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THE ACTIONS OF LEUKOTRIENES C_4 AND D_4 ON GUINEA-PIG ISOLATED HEARTS

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1 Infusions of leukotrienes C_4 (LTC_4) and LTD_4 (5 min; 4×10^{-10}–2×10^{-8} M) produced dose-dependent decreases in coronary flow in Langendorff preparations of guinea-pig isolated hearts.

2 LTC_4 was more active than LTD_4 and produced a greater reduction in coronary flow.

3 LTC_4 and LTD_4 also caused reduction in contractility in perfused hearts but not in isolated atria or driven ventricular strips.

4 There was a greater reduction in contractility to LTD_4 compared with LTC_4 at doses which produced approximately 50% reduction in coronary flow.

5 Indomethacin (1.4×10^{-5} M) inhibited the effects of LTC_4 but only reduced the initial LTD_4-induced effects.

6 The effects of FPL 55712 (3.8×10^{-6} M) were similar to those of indomethacin.

7 LTC_4 and LTD_4 may therefore contribute to the abnormalities of cardiac function that occur in immediate hypersensitivity reactions, particularly reduction in coronary flow.

Introduction

Antigen challenge of isolated perfused hearts from sensitized guinea-pigs causes cardiac disorders including tachycardia, reduction of coronary flow, arrhythmia and changes in contractility (Wilcox & Andrus, 1938; Greef & Heeg, 1964; Feigen & Prager, 1969; Levi, 1972). In addition, the cardiac immediate hypersensitivity reaction, cardiac anaphylaxis, results in release of mediators which include histamine (Giotti, Guidotti, Mannaioni & Zilletti, 1966; Levi, 1972), slow-reacting substance of anaphylaxis (SRS-A) (Brocklehurst, 1960; Chakravarty, 1960), prostaglandins and thromboxane A_2 (Anhut, Bernauer & Peskar, 1978a; Allan & Levi, 1981).

The major biological activity of guinea-pig SRS-A is leukotriene D_4 (LTD_4) (Morris, Taylor, Piper & Tippins, 1980) and there is evidence to suggest that a primary mechanism and site of action of peptido-lipid leukotrienes is a potent constriction of small-diameter blood vessels such as, for example, those of the microvasculature of the skin (Drazen, Austen, Lewis, Clark, Goto, Marfat & Corey, 1980; Hedqvist, Dahlen, Gustafsson, Hammarstrom & Samuelsson, 1980; Peck, Piper & Williams, 1981). The purpose of this investigation was to assess the actions of synthetic LTC_4 and LTD_4 on the guinea-pig isolated heart. Part of this work has been communicated to the Physiological Society and the British Pharmacological Society.

Methods

Male guinea-pigs (Dunkin-Hartley, 400–450 g) were killed by stunning and cervical dislocation and their hearts immediately removed for either of the following preparations:

(a) *Isolated spontaneously beating paired atria*
Guinea-pig paired atria were set up in a 10 ml organ bath, bathed in Krebs-Henseleit solution maintained at 37°C and aerated with 95% O_2 and 5% CO_2. A resting tension of 1 g weight was applied and the spontaneous rate and force of contraction were measured with a force displacement transducer (Grass model FTO 3c) connected to a recorder (Servoscribe model 330). Preparations were allowed an equilibration period of 45 min before the start of each experiment.

(b) *Electrically-driven right ventricular muscle strips*
The method was essentially as described by Bolton & Raper (1966). Right ventricular muscle strips ($15 \times 3 \times 2$ mm) were set up in a 10 ml organ bath, bathed in Krebs-Henseleit solution maintained at 37°C and aerated with 95% O_2 and 5% CO_2. Each strip was stimulated with square-wave pulses of 5 ms duration at 3 Hz using a Bell stimulator. A resting tension of 0.5 g weight was applied and contractions were recorded with a force displacement transducer (Grass model FTO 3c) and displayed on a Servoscribe (model 330) recorder.

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(c) *Langendorff perfused heart preparation*

The coronary vasculature of spontaneously beating whole hearts from guinea-pigs was perfused with Ringer-Locke solution at constant pressure (50 cmH_2O) via retrograde cannulation of the aorta. The perfusate was prewarmed to 37°C, aerated with pure O_2 and the heart suspended in a water-jacketed organ bath (maintained at 37°C). A thread hooked to the apex of the left ventricle was attached to a force displacement transducer (Grass, model FTO 3c). A basal tension of 4 g weight was applied and the spontaneous rate and force of contraction was displayed on a multi-channel recorder (Servoscribe, model 330). Coronary flow was measured, by means of a drop-counter (Devices, type 3210) situated directly below the heart, and recorded on another channel of the recorder. In addition, the perfusate was collected each minute and the volume measured. Each heart was allowed a 45 min equilibration period and received only one 5 min infusion of leukotriene (Watson-Marlow pump, type MHRE 7). In some experiments indomethacin, FPL-55712, mepyramine, cimetidine or propranolol were added to the Ringer-Locke solution.

Statistical analysis

The arithmetic mean and their standard error are quoted in the text and differences were analysed with Student's t test for unpaired data. The criterion for statistical significance was $P<0.05$.

Materials

Synthetic LTC_4 and LTD_4 were a gift from Dr J. Rokach, Merck Frosst Laboratories. FPL-55712 (sodium 7-[3(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxy propoxy]-4 oxo-8-propyl-4H-1-benzopyran-2-carboxylate), mepyramine, cimetidine, indomethacin and propranolol were gifts from Fisons Pharmaceuticals; May & Baker; Smith, Kline & French; Merck Sharp & Dohme and I.C.I. respectively. Isoprenaline and acetylcholine were obtained from Sigma.

Results

Isolated atria

LTC_4 (2.0×10^{-10} to 2.0×10^{-7} M) and LTD_4 (2.6×10^{-10} to 2.0×10^{-6} M) had no effect on either the spontaneous rate or the force of contraction of the isolated atria ($n=4$) (Figure 1).

Electrically-driven right ventricular strips

In three preparations, LTC_4 (2×10^{-7} M) and LTD_4 (4.0×10^{-7} M) following a 10 min equilibration period, produced no actions on the developed force of contraction of the isolated ventricular muscle strips. Isoprenaline (1×10^{-8} M) and acetylcholine (5×10^{-7} M) were used as positive- and negative-inotropic agonists (Figure 1).

Langendorff preparation

Both LTC_4 and LTD_4 produced dose-dependent decreases of coronary flow of isolated working hearts of the guinea-pig (Figure 2). However, qualitative and quantitative differences were observed. LTC_4 had both a greater efficacy in reducing the coronary flow and a steeper log dose-response relationship when compared with the actions of LTD_4; $71\pm6\%$ compared with $45\pm2\%$ (Figure 2). Also the peak effect of the LTC_4-induced reduction in coronary flow was slower in onset than that caused by LTD_4 (Figures 3a, 4a).

Indomethacin (1.4×10^{-5} M) delayed the LTD_4-induced decrease in coronary flow but did not alter the absolute reduction during a 5 min infusion. The reduction in flow in the first minute of the LTD_4 infusion was significantly reduced (Figure 3a). The dose-response relationship of the peak decrease in flow was not affected by indomethacin (Figure 5). The effects of 5 min infusions of a near-maximal dose of LTC_4 were significantly blocked by indomethacin (Figure 4a).

FPL-55712 (3.8×10^{-6} M) fully antagonized the effects of a near-maximal dose of LTC_4 (Figure 4b). However, FPL-55712 was not as effective against LTD_4 and only significantly inhibited the reduction in coronary flow during the first minute (Figure 3b).

Mepyramine (1×10^{-7} M, $n=3$) and cimetidine (5×10^{-6} M, $n=3$) had no effect on the LTD_4-induced reductions in coronary flow. The peak decrease in flow in the presence of mepyramine was slightly, but not significantly, enhanced. Propranolol (1×10^{-6} M, $n=6$) partially blocked the LTD_4-induced decrease in coronary flow. There was no significant difference in the treated/untreated flow rates nor was there any change in the time to the greatest effect.

Contractility

In contrast to their actions on isolated atrial and electrically-driven ventricular muscle strips, the leukotrienes produced decreases in contractility of the isolated Langendorff perfused hearts. Comparison of doses of the leukotrienes LTC_4 and LTD_4 producing similar reductions in coronary flow showed that LTD_4 had a greater efficacy in reducing the contractility (Figures 3a, 4a). As illustrated during infusion of a low dose of LTD_4 in the Langendorff

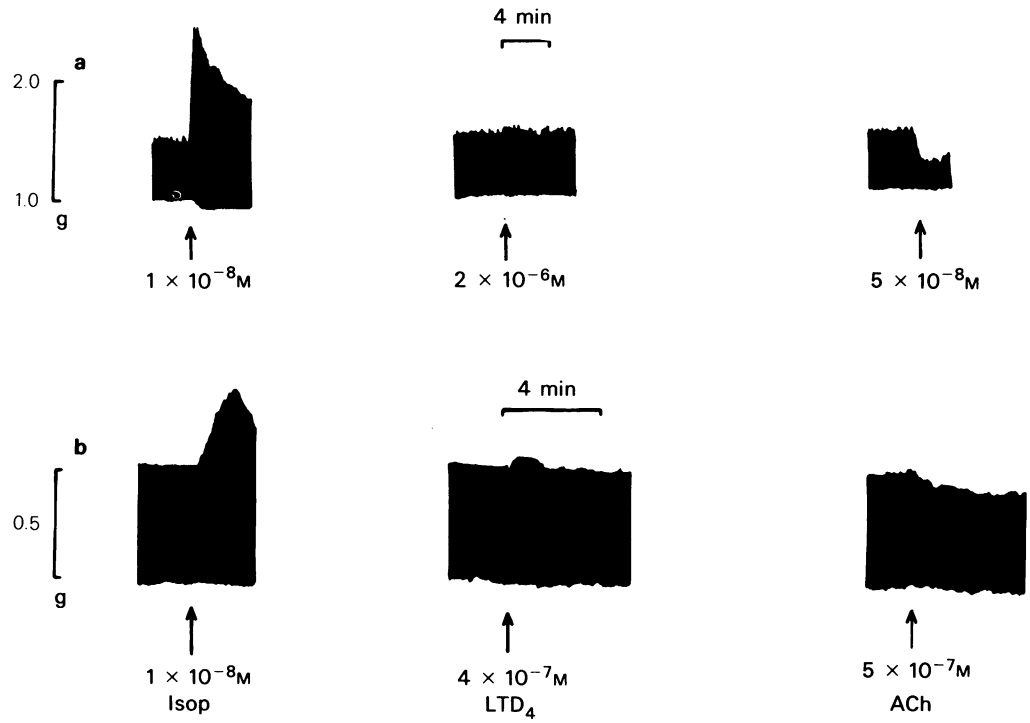

Figure 1 (a) Effects of isoprenaline (Isop), leukotriene D_4 (LTD_4) and acetylcholine (ACh) on spontaneously beating atria from guinea-pig heart; (b) effects of isoprenaline, LTD_4 and acetylcholine on electrically-driven right ventricular strips from guinea-pig heart.

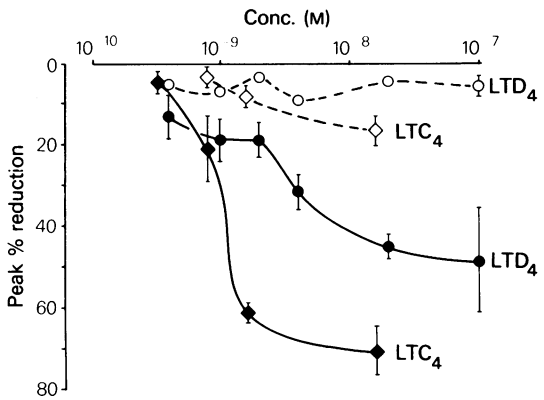

Figure 2 Actions of leukotriene C_4 (LTC_4) and LTD_4 on guinea-pig isolated heart. LTC_4 (◆) and LTD_4 (●) caused dose-related reduction in coronary flow. LTC_4 (2.0×10^{-8} M) caused a maximum reduction of $71 \pm 6\%$ while the maximum reduction produced by LTD_4 (1.6×10^{-8} M) was $45 \pm 2\%$. Effects on rate: LTC_4 (◇); LTD_4 (○). Vertical axis peak percentage reduction. Horizontal axis log dose of LTC_4, LTD_4. Vertical bars show s.e.mean ($n = 3-5$ per dose).

perfused heart preparation (Figure 6) there was a sharp reduction in contractility. The decrease in contractility with the low dose of LTD_4 is associated with a decrease in the resting tension since during this infusion a momentary readjustment of the resting tension to the pre-infusion level showed no decrease in the developed tension. Infusion of a higher dose of LTD_4 also produced a reduction in contractility associated with a decrease in the resting tension. However, after readjustment of the resting tension as described above, a reduction in contractility persisted. In both cases upon cessation of leukotriene infusion, coronary flow, resting tension and contractility returned to pre-infusion levels. Infusion of a near maximal dose of LTD_4 (above 1×10^{-8} M) produced effects as already described. In these cases, the decrease in contractility as well as coronary flow did not fully recover (observations over one hour).

Indomethacin (1.4×10^{-5} M) appeared to antagonize the LTD_4-induced reductions in contractility (Figures 3a, 6). There was an inherent variability in the contractility of the hearts and the apparent inhibition by indomethacin was close to significance ($0.05 < P < 0.1$; 7 d.f.). Similarly, LTD_4-induced

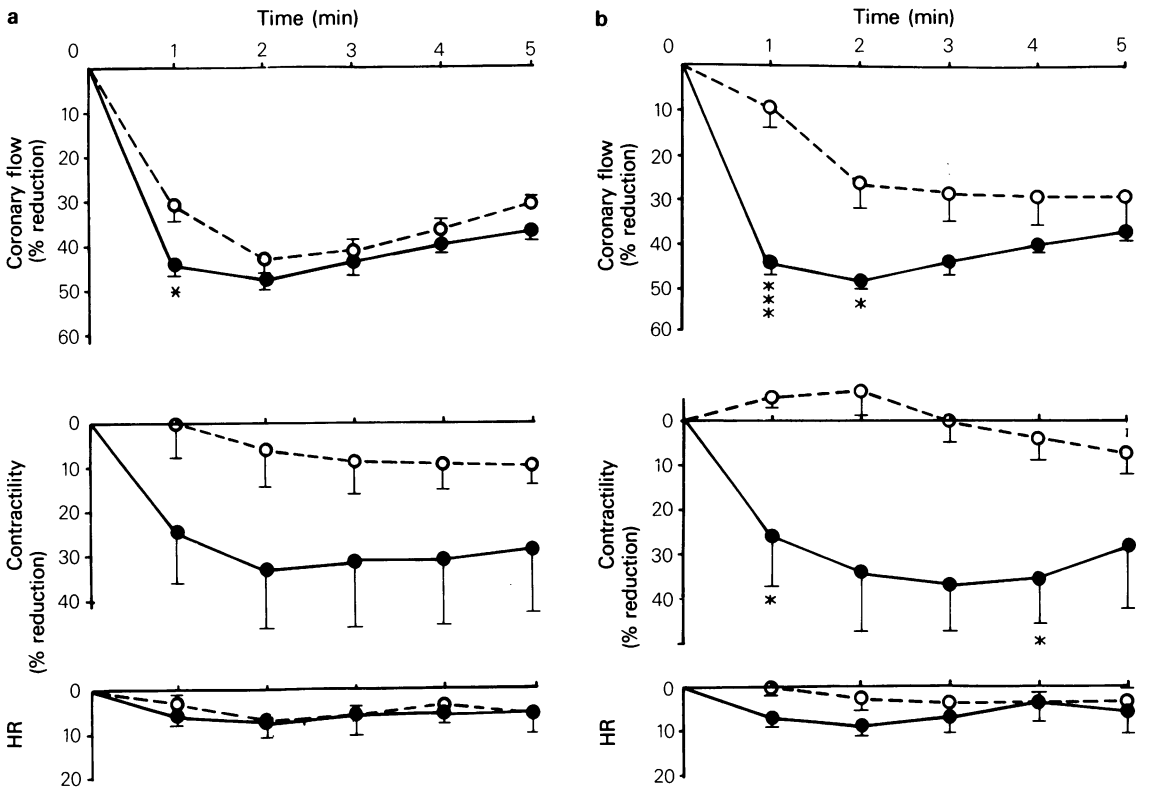

Figure 3 The actions of leukotriene D_4 (LTD_4) on coronary flow, contractility and heart rate (HR) and the effects of indomethacin and FPL 55712. (a) LTD_4 (2.0×10^{-8} M, ●) reduced coronary flow by a maximum of $48 \pm 2\%$ during the first 2 min of infusion, reduced contractility by a maximum of $37 \pm 11\%$ but had little effect on heart rate. Indomethacin (1.4×10^{-5} M, ○) caused a slight but significant inhibition of LTD_4-induced reduction of coronary flow at 1 min only and did not significantly inhibit the reduction in contractility or affect heart rate. (b) LTD_4 (2.0×10^{-8} M) alone (●); FPL 55712 (3.8×10^{-6} M, ○) significantly inhibited the LTD_4-induced fall in coronary flow at 1 and 2 min, inhibited the fall in contractility but did not affect heart rate.
In Figures 3a, b; 4a, b vertical axes; percentage reduction; horizontal axes; time in min. $^{***}P<0.001$; $^{**}P<0.01$; $^{*}P<0.05$.

contractility changes were antagonized by the same concentration of indomethacin. The LTC_4-induced blockade by indomethacin was significant (Figure 4a).

FPL-55712 (3.8×10^{-6} M) totally blocked the LTC_4-induced decreases in contractility (Figure 4b) and partially blocked the LTD_4-induced decreases (Figure 3b). The rapid decrease in contractility seen in the initial 2 min of an LTD_4 infusion was also antagonized.

Spontaneous rate

Neither LTC_4 nor LTD_4 produced significant changes in the basal rate of the isolated working hearts. Indomethacin (1.4×10^{-5} M) or FPL-55712 (3.8×10^{-6} M) pretreatments did not alter the results.

Although the surface electrocardiogram was not monitored, the leukotrienes did not induce arrhythmias.

Discussion

The above results show that LTC_4 and LTD_4 are potent constrictors of the coronary vasculature of the guinea-pig. It is noteworthy that the leukotrienes have also been shown to be potent constrictors of the vascular smooth muscle in the skin (Drazen *et al.*, 1980; Hedqvist *et al.*, 1980; Peck *et al.*, 1981), cerebral (Beckett & Boullin, 1981) and hamster cheek pouch (Dahlen, Bjork, Hedqvist, Arfors, Hammarstrom, Lindgren & Samuelsson, 1981) vascular beds. Although the two leukotrienes investigated had similar actions on the heart, qualitative and

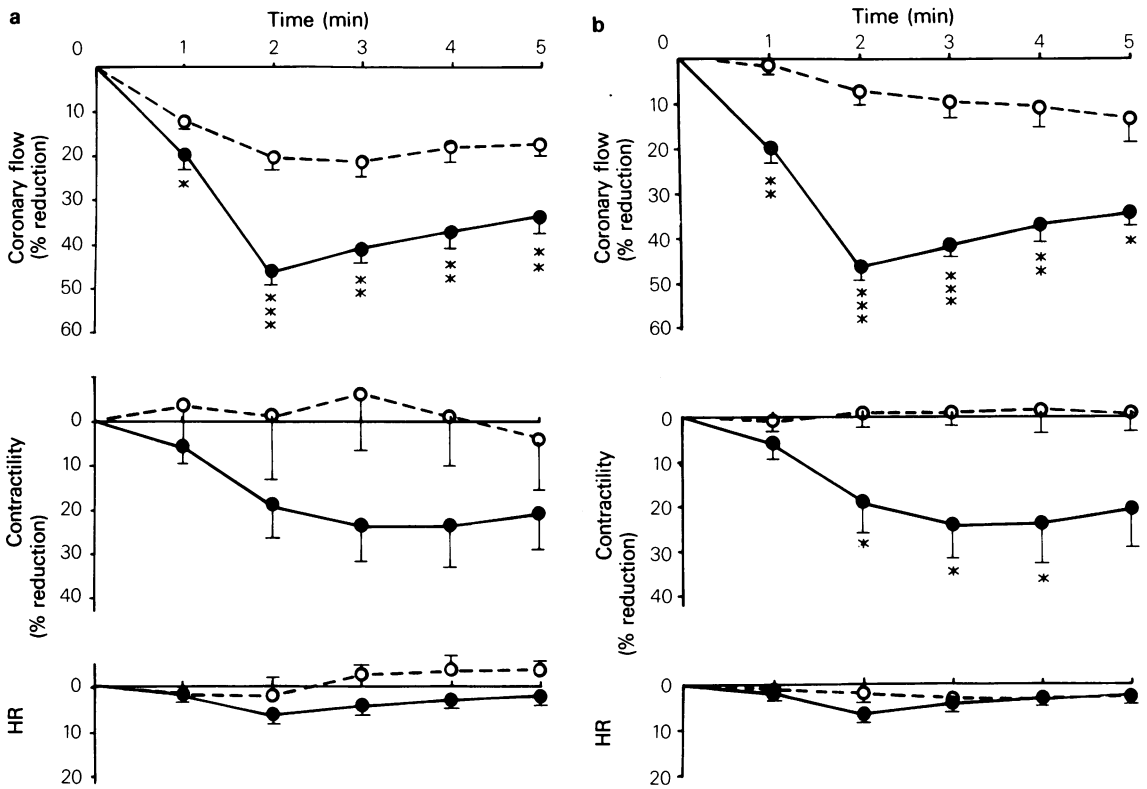

Figure 4 The actions of leukotriene C_4 (LTC_4) on coronary flow, contractility and heart rate (HR) and the effects of indomethacin and FPL 55712. (a) LTC_4 (1.6×10^{-9} M, ●) caused reduction in coronary flow by a maximum of $46 \pm 3\%$ at 2 min. Indomethacin (1.4×10^{-5} M, ○) significantly inhibited the LTC_4-induced reduction in flow but did not affect heart rate. (b) LTC_4 (1.6×10^{-9} M) alone (●); FPL 55712 (3.8×10^{-6} M, ○) significantly inhibited the LTC_4-induced reduction in coronary flow and contractility but had no effect on heart rate.

quantitative differences were detected.

In guinea-pig isolated hearts LTC_4 had the slower onset of action when it was compared with LTD_4. It had the greater efficacy in reducing coronary flow and a steeper slope in the logarithmic dose-response relationship. A similar lack of parallelism in the log dose-response relationships for their vasoconstrictor activity in the microcirculation of the guinea-pig skin has been observed (Peck *et al.*, 1981).

The experiments described show that the vascular actions of the leukotrienes in the guinea-pig involve the effects of released vasoconstrictor cyclo-oxygenase metabolites of arachidonic acid. At the dose-level investigated, the LTC_4-induced coronary flow reductions were halved by the prior addition of indomethacin. Only the initial LTD_4-induced reduction in coronary flow was affected by an identical pretreatment with indomethacin. This dose of indomethacin has been shown to inhibit cyclo-oxygenase effectively (Flower, 1974); thus these results also indicate the vascular effects of LTC_4 are more dependent on the release of cyclo-oxygenase products than LTD_4. The cyclo-oxygenase products, thromboxane A_2 (TxA_2), prostaglandin D_2 (PGD_2), $PGF_{2\alpha}$ and PGI_2 had been shown to be released from guinea-pig isolated hearts (Anhut, Bernhauer & Peskar, 1977; Liebig, Bernauer & Peskar, 1975; Levi, Allan & Zavecz, 1976; Allan & Levi, 1981; Anhut *et al.*, 1978a; Schror, Moncada, Ubatuba & Vane, 1978). TxA_2, PGD_2 and $PGF_{2\alpha}$ have been shown to have coronary vasoconstrictor activities (Terashita, Fukui, Nishikawa, Hirata & Kikuchi, 1978; Schror, 1978; Liebig *et al.*, 1975; Allan & Levi, 1980) and it is interesting that indomethacin has been reported to block (Levi *et al.*, 1976; Anhut *et al.*, 1977) or have no effect (Liebig *et al.*, 1975) on the coronary vasoconstriction in cardiac immediate hypersensitivity reactions in guinea-pig hearts *in vitro*. Also inhibition of TxA_2 synthesis has been shown to be either effective (Allan & Levi, 1980) or non-effective (Anhut, Bernauer & Peskar, 1978b) in antagonizing the vasoconstriction.

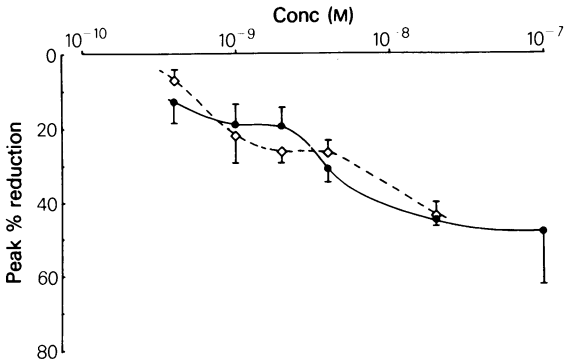

Figure 5 The lack of effect of indomethacin (1.4×10^{-5} M, ◇) on the leukotriene D_4 (●)-induced reduction in coronary flow. Vertical bars show s.e.mean ($n = 3-5$ per dose).

FPL 55712, an SRS-A (leukotriene) antagonist (Augstein, Farmer, Lee, Sheard & Tattershall, 1973), effectively antagonized the actions of LTC_4 on the coronary vasculature but the doses required were higher than those used to block leukotriene-induced contraction of guinea-pig ileum. However, the dose of LTD_4 was only partially antagonized. FPL 55712 was most effective during the first to second min of the LTD_4 infusion, suggesting that the second action of LTD_4, which took longer to reach equilibrium, may reflect a gradually increasing effect of the agonist by competitive displacement of the antagonist.

The actions of the leukotrienes on contractility of the guinea-pig isolated working heart were complex. Although several distinct dose-related effects were apparent, the relative contribution and importance of each was not determined. The results of the experiments with isolated atrial and ventricular muscle strips show that concentrations of the leukotrienes, 100 fold greater than those that produce maximal reductions in coronary flow, have no direct effect on the developed tensions (10 min observation periods). In contrast, in isolated perfused hearts, doses of the leukotrienes reducing coronary flow also produced rapid decreases in contractility. These decreases in contractility were associated (in part) with decreases in the resting tension of the working heart. A similar profile of action with TxA_2 in the guinea-pig isolated heart has been reported (Terashita *et al.*, 1978). The reason(s) for this action could be a reversible 'direct' effect on the ventricular 'extravascular support', the release of another mediator, or responses 'secondary' to the coronary constriction. For example, the constriction of the coronary vessels would decrease the total volume of fluid within the ventricle wall. This would lead to a 'thinning' of the ventricle wall which (particularly during diastole) would lead to a decrease in the 'hydrostatic component of ventricular rigidity' and express itself by a ventricular dilatation or decrease in the resting tension. In isolated Langendorff hearts perfused at constant flow (unpublished observation), the leukotrienes first induce an increase in perfusion pressure with either no change or a slight increase in contractility with high doses. This is followed by a gradual decrease in the developed tension.

In most cases the leukotriene-induced effects on contractility were antagonized by pretreatment with

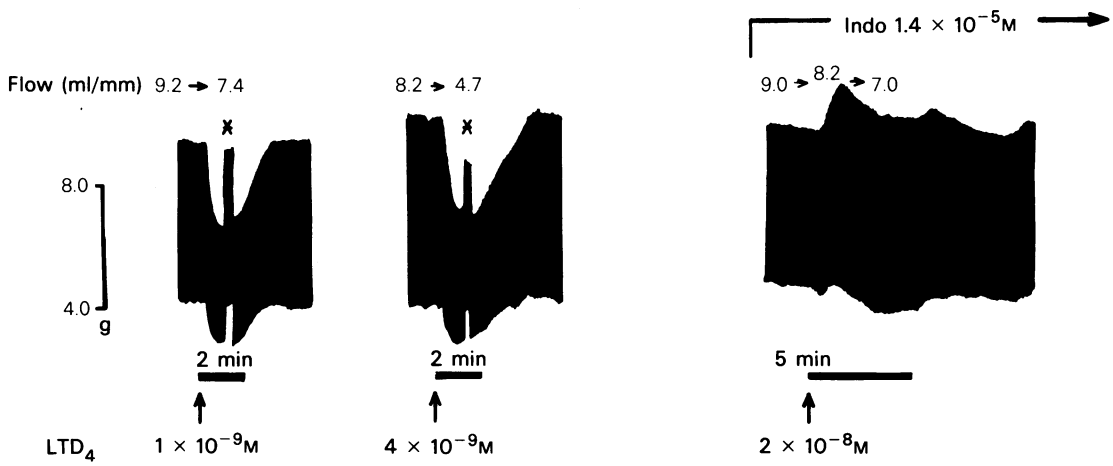

Figure 6 The actions of leukotriene D_4 (LTD_4) on guinea-pig perfused heart and the effects of indomethacin (Indo). Infusions of LTD_4 (1×10^{-9} M and 4×10^{-9} M, solid bars) decreased coronary flow, resting tension and contractility. The asterisks show the effects of a momentary readjustment of the resting tension to the pre-infusion level. Indomethacin (1.4×10^{-5} M) delayed the reduction in coronary flow and blocked the effects on contractility to 5 min infusion of LTD_4 (2×10^{-8} M).

indomethacin. These results indicate an additional and variable action of cyclo-oxygenase products. The total blockade of the actions of LTC_4 with indomethacin and FPL 55712, but only partial blockade of the actions of LTD_4, again suggests different mechanisms of action. Complex actions and interactions of cyclo-oxygenase products on isolated working hearts have been reported (Karmazyn, Leung & Dhalla, 1979; Allan & Levi, 1980). Concentrations of the leukotrienes causing maximal coronary vasoconstriction produced, in addition to the effects already discussed, a prolonged negative inotropic action. Whereas the decreases in the resting tension tended to recover within 3 min after cessation of the leukotriene infusions, the contractility did not fully recover. It should be noted that in each of these cases the coronary flow rates also never returned to pre-infusion values. Again this action was more apparent with LTD_4.

In all of the experiments in this study, the leukotrienes did not produce significant changes in the spontaneous rate of contractions of either isolated atria or of the isolated working hearts, neither did they produce arrhythmias. There was no evidence to suggest that β-adrenoceptor or histamine H_1- or H_2-receptor actions were involved in the actions of LTC_4 or LTD_4.

In conclusion, these experiments have shown that the leukotrienes are potent constrictors of the coronary vasculature, LTC_4 having the greater activity than LTD_4. The results described suggest the leukotrienes would have profound effects on the heart *in vivo*, consequent upon either intra-cardiac release or as a circulatory 'target organ'. Leukotrienes would probably contribute to the primary cardiac participation observed in systemic anaphylaxis (Capurro & Levi, 1975), particularly the decrease in cardiac output which has frequently been described. Indeed SRSs can be formed by non-immunological means (Piper & Seale, 1979) and there is growing evidence that a main site of action of the peptido-lipid leukotrienes is associated with microvascular beds. They may also therefore, play a role in cases of variant angina and myocardial ischaemia and further investigation seems warranted.

While this manuscript was in preparation some of the findings reported in this paper have been described by Terashita *et al.* 1981.

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