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The role of blood vessels in the bioconversion of leukotrienes in the pig

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1 Porcine pulmonary artery has the ability to convert leukotriene C_4 (LTC_4) to LTD_4 and then to LTE_4.

2 In this vessel, there appears to be no further metabolism beyond LTE_4.

3 LTC_4 (1 nM) is converted rapidly to LTD_4, whereas the conversion of LTD_4 to LTE_4 is somewhat slower.

4 The conversion of LTC_4 to LTD_4 is inhibited by the γ-glutamyl transpeptidase inhibitor, serine-borate (45 mM).

5 The conversion of LTD_4 to LTE_4 is inhibited by the aminopeptidase inhibitor, L-cysteine (10 mM).

6 LTB_4 did not appear to be metabolized by porcine pulmonary artery.

7 These results suggest that the vessel wall may play a role in the early stages of leukotriene metabolism.

Introduction

The leukotrienes are a group of recently characterized compounds derived from arachidonic acid (Murphy *et al.*, 1979; Morris *et al.*, 1980). Slow-reacting substance of anaphylaxis (SRS-A), which has been suggested as a mediator of bronchospasm in asthma (Dahlén *et al.*, 1980), has been shown to consist of leukotriene C_4 (LTC_4), LTD_4 and LTE_4 (Morris *et al.*, 1980; Lewis *et al.*, 1980). LTC_4 is converted to LTD_4 by the removal of glutamic acid by the enzyme γ-glutamyl transpeptidase, and LTD_4 is subsequently converted to LTE_4 by removal of glycine by an aminopeptidase. This conversion has been demonstrated in a number of systems including guinea-pig ileum (Krilis *et al.*, 1983), guinea-pig trachea (Snyder *et al.*, 1984), guinea-pig lung (Hammarström, 1981), rat perfused lung (Harper *et al.*, 1984) and in the mouse *in vivo* (Appelgren & Hammarström, 1982). Further metabolism beyond LTE_4 has been demonstrated (Hammarström, 1981) and in some instances the metabolites have been identified. N-acetyl LTE_4 has been detected in the plasma and bile of rats after trauma (Denzlinger *et al.*, 1985).

In addition to their potent bronchoconstrictor actions, the peptido-leukotrienes (LTC_4, LTD_4 and LTE_4) also have powerful vasoconstrictor activity *in vitro* (Letts & Piper, 1982) as well as *in vivo* (Ezra *et al.*, 1983; Piper *et al.*, 1984). In addition, blood vessels have been shown to have the capacity to generate these leukotrienes (Fleisch & Haisch, 1982; Piper *et al.* 1983; Wölbling *et al.*, 1983; Lefer *et al.*, 1984) and, as such, may play a role in vasospastic diseases such as angina. Porcine pulmonary and other arteries have been shown to release LTC_4, LTD_4 and LTE_4 upon stimulation with calcium ionophore A23187 (Piper & Galton, 1984) and the present investigation extends this work to determine whether further metabolism occurs in this preparation and to study the effects of inhibition of the enzymes involved. Porcine pulmonary arteries also generate LTB_4 (Piper & Galton, 1984), a powerful chemotactic and chemokinetic agent (Smith *et al.*, 1980). Study of the metabolism of LTB_4 in human polymorphonuclear leukocytes has shown that it is first converted to the 20-hydroxy and then to the 20-carboxy compound (Powell, 1984). However, in rat perfused lung, there appears to be no metabolism, the LTB_4 remaining unchanged (Harper *et al.*, 1984). Part of this work has been presented at the meeting of the British Pharmacological Society in Bath, April 1986 (Galton & Piper, 1986).

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Methods

Porcine pulmonary arteries were obtained from freshly slaughtered pigs. These were weighed, chopped, suspended in Tyrode solution (6 ml g^{-1}) and shaken in a water bath at 37°C. To study the conversion of the leukotrienes, LTB_4, LTC_4 or LTD_4 was added to give a final concentration of 1 nM. [^{3}H]-LTB_4, C_4 or D_4 (0.1 μCi) was added as a tracer. At timed intervals, fractions (2 ml) were removed and partially purified using C_{18} Sep-Paks (Waters), primed with methanol (5 ml) followed by water (5 ml). After washing with water (5 ml), the leukotrienes were eluted from the column with methanol (5 ml), and the eluant evaporated to dryness under vacuum. The samples were then subjected to reverse phase high performance liquid chromatography (r.p.-h.p.l.c.) using a Spherisorb 5ODS column and a solvent system of MeOH-: water : acetic acid 80 : 20 : 0.02 v/v/v, pH adjusted to 5.4 with NH_4OH. The flow rate was maintained at 1 ml min^{-1}. Authentic standards were co-injected with the samples to assess the retention times of the leukotrienes. Samples (1 ml) were collected and LKB Optiphase Safe (4 ml) was added and the fractions counted on a Packard Tricarb liquid scintillation counter. In all experiments, the aqueous effluents from the Sep-Paks were concentrated and counted for radioactivity, as polar metabolites produced could have passed through the Sep-Pak in the aqueous phase. In later experiments, fractions (200 μl) of the incubation medium were added to methanol (300 μl) at − 20°C in Eppendorf tubes. After centrifugation at 2000 *g* for 15 min, samples were purified on r.p.-h.p.l.c. as described above. Control experiments were carried out using chopped pulmonary arterial tissue that had been boiled for 10 min. In some experiments, large-scale incubations were performed and the h.p.l.c. fractions (after removing 50 μl for counting) were

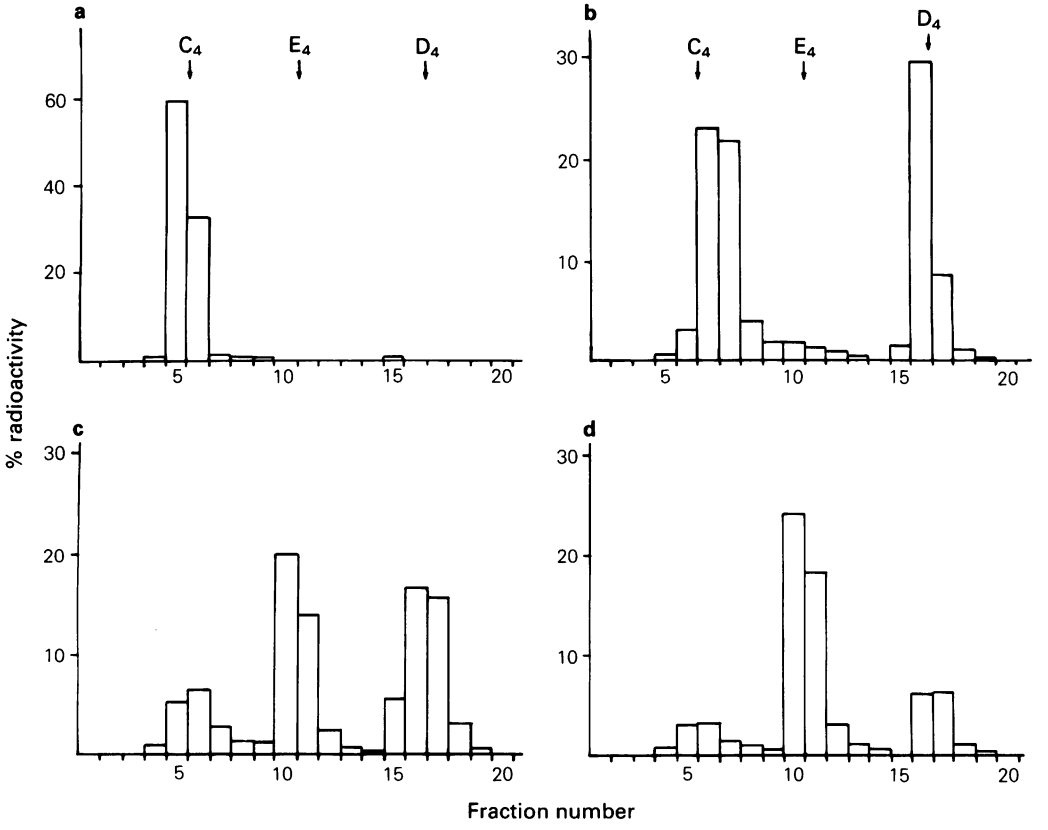

Figure 1 Typical h.p.l.c. trace showing the radioactive profile resulting from the incubation of leukotriene C_4 (LTC_4, 1 nM) + tracer [^{3}H]-LTC_4 with porcine pulmonary artery for different time intervals, (a) 0 min, (b) 2.5 min, (c) 30 min and (d) 70 min.

assayed for biological activity on superfused strips of guinea-pig ileum smooth muscle (Samhoun & Piper, 1984). FPL55712 (2.3×10^{-6}M), a leukotriene receptor antagonist, was infused over the lowest tissue to increase the specificity of the assay.

Effect of inhibitors on leukotriene metabolism

To investigate the effect of γ-glutamyl transpeptidase and aminopeptidase inhibitors on the metabolism of leukotrienes, serine-borate (45 mM) or L-cysteine (10 mM) respectively was added to the Tyrode solution in which the vessels were suspended. After a pre-incubation period of 30 min at 37°C, the leukotrienes were added and the experiment conducted as above.

Materials

All chemicals 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: FPL55712 (sodium-7-[3-(4-acetyl-3-hydroxy-2-propyl phenoxy)-2-hydroxypropoxy]- 4-oxo-8-propyl-4-H-1-benzopyran-2-carboxylate), Fisons Pharmaceuticals; L-cysteine, Sigma; L-serine, BDH; sodium tetraborate, BDH; synthetic LTB_4, LTC_4, LTD_4 and LTE_4, Merck Frosst Laboratories, Canada; 14,15-[^{3}H]-LTB_4 (32 Ci mmol^{-1}), 14,15-[^{3}H]-LTC_4 (40 Ci mmol^{-1}), New England Nuclear.

Results

When LTC_4 (1 nM) was incubated with porcine pulmonary artery, it was converted to LTD_4 and LTE_4, as determined by the separation of the labelled metabolites on h.p.l.c. A typical set of h.p.l.c. chromatograms is shown in Figure 1. The time course of conversion of LTC_4 is shown in Figure 2. At a concentration of 1 nM, LTC_4 had a half-life of about 3 min when incubated with pulmonary artery. LTD_4 reached a maximum concentration at 10 min, after which it slowly declined with a concomitant increase in the levels of LTE_4. All the radioactivity added to the incubation medium could be accounted for by these three leukotrienes, suggesting that no further metabolism was taking place. Experiments with boiled tissue showed no conversion of LTC_4. Biological assay of the h.p.l.c. fractions resulting from a large-scale incubation of LTC_4 showed biological activity in the fractions with the same retention times as those of synthetic LTC_4, LTD_4 and LTE_4. The profiles of contractions were identical to those of the standard leukotrienes and were blocked by FPL55712, confirming the identity of the peaks.

When LTD_4 was incubated with porcine pulmonary artery, it was converted to LTE_4 (Figure 3). As with LTC_4, there appeared to be no metabolism beyond LTE_4. After incubation for 180 min, 90% of the radioactivity could be associated with LTE_4. No

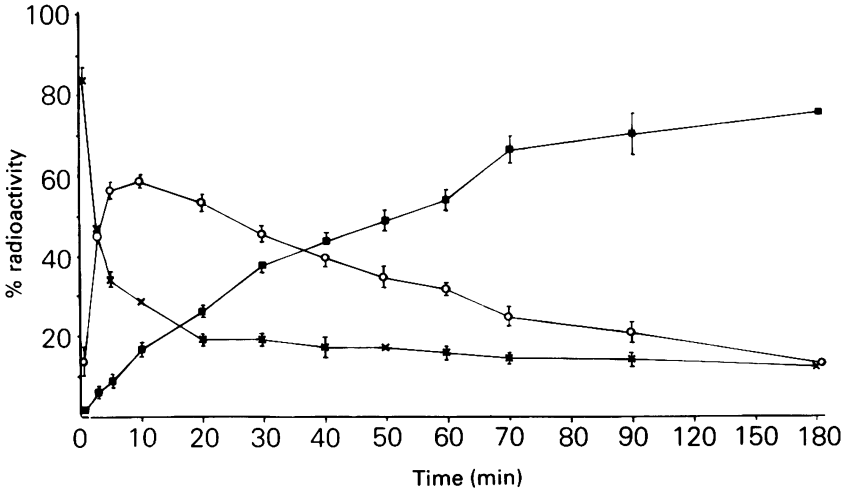

Figure 2 Time course of metabolism of leukotriene C_4 (LTC_4) resulting from the incubation of LTC_4 (1 nM) + tracer [^{3}H]-LTC_4 with porcine pulmonary artery. Samples were removed at time intervals and the leukotrienes separated by h.p.l.c. (×) LTC_4, (○) LTD_4, (■) LTE_4. Each point represents the mean with vertical lines showing s.e.mean ($n = 5$).

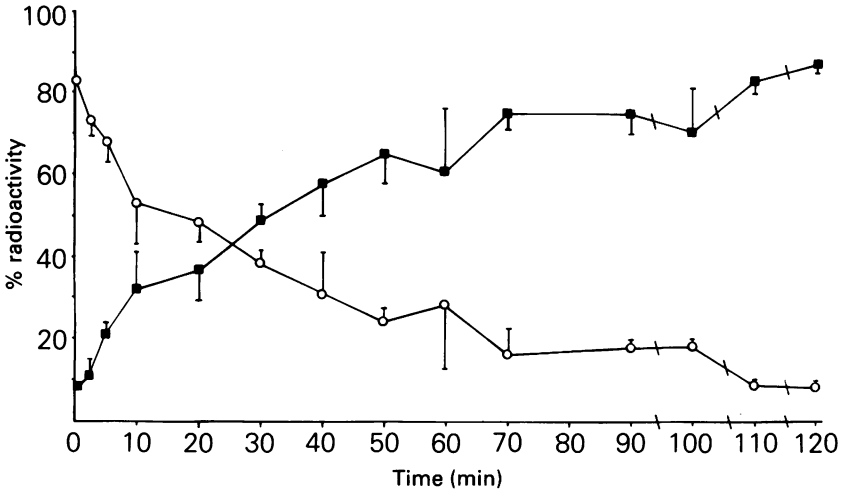

Figure 3 Time course of metabolism of leukotriene D_4 (LTD_4) by porcine pulmonary artery incubated with LTD_4 (1 nM) + tracer [^{3}H]-LTD_4. Samples were removed at time intervals and the leukotrienes separated by h.p.l.c. (O) LTD_4 (■) LTE_4. Each point represents the mean with vertical lines showing s.e.mean ($n = 4$).

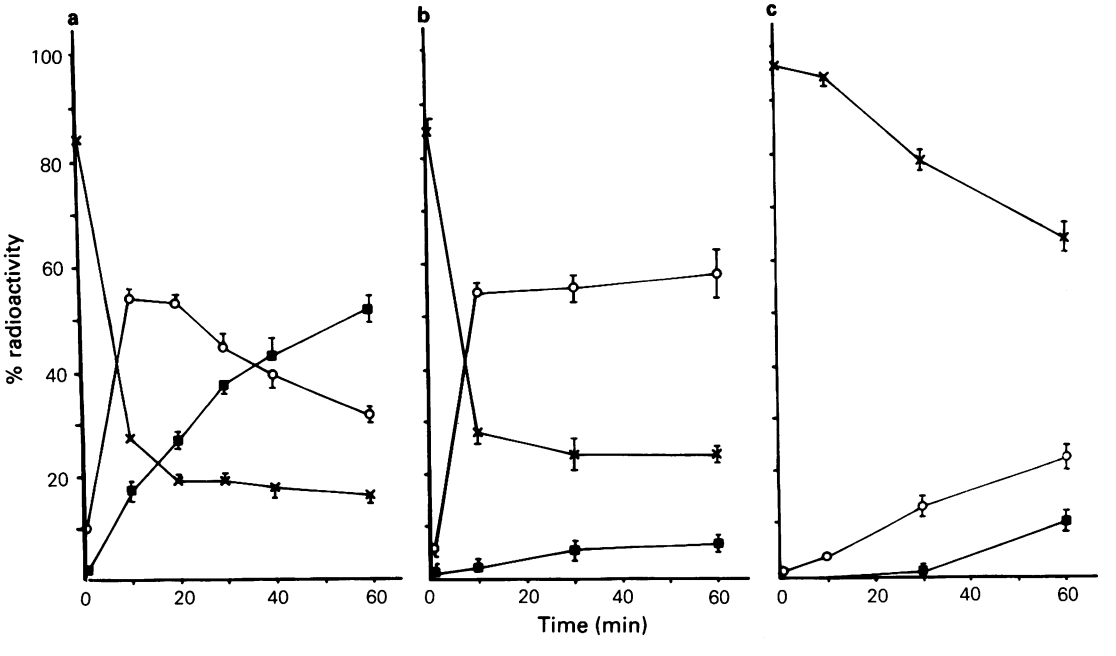

Figure 4 Effect of inhibitors on the metabolism of leukotriene C_4 (LTC_4) by porcine pulmonary artery. LTC_4 (1 nM) + tracer [^{3}H]-LTC_4 was incubated with porcine pulmonary artery in the presence of an inhibitor. Samples were removed at time intervals and the leukotrienes separated by h.p.l.c. (a) No inhibitor, (b) L-cysteine (10 mM) and (c) serine-borate (45 mM). (×) LTC_4, (O) LTD_4. (■) LTE_4. Each point represents the mean with vertical lines showing s.e.mean ($n = 5$).

conversion occurred during incubation with boiled tissue.

The aminopeptidase inhibitor L-cysteine (10 mM) had no effect on the conversion of LTC_4 to LTD_4 (Figure 4) but markedly inhibited the conversion of LTD_4 to LTE_4. The inhibitor of γ-glutamyl transpeptidase, serine-borate (45 mM), reduced the formation of LTD_4 (Figure 4). Production of LTE_4 was also virtually abolished as a consequence.

Leukotriene B_4 does not appear to be metabolized in the system studied, since all the radioactivity in the h.p.l.c. profile could be associated with the LTB_4 peak.

No radioactivity was detected in the concentrated aqueous Sep-Pak effluents in any of the above experiments.

Discussion

In this study, we have demonstrated the ability of the porcine pulmonary artery to metabolize LTC_4 and LTD_4. A concentration of 1 nM was used, as this was the concentration generated by guinea-pig hearts during experimental cardiac anaphylaxis (Aehringhaus *et al.*, 1984). Metabolism occurred to the extent that both LTC_4 and LTD_4 were converted to LTE_4 which, though longer-lasting, is in general less active in various vascular beds (Ezra *et al.*, 1983; Letts & Piper, 1983; Feigen, 1983). The metabolism of leukotrienes by other components of the circulation has been reported. Human blood plasma has been shown to metabolize leukotrienes in a similar way (Köller *et al.*, 1985) and human umbilical endothelial cells have been demonstrated to bring about this conversion (Pologe *et al.*, 1984; Johnson *et al.*, 1985), though their ability to form LTE_4 is considerably less than in the present investigation using whole vessels. This is also the case with porcine aortic endothelial cells (Fan, Galton, Lewis, Piper & Stewart, unpublished observations), suggesting that other parts of the vessel are responsible for the conversion of LTD_4 to LTE_4. Leukotriene E_4 appears to be the final metabolite and no further degradation occurs. The liver seems to be the main site of further metabolism and the production of N-acetyl LTE_4 from LTE_4 has been demonstrated in rat liver homogenates (Bernstrom & Hammarström, 1986). It is interesting to note that, as LTC_4 is not converted to LTD_4 by the liver to any great extent (Hammarström, 1981), the role played by the vessel and blood may be a necessary pre-requisite for leukotriene excretion.

The γ-glutamyl transpeptidase inhibitor, serine borate, and the aminopeptidase inhibitor, L-cysteine, were both effective in preventing leukotriene metabolism, demonstrating that, as in many other systems, the accepted metabolic pathway of LTC_4 being converted to LTD_4 and then to LTE_4 is correct. There have been reports of the conversion of LTC_4 to 5S,12S, 6 trans and 5S,12R metabolites of LTB_4 (Lewis *et al.*, 1983) but there was no evidence of this occurring in the present study.

In view of the fact that the pulmonary artery is able to generate leukotrienes as well as cause their metabolism, it is interesting to speculate as to the site of action of these compounds (if any). The pulmonary artery of various species has been shown to contract to leukotrienes, for example in the guinea-pig (Hand *et al.*, 1981; Berkowitz *et al.*, 1984) but, in general, it is fairly unresponsive. Another possibility is that the leukotrienes may be transported (and metabolized) by the circulation to act on the pulmonary microvasculature, which is responsive to leukotrienes in the neonatal pig (Leffler *et al.*, 1984) and in the adult sheep (Kadowitz & Hyman, 1984; Malik *et al.*, 1985). As a number of the larger vessels can produce leukotrienes (Piper *et al.*, 1983), it would be interesting to see if microvessels have this capacity.

In view of the growing evidence that leukotrienes may play some role in cardiovascular disease (Lefer, 1986), a knowledge of the release and metabolism of these compounds in the circulation may provide further information as to their role in the disease state or even in homeostatic mechanisms.

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