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Leukotriene C_4 stimulates TXA_2 formation in isolated sensitized guinea pig lungs

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The leukotrienes constitute a new group of biologically active compounds derived from polyunsaturated fatty acids [1–5]. Leukotriene A_4 (LTA_4), an unstable epoxide intermediate, is formed from arachidonic acid via 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid. It can be transformed enzymatically by hydrolysis into LTB_4 and by addition of glutathione into LTC_4 (5(S)-hydroxy-6(R)-*S*-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid). LTC_4 is converted into the corresponding cysteinylglycine derivative (LTD_4) by γ-glutamyl transpeptidase [6]. Slow Reacting Substance of Anaphylaxis (SRS-A) which is an important mediator in immediate hypersensitivity reactions has been shown to be due to LTC_4 and LTD_4 [5, 7].

Crude preparations of SRS-A have been found to stimulate release of thromboxane A_2 (TXA_2) from guinea pig lungs [8, 9]. Sensitized lungs release more TXA_2 than normal lungs following stimulation by crude SRS-A [9]. It was therefore of interest to study the effect of chemically pure LTC_4 on the release of thromboxane from guinea pig lungs. The effect of an SRS-A antagonist (FPL55712) on the SRS-A stimulated release of thromboxane was also studied [10].

Methods

Lungs from actively sensitized guinea pigs [11] (300–400 g) were used. The organs were removed and perfused through the pulmonary artery with Krebs-bicarbonate solution at a flow rate of 10 ml min^{-1}. The pulmonary outflow continuously superfused two spirally cut rabbit aortas (RbA), in order to detect TXA_2-like material. The bioassay tissues were first challenged with a single dose of noradrenaline (NA, 1 nmole) in order to assess their reactivity and subsequently treated, throughout the experiment, with a mixture of antagonists [12] and with indomethacin (1 μg $ml^{-1}min^{-1}$) to increase their sensitivity. Changes in the tone of the tissues were recorded with isometric transducers (Grass model FT 03).

After passage over the assay organs, the pulmonary effluent was collected for periods of 1 min directly into excess methanol and subsequently assayed for mono-*O*-methyl-TXB_2 [13]. This compound is an indicator of TXA_2. The collection of the perfusate was started directly after administration of LTC_4 to the lungs; the maximal biological activity was registered during the collection time.

Chemically pure leukotriene C_4 was prepared at the Karolinska Institutet, Stockholm, Sweden, as previously described [2].

Experimental data were processed according to the method of factorial analysis of variance for completely randomized design with two factors at two levels. Multiple comparison according to Duncan was also performed [14].

Results

When LTC_4 (0.55–2.2 pmoles) was injected as a bolus into the isolated lungs of ovalbumin sensitized guinea pigs, a dose dependent formation of vasoactive material was observed (Fig. 1). No direct effect of LTC_4 on the isolated vessels was observed (Fig. 1) and pretreatment of the isolated lungs with eicosatetraynoic acid (1 μg ml^{-1} min^{-1}) prevented formation of the vasoactive material.

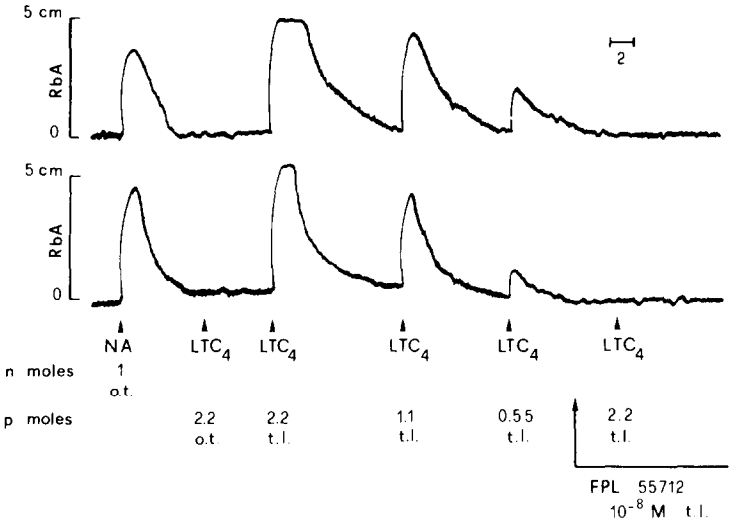

Fig. 1. Effect of Leukotriene C_4 (LTC_4) on the generation of thromboxane A_2-like material in ovalbumin sensitized lungs from guinea-pig. The bank of tissues in cascade consists of strips of rabbit aortas. (o.t.) Denotes an injection directly over the tissues in cascade. (t.l.) Denotes an injection through the lungs. Noradrenaline (NA, 1 nmole injected o.t) was used to check the reactivity of the RbA which were subsequently treated with a mixture of antagonists to prevent the effect of unwanted substances.

The presence of TXA_2 in the lung perfusate was also proven and quantitated by radioimmunoassay. The results of these experiments, which are in agreement with those obtained by bioassay, are reported in Fig. 2. Basal release of TXA_2 was low, amounting to around 4 pmole/min of mono-*O*-methyl-TXB_2 after treatment of the sample with methanol. LTC_4, given as bolus injections, strongly increased the output of TXA_2 (Fig. 2). After an injection of 0.55 pmoles of LTC_4, the measured level of mono-*O*-methyl-TXB_2 increased to 20.6 pmoles, whereas doses of 1.1 and 2.2 pmoles or LTC_4 stimulated the TXA_2 formation even further (measured amounts of mono-*O*-methyl-TXB_2, 71.6 pmoles and 173.4 pmoles, respectively, in the methanol treated samples).

Pretreatment of the guinea pig lungs with FPL 55712 at the concentration of 10^{-8} M inhibited the response to LTC_4. The blockade was rapid in onset and recovery took place within minutes after stopping the perfusion with FPL 55712 (Figs. 1 and 2).

Discussion

The recent discovery that leukotrienes are a new group of biologically active compounds (including SRS-A), derived from arachidonic acid indicates that this precursor fatty acid has a pivotal role as modulator of various cell functions. SRS-A is an important mediator in asthma and other immediate hypersensitivity reactions [15] and its capacity to promote formation of TXA_2 in lungs has been reported [8, 9]. Indeed it appears that SRS-A owes part of its bronchoconstrictor effect to the augmented synthesis of TXA_2 which is one of the most potent contractile agents of smooth muscle of vascular and respiratory origin [16, 17].

The capacity of LTC_4 to stimulate formation of TXA_2 in isolated sensitized guinea pig lungs, indicates the existance of interrelationships between these biosynthetic pathways within the eicosanoid system. Therefore LTC_4, together with its own effects on peripheral pulmonary mechanics [7], is able to trigger a process of bioamplification through TXA_2. The fact that FPL 55712 antagonizes the TXA_2-releasing ability of LTC_4 seems to to suggest that

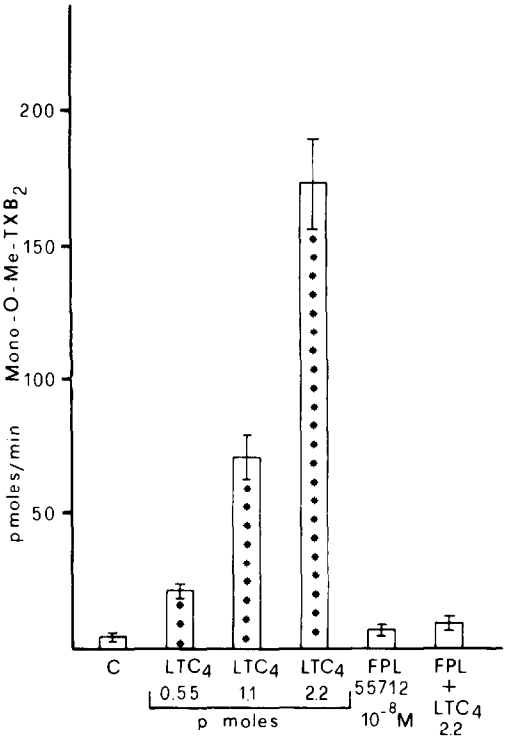

Fig. 2. Effect of Leukotriene C_4 (LTC_4) on thromboxane A_2 generation in ovalbumin sensitized lungs of guinea-pig during the first minute after exposure of the lung to LTC_4. TXA_2 was measured as mono-*O*-methyl thromboxane B_2. C denotes control conditions and each bar represents the S.E. mean of 5 experiments. Duncan test gives the following results for the comparison between each two means: C vs LTC_4 0.55 pmoles = highly significant ($P < 0.01$); C vs FPL 8 LTC_4 2.2 pmoles = non significant ($P > 0.5$).

the activation of the eicosanoid system may involve specific receptors for LTC_4 in guinea pig lung. In fact pyrilamine, a histamine H_1-receptor antagonist, does not prevent TXA_2 generation in nonsensitized lungs due to LTC_4 (G. C. Folco, unpublished observation).

The remarkable potency of LTC_4 in triggering TXA_2 generation in sensitized lungs, might be partly due to concomitant release of histamine from sensitized cells caused directly by LTC_4. It is of interest in this context that lipoxygenase products have been shown to increase the release of histamine from perfused guinea pig lung [18]. Further studies are required to settle this point.

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On the nature of the interaction between chlorpromazine and the muscarinic receptor

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Chlorpromazine is an effective antagonist on a remarkable range of drug receptors and biochemical systems. At receptors for which it has a high affinity, such as histamine-H_1, α_1-adrenergic and dopaminergic, it seems very likely that it acts as a competitive inhibitor. However, on receptor systems for which chlorpromazine has a lower affinity it is not so certain that the interaction is competitive and there is seldom any experimental evidence which allows a firm conclusion to be drawn. Hill coefficients of unity for curves of the inhibition of the binding of ^{3}H-receptor ligands are consistent with either competitive or non-competitive inhibition. Similarly, a parallel shift of agonist log dose–response curves in intact tissues with an appreciable 'spare' receptor population could indicate either a competitive or an effectively irreversible blockade [1]. Chlorpromazine has potent membrane actions [2] and it is possible that with some receptors inhibition could be the result of a perturbation of the receptor–membrane interface, particularly at higher concentrations of the drug. There is some suggestive evidence that such could be the case with the muscarinic receptor.

Low concentrations of chlorpromazine produce a parallel shift of the log dose–response curve for the acetylcholine-induced contraction of the rabbit ileum, but at higher concentrations the curve is flattened [3]. This effect is similar to that of tetracaine [4], and for this local anaesthetic a study of the inhibition of the binding of [^{3}H]quinuclidinyl benzilate, [^{3}H]QNB, a selective ligand for the muscarinic receptor [5], indicated that at low concentrations the inhibition is competitive, but that at higher