).

ileum, although less intense (data not shown). A nonspecific agonist, calcium ionophore A23187, at the dose of 10 μg also caused release of LTC_4-like substance estimated to be less than one-fifth the strength of 2 μg of PAF (Fig. 1 *C*). Other humoral vasoactive agents, such as histamine (0.2–2 μg) or serotonin (0.4–2 μg), were ineffective.

Another interesting observation is the release of PGE-like material by the perfused intestine. After pretreatment with FPL 55712, the rat stomach strip still responded to substances released from PAF-stimulated perfused intestine (Fig. 3 *A*). Furthermore, when the perfused intestine was pretreated with indomethacin (100 μg/min), no contraction of the rat stomach strip was observed after PAF injection (Fig. 3 *B*). These observations indicate that PGE-like material was also released in addition to LTs after PAF stimulation. HPLC analysis of the venous effluent showed that the major PG released by intestinal tissue was PGE_2 (Fig. 4 *B*). The second largest peak was $PGF_{2\alpha}$. Thromboxane B_2 was almost undetectable by HPLC (Fig. 4 *B*). When both TLC and HPLC were used to purify the sample, it was confirmed that PGE_2 was the major PG produced. After the zone that co-mi-

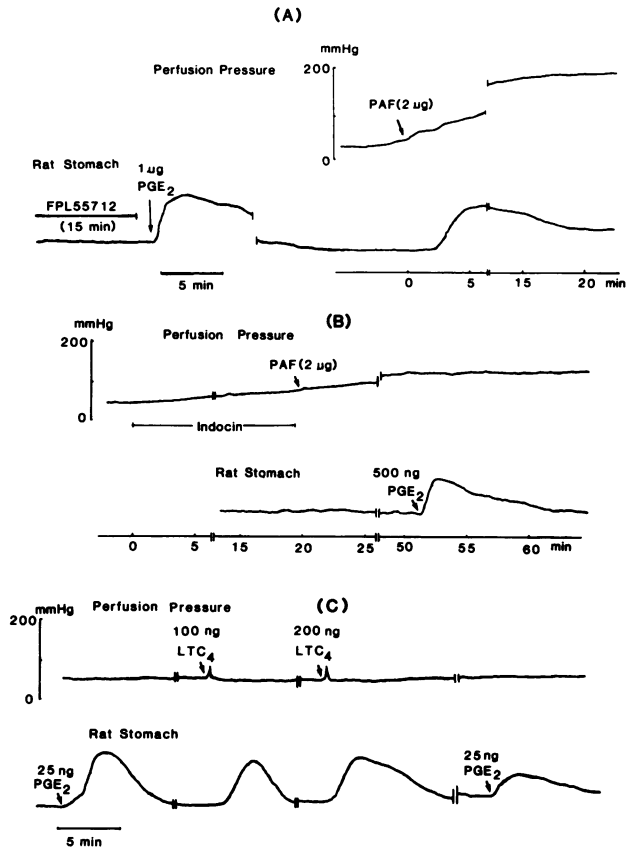

Figure 3. Release of PGE-like substance by isolated, perfused small intestine after PAF injection. The venous effluent continuously dripped on the bioassay organ (n = 7, a typical experiment is shown). (*A*) Contractile response of rat stomach strip to intestinal venous effluent after PAF injection was compared with that caused by PGE_2 standard. The stomach strip had been pretreated with FPL 55712 (0.5 μg/ml) for 15 min to abolish the effect of LTs. (*B*) After pretreatment of perfused intestine with indomethacin (10 μg/ml) for 20 min, the release of PGE_2-like substance from the perfused intestine was completely abolished. (*C*) Effect of LTC_4 on PGE release by perfused intestine (n = 3, a typical experiment is shown).

grated with authentic PGE_2 (and TXB_2) on TLC was scraped, extracted, and injected into reverse-phase HPLC, only the peak with the same elution time as PGE_2 appeared (Fig. 4 *D*). TXB_2 was not detected. Production of $PGF_{2\alpha}$ was also demonstrated by this method. However, its peak was much smaller than that of PGE_2 on HPLC (data not shown). There was a great variation of the quantities of PG release between animals. It was estimated by HPLC that 1.05–5 μg of PGE_2 were released by the intestinal tissue in response to 2 μg of PAF (n = 8). The contractile activity of the PGE_2 peak on rat stomach strips was confirmed by bioassay (data not shown). The amount of PGE_2 released was estimated to be 2.4±0.6 μg in 30 min by organ bath bioassay (n = 3). Pretreatment of the intestine with indomethacin (100 μg/min) for 20 min markedly diminished the production of PGE_2 and $PGF_{2\alpha}$ (Fig. 4 *C*).

Pretreatment of the perfused intestine with indomethacin (100 μg/min) (Fig. 3 *B*) or with OKY-046 (0.3–50 μg/min) (Fig. 5) failed to abolish the mesenteric vasoconstriction and sustained increase in perfusion pressure, which indicates that thromboxane was not the mediator responsible for the sustained vasoconstriction of the mesenteric and intestinal vasculature. In fact, indomethacin pretreatment itself resulted in mesenteric vasoconstriction shown by an increase of perfusion pressure (Fig. 3 *B*), which suggests that a small amount of vasodilating PGs, e.g., PGE_2 and perhaps PGI_2, were released continuously in the resting state. This release was enhanced as a defense mechanism of the system to counteract the severe vasoconstricting effects of LTs. In contrast, pretreatment of the perfused intestine with FPL 55712 (0.5 or 1 μg/min) completely abolished the vasoconstriction (Fig. 6), indicating that the release of LTC_4 was directly or indirectly responsible for the prolonged vasoconstriction of mesenteric and intestinal vasculature. Interestingly, the contraction of rat stomach strip was also markedly reduced, which suggests that the production of PGE_2 was secondary to LT release. However, direct injection of 100 ng LTC_4 into the perfused intestine resulted in release of PGE in a much smaller quantity, estimated by HPLC and bioassay to be 15–25 ng (Fig. 3 *C*). Direct injection of 1 μg LTC_4 resulted in release of 500–800 ng of PGE_2.

Isolated perfused mesenteric vessels without intestine did not release any substance that contracted guinea pig ileum when challenged with PAF either as a bolus or by slow infusion (0.5 μg/min, 5 min) (Three experiments were done, data not shown). This observation suggests that it was the intestinal tissue, including intraintestinal vasculature, that synthesized LTC_4 in response to PAF.

Each experiment was repeated several times as indicated. The reproducibility was good, although the quantitation of the mediator released varied considerably as stated above. Only representative experiments are shown.

Discussion

Platelet activating factor is a naturally occurring phospholipid that possesses potent biological effects (2, 3). Although there have been numerous studies on the in vivo pharmacological effects of PAF, the mechanism of its action remains elusive. A previous report has suggested that sulfidopeptide LTs are produced when PAF is injected into isolated perfused rat lungs and that the LTs produced may account for the pulmonary vasoconstriction and edema caused by PAF (10). In the present study, we have provided evidence in favor of the following conclusions in the system of isolated perfused small intestine: (*a*) Isolated perfused intestine devoid of intravascular white blood cells and plasma was able to synthesize leukotrienes in response to PAF injection. (*b*) LTC_4 was the predominant LT released in rat small intestine. (*c*) The initial phase of vasoconstriction and increased perfusion pressure after PAF injection was due to a transient release (30–40 min) of LTC_4 because the increase in perfusion pressure could be blocked by pretreatment of the perfused bowel with FPL 55712. Thus, LTC_4 release is transient but the vasoconstriction is sustained, which suggests release of other mediator(s). However, blocking of peptide LT receptor in the tissue by FPL 55712 totally abolished the sustained rise in perfusion pressure. Hence, (*d*) the peptide LTs are not only the cause of the initial vasoconstriction, but may, in addition, be responsible for the sustained vasoconstriction response, probably by inducing the release of another secondary mediator. (*e*) The mediator that sustained the mesenteric and intestinal vasoconstriction is unknown, but is probably not thromboxane A_2, another arachidonic acid metabolite, because administration of OKY-046 failed

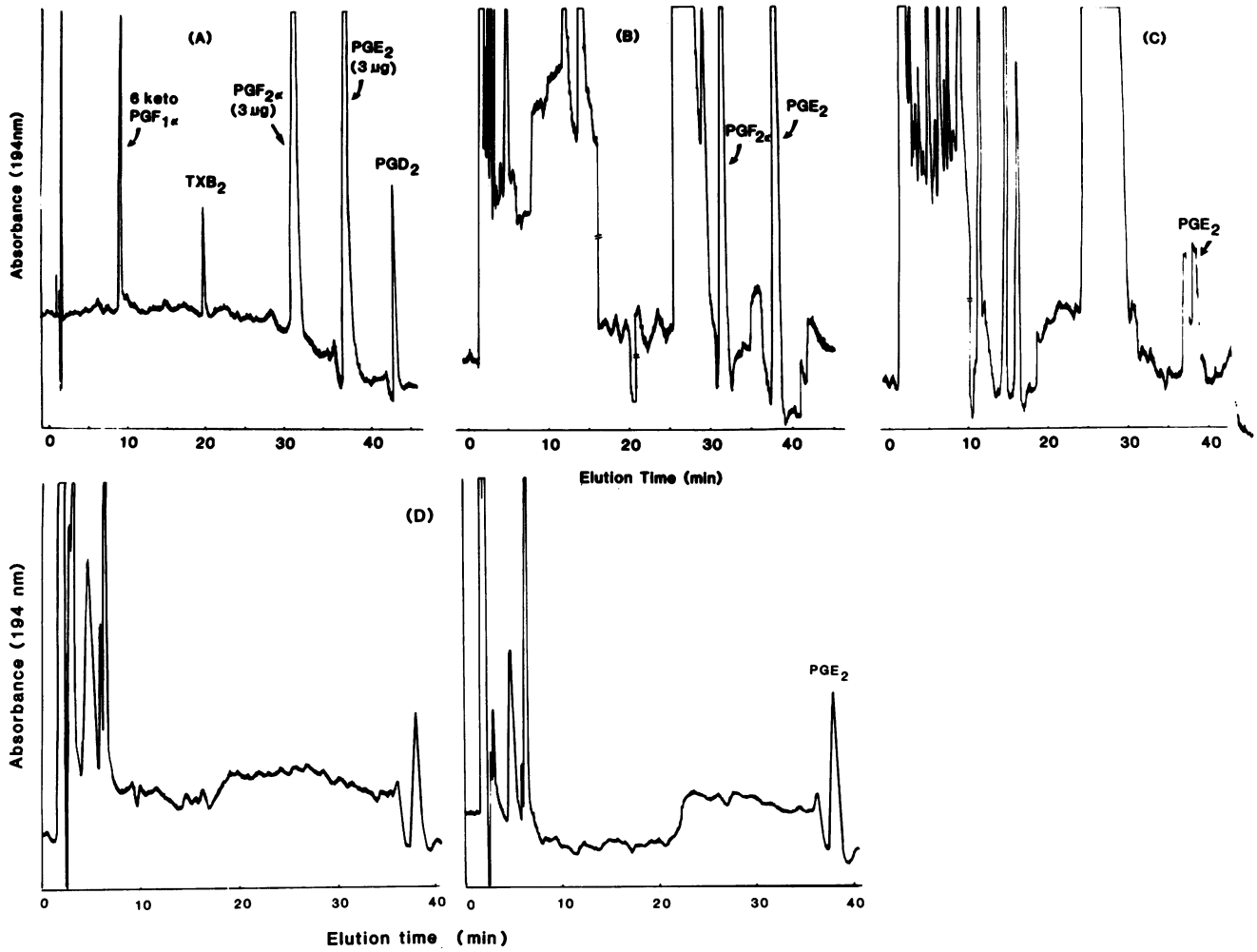

Figure 4. HPLC separation of PGs in the intestinal venous effluent after PAF injection (see text for solvent system) (n = 8, one experiment is shown). (*A*) Standards. (*B*) HPLC profile of extracted venous effluent. (*C*) HPLC profile of venous effluent from perfused intestine pretreated with indomethacin (10 μg/ml) for 20 min (n = 2, one experiment is shown). (*D*) HPLC profile of extracted PGE_2-TXB_2 zone from TLC. The sample, after being extracted by ethyl acetate, was purified by silicic column chromatography and TLC (see Methods). The zone co-migrating with PGE_2 and TXB_2 standards was scraped, extracted, and injected into HPLC. *Left,* extracted PGE_2 zone from TLC. *Right,* PGE_2 standard extracted from the same TLC plate (n = 6, one representative experiment is shown).

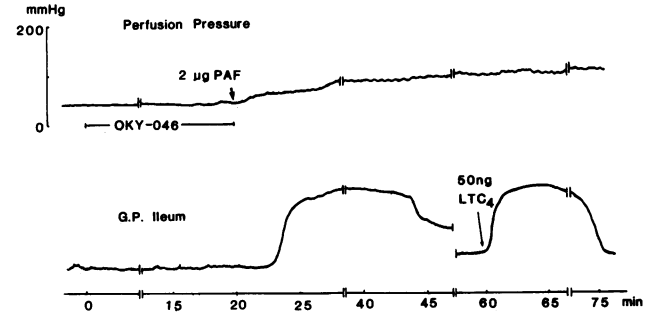

Figure 5. Lack of effect of OKY-046 on perfusion pressure and LT release by PAF-stimulated perfused intestine. The isolated perfused intestine was pretreated with OKY-046 (10 μg/min) for 20 min. This pretreatment failed to abolish the release of guinea pig ileum contracting substance, it also did not prevent the rise in perfusion pressure. Six experiments were done: two treated with 0.3 μg/min OKY-046, two with 10 μg/min, two treated with 50 μg/min. One experiment with 10 μg/min OKY-046 is shown. Results are similar in all six experiments.

to abolish the sustained increase in perfusion pressure. (*f*) The majority of the peptide LTs were released from the intestinal tissue itself (including the intraintestinal microvasculature). The mesenteric vessels and tissue only played a minor role, if any. (*g*) Vasodilating PGs, e.g., PGE_2, and probably prostacyclin, were also released, probably as a defense mechanism to counteract the severe vasoconstriction after PAF injection. This finding and our conclusion from a previous study in rats (7) showing aggravation of PAF-induced bowel necrosis by pretreatment with indomethacin are mutually supportive. It is not known if release of PGs was a direct effect of PAF, or was secondary to the release of LTs. The stimulatory effect of LTs on cyclooxygenase has been observed in other systems. The actions of LTC_4 and LTD_4 on the guinea pig lung are largely mediated by thromboxane A_2 (20). Furthermore, PG was reported to be released from isolated guinea pig lung perfused with LTC_4 (21) and prostacyclin has been reported to be released by isolated guinea pig heart (22) and by cultured endothelial cells when LTC or LTD was given as stimulus (23). Our results suggest that at least a large portion

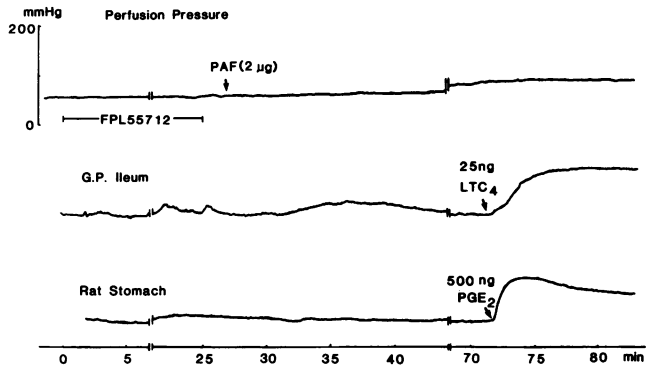

Figure 6. Effect of FPL 55712 on perfusion pressure and release of LTs and PGs by perfused intestine. FPL 55712 (0.5 μg/min) was infused into the mesenteric artery of isolated, perfused intestine for 20 min. (During this pretreatment period, the stream of intestinal effluent was deflected so that the guinea pig ileum would not be affected by FPL 55712). PAF was then injected into the intestine. The increase in perfusion pressure was prevented. The release of PG-like substances was abolished as shown by the lack of contraction of rat stomach strip. Contraction of guinea pig ileum also decreased. (n = 4, one typical experiment is shown.)

of the PG produced was secondary to LT release. However, direct injection of LTC_4 into the perfused intestine elicited a much weaker PGE releasing response compared with PAF. It is possible that PAF causes release of bioactive substances which either contract rat stomach or potentiate PGE release by the intestine.

The cell source of arachidonic acid metabolites is still unknown, although histological examination of the perfused organ allows us to rule out white blood cells in the blood vessels. Rat jejunal tissue in vitro synthesized large amounts of PGD_2 and small amounts of PGE_2 and 6-keto-$PGF_{1\alpha}$ (24). $PGF_{2\alpha}$ and thromboxane have also been demonstrated (25–28). Release of LTC_4-like immunoreactive material has also been shown to occur in the anaphylactic guinea-pig mesenteric vascular bed (29). Other possible sources of LTs are the inflammatory cells in the intestinal mucosa, e.g., mast cells, eosinophils, and macrophages, all of which have been shown to release LTs when stimulated (30–33). The interplay of various inflammatory cells and the complex interactions of these lipid mediators probably contribute to the pathogenesis of lesions in clinical diseases. The present study indicates that the net effect of these complex actions may be of immediate relevance to the development of ischemic bowel necrosis.

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