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Vascular responses to leukotriene B_4, C_4 and D_4 following FPL 55712, indomethacin, saralasin, phentolamine and verapamil in the conscious rat

János Filep*,†,[1], Éva Földes-Filep*,† & Jürgen C. Frölich†

Department of Physiology*, Semmelweis University Medical School, Budapest, Hungary and Department of Clinical Pharmacology†, Hannover Medical School, Hannover, FRG

1 The pressor and vascular permeability effects of leukotrienes B_4 (LTB_4), C_4 and D_4 were investigated in conscious unrestrained rats.

2 Leukotrienes C_4 and D_4 (3.2–51 nmol kg^{-1} i.v.) caused an acute dose-dependent elevation of the mean arterial pressure, which was maximal after 2 min and returned to control levels within 14 min. Heart rate was significantly reduced by the higher doses of LTC_4 and LTD_4. LTB_4 (up to a dose of 51 nmol kg^{-1}) was essentially inactive.

3 These effects of LTC_4 and LTD_4 were abolished by FPL 55712, a putative antagonist of sulphidopeptide leukotrienes and by verapamil, a calcium channel blocker.

4 Indomethacin, phentolamine or saralasin pretreatment failed to modify the pressor response to LTC_4 and LTD_4.

5 LTC_4 and LTD_4 furthermore caused an increase in haematocrit values, which was significantly attenuated by FPL 55712, indomethacin and verapamil.

6 The present findings show that the pressor effect of LTC_4 and LTD_4 is not related to prostanoid release and can be reversed by calcium channel blockade; whereas the effect on vascular permeability seems to require the presence of both cyclo-oxygenase product(s) and calcium.

Introduction

Leukotrienes are naturally occuring compounds derived from the 5-lipoxygenase pathway of arachidonic acid. Leukotriene C_4 (LTC_4) and leukotriene D_4 (LTD_4) were identified as the major active components of slow reacting substance of anaphylaxis (SRS-A) (Murphy *et al.*, 1979; Lewis *et al.*, 1980). These leukotrienes have been implicated in asthma, oedema and cardiovascular disorders (for reviews see Hammarström, 1983; Lewis & Austen, 1984). Leukotriene B_4 (LTB_4) has different properties with prominent chemotactic activity (Ford-Hutchinson *et al.*, 1980).

Acute administration of LTC_4 and LTD_4 has been shown to produce an acute pressor response followed by a more prolonged hypotensive period in various experimental animals (for review see Feuerstein, 1984). However, the majority of these investigations were performed on guinea-pigs, in which the systemic effects of leukotrienes are always accompanied by severe respiratory distress. These pulmonary effects of leukotrienes might cause secondary cardiovascular responses. Recent investigations on systemic effects of LTC_4 and LTD_4 in the rat, a species in which leukotrienes do not cause hypoxaemia and respiratory distress (Krell *et al.*, 1981) have also demonstrated a pressor effect either followed by hypotension (Zukowska-Grojec *et al.*, 1982a; Iacopino *et al.*, 1983; Feuerstein *et al.*, 1983) or without subsequent decrease in blood pressure (Badr *et al.*, 1984; Filep *et al.*, 1985).

The pressor response of guinea-pigs to leukotrienes is generally thought to be mediated in part by release of thromboxane because the vasoconstrictor effect can be blocked by either indomethacin (Schiantarelli *et al.*, 1981; Sirois *et al.*, 1981; Ueno *et al.*, 1982; Samhoun & Piper, 1984) or by OKY 1581, a specific thromboxane synthetase inhibitor (Ueno *et al.*, 1982). Investigations in rats showed different results: indomethacin did not affect the pressor response (Zukowska-Grojec *et al.*, 1982a) and shortened or prevented the depressor

[1] Author for correspondence at Department of Clinical Pharmacology, Hannover Medical School, Konstanty-Gutschow-Str. 8, D-3000 Hannover 61, FRG.

phase (Zukowska-Grojec *et al.*, 1982a; Iacopino *et al.*, 1983). On the other hand, potentiation of the pressor response to LTD_4 by indomethacin has also been observed (Zukowska-Grojec *et al.*, 1982a). Studies with FPL 55712, a putative antagonist of leukotrienes (Augstein *et al.*, 1973) also led to controversial results as significant attenuation of pressor responses to LTC_4 (Badr *et al.*, 1984; Filep *et al.*, 1985) but not to LTD_4 (Zukowska-Grojec *et al.*, 1982b) were found to occur. The interpretation of these findings is further complicated by the fact that the majority of the above mentioned experiments were performed on anaesthetized rats, under conditions which are known to alter cardiovascular control mechanisms (Cox & Bagshaw, 1980; Zerbe & Feuerstein, 1985).

The objectives of the present study were to characterize the vascular responses to LTB_4, LTC_4 and LTD_4 in conscious rats and to investigate the possible mechanism(s) contributing to these responses.

Methods

The experiments were carried out on 9 conscious, female Sprague-Dawley rats weighing 217–255 g. The animals were kept in individual metabolic cages and were prepared according to the methods of Fejes-Tóth *et al.*, (1984) with modifications. Briefly, under anaesthesia (ketamine, 75 mg kg^{-1} and sodium pentobarbitone, 15 mg kg^{-1}, i.p.) catheters were implanted into the abdominal aorta and vena cava through the ventral tail artery and femoral vein, respectively. The venous catheter was led subcutaneously to the root of the tail. The catheters emerging from the tail and the wounds were protected by an acrylic cuff glued to the tail. The cuff was connected to a stainless steel spiral that was fed through the top of the metabolic cage. At least 4 days were allowed for the animals to recover completely before the first experiment was performed. Before and during the experiments the rats could move freely and had free access to food and water. Mean arterial pressure was monitored with an electromanometer (EM 61, Medicor, Budapest, Hungary) using a Statham P 23 dB strain gauge. Heart rate was measured with a rate-meter connected to the manometer.

After an equilibration period of 3 h, various doses of different leukotrienes were injected intravenously. Doses ranged from 3.2×10^{-9} to 5.1×10^{-8} mol kg^{-1} and leukotrienes were injected in a volume of 6.25 µl 100 g^{-1} body wt. followed by 30 µl of sterile 0.9% w/v NaCl solution. On any one day each animal received only three doses of leukotrienes at 70–80 min intervals. Both the order in which leukotrienes were injected and the doses within each dose-response curve were randomized.

In the second set of experiments we investigated the pressor effect of 6.4×10^{-9} and 12.8×10^{-9} mol kg^{-1} LTC_4 and LTD_4 without and with prior intravenous adminstration of FPL 55712 (4 mg kg^{-1} 2 min before the leukotrienes), indomethacin (3 mg kg^{-1} plus 0.3 mg kg^{-1}, h^{-1}), saralasin (4 µg kg^{-1} min^{-1}) or verapamil (1.25 mg kg^{-1} 20 min before administration of leukotrienes). In these experiments on the same day each animal was pretreated with only one of the above mentioned drugs and received only one dose of leukotriene. At least two days were allowed to elapse between two experiments on the same rat.

In the third series of experiments a similar protocol was followed to that in the second series except that 60 min before and 15 min after the injection of LTC_4 or LTD_4 (1.28×10^{-8} mol kg^{-1}) approximately 20 µl of blood was obtained through the arterial catheter to determine haematocrit (Hct) values. At the end of the experiments 50 µl of donor blood was injected intravenously to all animals.

Synthetic leukotrienes were kept under N_2 at −80°C in aqueous ethanol (50%). On the experimental day an aliquot was removed, evaporated under N_2 at 0°C and was redissolved in ice-cold phosphate buffer (40 mM, pH 7.2 equilibrated with N_2) containing glucose (40 g l^{-1}) in a final concentration of 41×10^{-8} M. This solution was further diluted in the same solvents to the appropriate concentration. FPL 55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxy propoxy] -4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate, Fisons Ltd, Leicestershire, England), 1-sarcosine, 8-alanine angiotensin II (saralasin, Calbiochem, La Jolla, Ca, USA) and verapamil (Knoll AG, Ludwighshafen, FRG) were dissolved in 4% glucose. Indomethacin (Chinoin, Budapest, Hungary) was dissolved freshly in phosphate buffer (50 mM, pH 7.2). All drugs were injected in a volume of 0.25 µl 100 g^{-1} body wt. as a bolus, and in 25 µl 100 g^{-1} body wt. h^{-1} as an infusion. Phentolamine (Regitin, Ciba-Geigy, Basel, Switzerland) was administered 30 min before the leukotrienes in a volume of 1 ml kg^{-1}.

Results are expressed as means ± s.e. Statistical evaluation of the data was performed by Wilcoxon's test and by Mann-Whitney's U test for paired and unpaired observations, respectively. A 0.05 level was considered significant.

Results

Five days after surgery mean arterial pressure was 102 ± 2 mmHg with a basal heart rate of 339 ± 7 beats min^{-1}.

LTC_4 and LTD_4 caused a dose-dependent increase in mean arterial pressure (Figure 1), while LTB_4 was essentially inactive. LTC_4 and LTD_4 appeared to be equipotent pressor substances. The pressor responses to these leukotrienes were rapid in onset, returning to

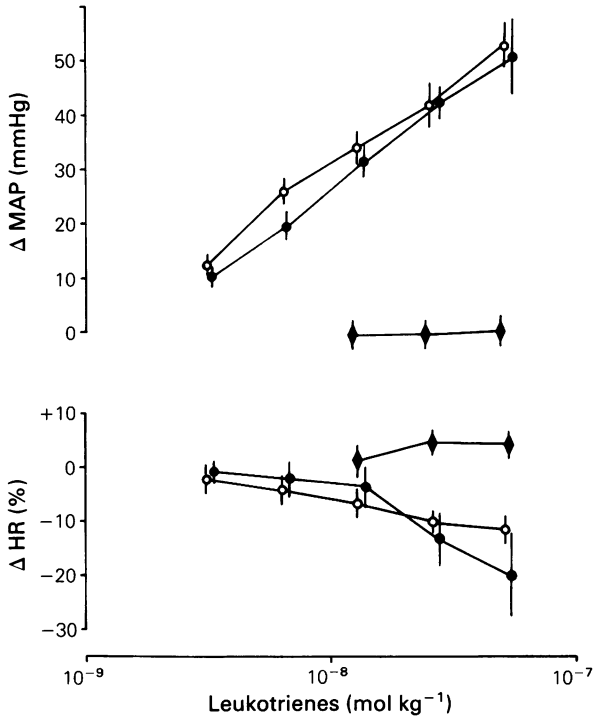

Figure 1 Pressor responses to intravenous injections of leukotriene B$_4$ (LTB$_4$, ◆), LTC$_4$ (○) and LTD$_4$ (●) in conscious rats. MAP, mean arterial pressure; HR, heart rate. Results show mean values ($n = 6$ rats) and vertical lines represent s.e.mean.

base line within 3–4, 4–6, 6–8, 8–10 and 10–14 min corresponding to the five doses, and were monophasic (Figure 2). When either LTC$_4$ or LTD$_4$ was injected repeatedly at 1 h intervals no tachyphylaxis could be observed (Figure 3). The pressor response to LTC$_4$ and LTD$_4$ was accompanied by a concomitant decrease in heart rate. The lower doses of leukotrienes had a highly variable effect on heart rate resulting in mean heart rates close to control values (Figure 1). Higher doses of LTC$_4$ and LTD$_4$, however, caused a significant decrease in heart rate (Figure 1). FPL 55712 by itself had no effect on mean arterial pressure (104 ± 3 versus 107 ± 2 mmHg, $n = 6$) or heart rate (341 ± 8 versus 339 ± 6 beats min^{-1}, $n = 6$), but it substantially reduced both the vasopressor and cardiodepressant effects of LTC$_4$ and LTD$_4$ (Figures 4 and 5). Indomethacin or saralasin infusions did not alter blood pressure or heart rate, and they also failed to modify cardiovascular responses to LTC$_4$ and LTD$_4$ (Figures 4 and 5). Although phentolamine pretreatment reduced basal mean arterial pressure by 6% and increased heart rate by 5%, the changes in mean arterial pressure and in heart rate after LTC$_4$ or LTD$_4$ were similar to those seen when these leukotrienes were administered without pretreatment (Figures 4 and 5). Verapamil alone caused a slight decrease in mean arterial pressure (from 104 ± 2 to 96 ± 1 mmHg, $P < 0.05$) with a concomitant increase in heart rate (from 331 ± 7 to 355 ± 7 beats min^{-1}, $P < 0.05$). Verapamil significantly attenuated the pressor effects of LTC$_4$ and LTD$_4$ (Figures 4 and 5).

Administration of the vehicle used to dissolve leukotrienes itself had no effect on Hct (40.8 ± 1.1

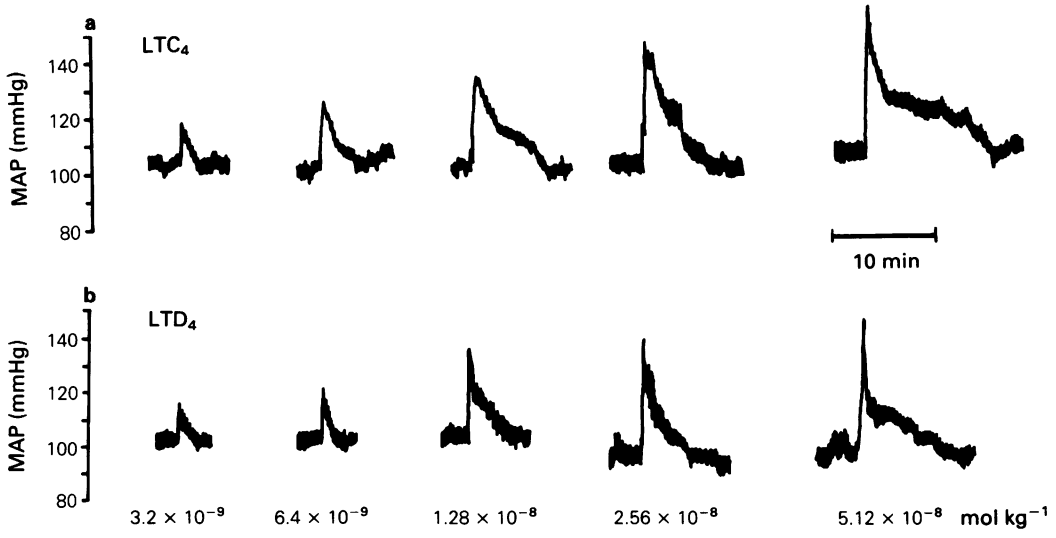

Figure 2 Representative mean arterial pressure (MAP) traces following intravenous administration of (a) leukotriene C$_4$ (LTC$_4$) and (b) LTD$_4$ in the conscious rat.

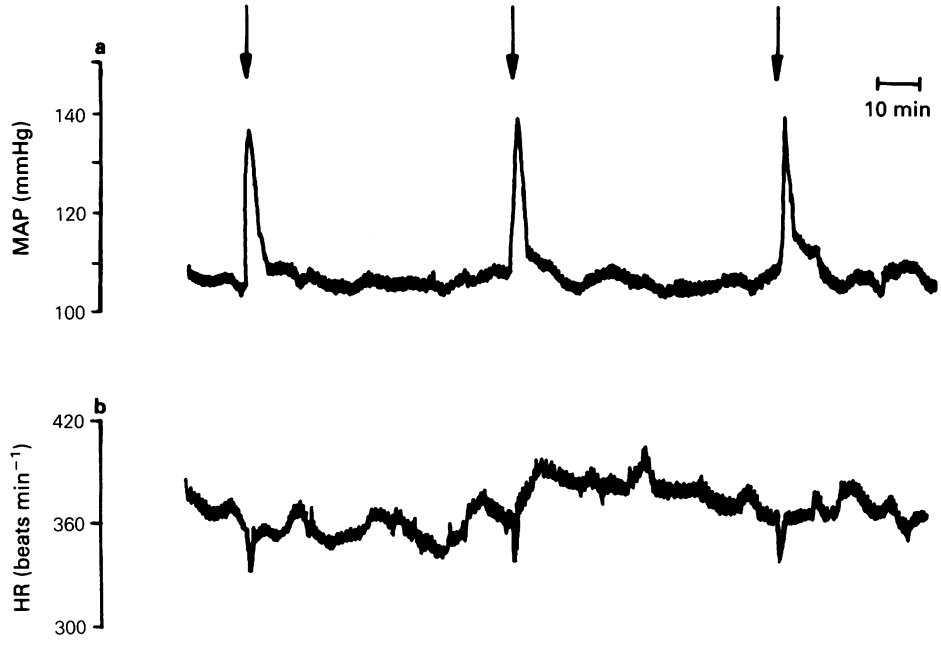

Figure 3 Authentic recordings of the (a) mean arterial pressure (MAP) and (b) heart rate (HR) response to repeated injections of leukotriene C_4 (LTC_4). Arrows indicate the injections of LTC_4 (1.28×10^{-8} mol kg^{-1}).

control versus 40.7 ± 1.2 vol. % experimental), whereas injection of either 1.28×10^{-8} mol kg^{-1} of LTC_4 or LTD_4 resulted in an increase in Hct (Table 1). The rise in Hct after LTC_4 or LTD_4 administration was also seen in saralasin or phentolamine pretreated animals (Table 1). When the animals were pretreated with FPL 55712, indomethacin or verapamil, no significant increase in Hct was observed after LTC_4 and LTD_4 (Table 1).

The effectiveness of the saralasin and phentolamine treatments as specific antagonists was tested in separate experiments. Saralasin at a dose of 4 μg kg^{-1} min^{-1} reduced the pressor response to 25 and 50 ng angiotensin II by about 95 and 92%, respectively. When 90 min after the initial injection of phentolamine an additional dose (10 mg kg^{-1}) was given, no further reduction in mean arterial pressure was observed. This indicates that α-adrenoceptors were completely blocked during the experiments. Indomethacin at the dose employed inhibited prostaglandin synthesis, as evidenced by the decrease in urinary excretion of prostaglandin E from 167 ± 22 pg min^{-1} to 44 ± 5 pg min^{-1} ($n = 5$, $P < 0.05$), as determined by radioimmunoassay (Fejes-Tóth *et al.*, 1983).

Discussion

The present study demonstrates that LTC_4 and LTD_4, but not LTB_4 can induce an immediate rise in mean arterial pressure and in haematocrit in conscious rats. These effects of sulphidopeptide leukotrienes were attenuated by the calcium channel blocker verapamil and FPL 55712, a putative antagonist of SRS-A (Augstein *et al.*, 1973). Furthermore, indomethacin pretreatment significantly reduced the rise in haematocrit by LTC_4 and LTD_4, but did not modify the pressor response to these leukotrienes.

Although we did not measure plasma volume before and after injection of leukotrienes, it is likely that the increase in haematocrit was mainly due to plasma volume losses, since administration of LTC_4 (10 μg kg^{-1}) has been shown to result in an immediate loss of approximately 20% of plasma volume in anaesthetized rats (Badr *et al.*, 1984).

Some recent observations have suggested that calcium ion fluxes might be important in mediating the effects of leukotrienes in non-vascular (Findlay *et al.*, 1982; Weichmann *et al.*, 1983; Kohrogi *et al.*, 1985) and in coronary vascular smooth muscle *in vitro* (Roth & Lefer, 1983; Fiedler *et al.*, 1984). The capacity of

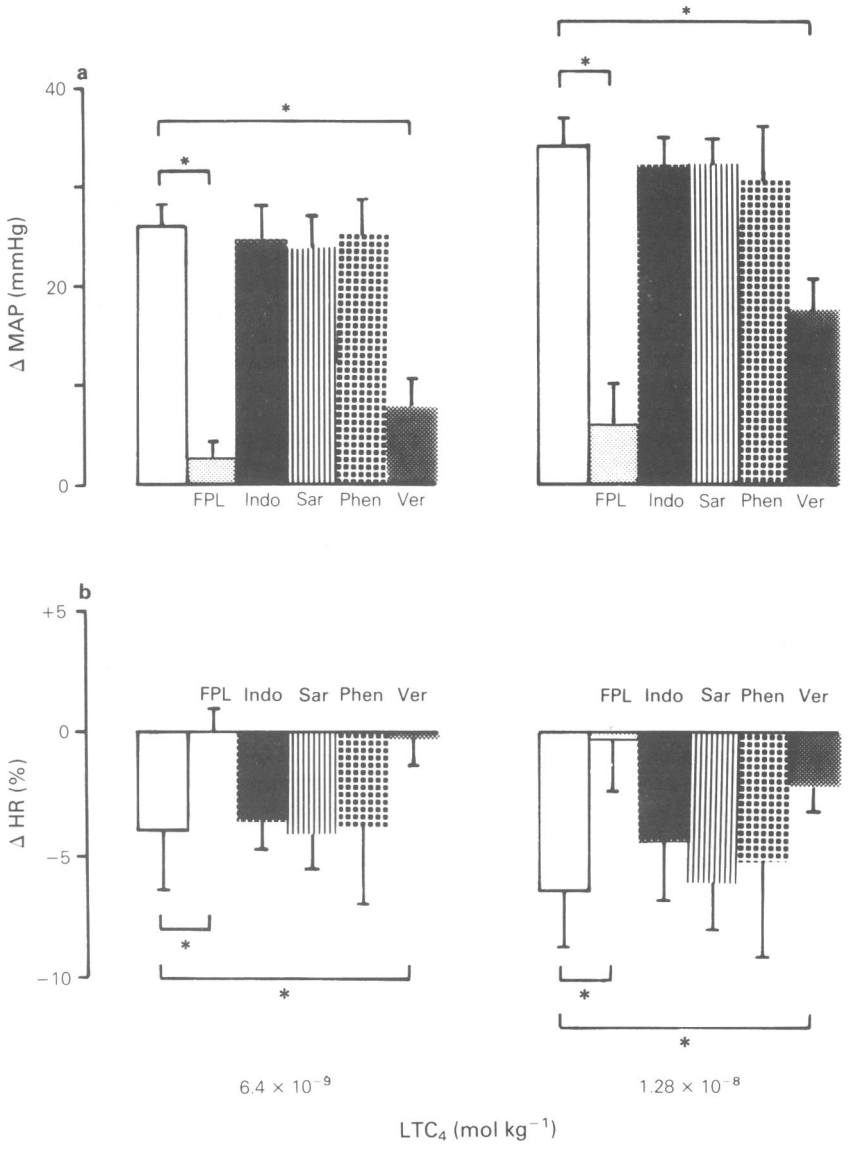

Figure 4 Effects of FPL 55712, indomethacin, saralasin, phentolamine and verapamil on leukotriene C_4 (LTC_4)-induced changes in (a) mean arterial pressure (MAP) and (b) heart rate (HR) in conscious rats. Abbreviations: FPL, FPL 55712; Indo, indomethacin; Sar, saralasin; Phen, phentolamine; Ver, verapamil. For doses of antagonists see Methods. Open columns represent control responses to LTC_4. $^*P < 0.05$ (probability level obtained by Mann-Whitney's U test). $n = 6$ rats.

verapamil to interfere directly with the pressor and haemoconcentration effects of LTC_4 and LTD_4, as demonstrated in the present study, suggests that these effects of cysteinyl leukotrienes might be, at least partly, mediated through activation of voltage-operated calcium channels. The failure of verapamil to block completely the pressor effect of leukotrienes is consistent with the presence of leukotriene receptor-operated calcium channels in smooth muscle (Weichmann *et al.*, 1983). As very little is known about the

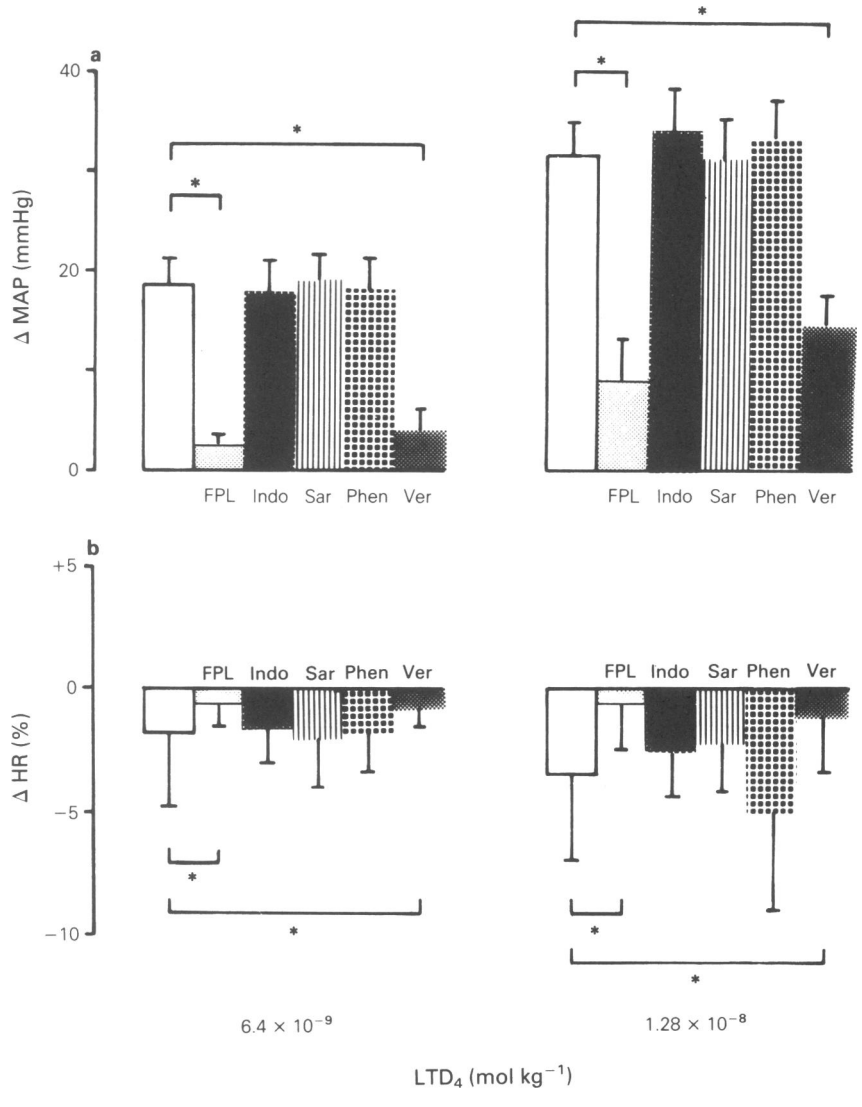

Figure 5 Effects of FPL 55712, indomethacin, saralasin, phentolamine and verapamil on leukotriene D_4 (LTD_4)-induced changes in (a) mean arterial pressure and (b) heart rate in conscious rats. Abbreviations are the same as in Figure 4. Open columns represent control responses to LTD_4. $^*P < 0.05$. $n = 6$ rats.

role of calcium in regulating capillary permeability, it is difficult to explain the effect of verapamil in preventing leukotriene-induced haemoconcentration.

Pressor responses to leukotrienes have been observed in guinea-pigs (Drazen *et al.*, 1980; Sirois *et al.*, 1981), primates (Smedegard *et al.*, 1982), anaesthetized rats (Iacopino *et al.*, 1983; Pfeffer *et al.*, 1983; Badr *et al.*, 1984) and in conscious rats (Filep *et al.*, 1985). In contrast to the biphasic response described in the majority of the previous papers (Drazen *et al.*, 1980; Iacopino *et al.*, 1983; Pfeffer *et al.*, 1983; Smedegard *et al.*, 1982), we could not detect any hypotension after LTC_4 and LTD_4 injections in conscious rats, not even if leukotrienes were injected in doses as high as 51 nmol kg^{-1}. Such discrepancies might be attributable to species differences or to the

Table 1 Effect of leukotrienes C_4 and D_4 (LTC_4 and LTD_4) on haematocrit values in the presence and absence of FPL 55712, indomethacin, saralasin, phentolamine and verapamil

		Hct (volume %)		
		LTC_4 (1.28×10^{-8} mol kg^{-1})		
Pretreatment		*Before*	*After*	P
Vehicle	(n = 6)	40.7 ± 1.4	44.3 ± 1.8	<0.05
FPL 55712	(n = 6)	40.3 ± 1.3	40.2 ± 0.7	NS
Indomethacin	(n = 6)	39.5 ± 0.9	41.0 ± 1.8	NS
Saralasin	(n = 6)	40.4 ± 0.9	43.3 ± 1.0	<0.05
Phentolamine	(n = 4)	39.8 ± 1.4	43.3 ± 1.1	<0.05
Verapamil	(n = 6)	39.8 ± 0.6	40.8 ± 0.6	NS
		LTD_4 (1.28×10^{-8} mol kg^{-1})		
Pretreatment		*Before*	*After*	P
Vehicle	(n = 6)	40.0 ± 1.1	43.0 ± 1.2	<0.05
FPL 55712	(n = 6)	40.0 ± 1.1	40.2 ± 1.4	NS
Indomethacin	(n = 6)	40.3 ± 0.7	40.3 ± 1.0	NS
Saralasin	(n = 6)	41.3 ± 0.8	44.2 ± 0.8	<0.05
Phentolamine	(n = 4)	40.8 ± 0.9	44.0 ± 1.1	<0.05
Verapamil	(n = 6)	41.3 ± 0.9	42.7 ± 0.4	NS

Values are means ± s.e. Doses of antagonists are given in Methods. Probability levels were obtained by Wilcoxon's test. Hct, haematocrit; NS, not significant.

different experimental conditions. When chronically catheterized rats were anaesthetized with Inactin (100 mg kg^{-1} i.p.) a biphasic response to LTC_4 was observed in two of three animals. When we repeated the experiments on anaesthetized, acutely catheterized rats, administration of LTC_4 (6.4×10^{-9}–25.5×10^{-9} mol kg^{-1}) elicited a transient increase followed by a decrease in mean arterial pressure in all animals (Filep *et al.*, unpublished observations). These latter findings suggest that, in the rat, the hypotensive phase observed after injection of leukotrienes might be due to the use of anaesthetics and/or presence of surgical stress.

The transient rise in blood pressure was associated with a decrease in heart rate. Whether this is the consequence of stimulation of the baroreceptors or of a direct effect on the heart (Letts & Piper, 1982; Letts *et al.*, 1983; Greenwald *et al.*, 1984) cannot be deduced from the present experiments.

Since in the present study FPL 55712 attenuated the pressor and haemoconcentration effects of both LTC_4 and LTD_4, it was assumed that these leukotrienes must have specific cardiovascular receptors. At present it is not clear whether FPL 55712 can be used to distinguish between LTC_4 and LTD_4 receptors. Indeed, FPL 55712 has been described as a selective antagonist for LTC_4 (Bach *et al.*, 1979) or for LTD_4 (c.f. Casey *et al.*, 1983), while others failed to find a selective antagonism (Palmer *et al.*, 1981; Letts *et al.*, 1983). We found that the cardiovascular actions of LTC_4 and LTD_4 were similar. As we did not investigate the metabolism of LTC_4 we cannot exclude the possibility that some effects observed after administration of LTC_4 were due to LTD_4 arising from the metabolism of LTC_4. However, the spasmogenic activity of LTC_4 was shown to be independent of its conversion to LTD_4 (Krillis *et al.*, 1983).

As all cardiovascular effects of leukotrienes can be reproduced in pithed rats (Zukowska-Grojec *et al.*, 1982a) it is likely that the pressor response to leukotrienes is of peripheral origin, and it seems to be a result of a generalized increase in total peripheral resistance (Smedegard *et al.*, 1982; Pfeffer *et al.*, 1983). Although leukotrienes have been shown to increase circulating catecholamine levels at the peak of their pressor effect in conscious rats (Zukowska-Grojec *et al.*, 1982a; Feuerstein *et al.*, 1983), the participation of catecholamines in mediating the pressor effect of LTC_4 and LTD_4 seems unlikely since (a) leukotrienes elevated blood pressure in pithed rats without altering plasma catecholamine levels (Zukowska-Grojec *et al.*, 1982a) and (b) α-adrenoceptor blockade failed to modify the pressor response to LTC_4 and LTD_4 in the present study. Similarly, angiotensin II receptor blockade did not alter the pressor effect of LTC_4 and LTD_4, excluding a possible involvement of the renin-angiotensin system.

The vascular action of leukotrienes has been found to be mediated by the release of thromboxane A_2 under certain conditions (Piper & Samhoun, 1981; Ueno *et al.*, 1982). In contrast, similar vascular effects of LTC_4 and LTD_4 were observed in the presence and absence of a prostaglandin synthesis inhibitor in rats and dogs (Feigen, 1983; Rosenthal & Pace-Asciak, 1983; Badr *et al.*, 1984). Our finding that the pressor effect of LTC_4 and LTD_4 was not affected by indomethacin gives further support to the notion that this effect of leukotrienes is not mediated by release of thromboxane or other prostaglandins in rats. On the other hand, indomethacin prevented leukotriene-induced plasma volume losses, as evidenced by stable haematocrit values. This finding suggests that the increased capillary permeability observed after administration of cysteinyl leukotrienes is, in part, mediated by prostanoids.

In conclusion, the present results demonstrate that the pressor action of LTC_4 and LTD_4 is not related to prostanoid release, but rather appears to be due to a calcium mediated event secondary to activation of specific leukotriene receptors, whereas their effect on vascular permeability seems to require the presence of both secondarily generated cyclo-oxygenase products and calcium.

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