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STRUCTURAL REQUIREMENT FOR THE ACTION OF LEUKOTRIENE B_4 ON THE GUINEA-PIG LUNG: IMPORTANCE OF DOUBLE BOND GEOMETRY IN THE 6, 8, 10-TRIENE UNIT

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SUMMARY

The action of four synthetic 5(S),12(R)-dihydroxy-6,8,10,14-eicosatetraenoic acids has been compared to the action of natural leukotriene B4 (LTB_4) in perfused guinea-pig lung and in the parenchymal strip preparations. Synthetic LTB_4 (Fig. 1) having the 6-cis, 8, 10-trans triene unit was found to be as powerful as natural LTB_4 both for contracting the parenchymal strip and for releasing prostaglandins and thromboxanes from the perfused lung while three other isomers were inactive. The results indicate that the action of LTB_4 on the lung is highly dependent on the geometry of the conjugated triene.

INTRODUCTION

The recent discovery of a 5,12-dihydroxyeicosatetraenoic acid (leukotriene B_4) by Borgeat and Samuelsson (1,2) has unravelled a completely new pathway of metabolism of arachidonic acid in polymorphonuclear leukocytes (3, see also ref. 4 for a review). Studies on the biological properties of purified LTB_4 have shown that the compound is a powerful chemotactic and chemokinetic agent (5), releases lysosomal enzymes and increases cyclic nucleotides levels (6), all key features of cellular inflammation. Recently. experiments from our laboratories showed that this dihydroxy acid also contracted various smooth muscles including the lung parenchymal strip (7), that it released prostaglandins and thromboxanes from the perfused guinea-pig lung (8) and that its effect on the lung was not affected by a mixture of inhibitors containing an anti-cholinergic, α and β-blockers, an antiserotonergic, an anti-histaminic and an anti-SRS-A (9).

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In the present studies, the biological activity of synthetic dihydroxy acids was compared in order to evaluate the importance of the geometry of the conjugated triene for the action of LTB_4. For this purpose, the effects of four geometric isomers were studied both on the perfused guinea-pig lung and on the parenchymal strip preparation and their activity was compared with pure native LTB_4.

MATERIALS AND METHODS

Preparation of natural LTB_4

LTB_4 was prepared from swine peripheral blood polymorphonuclear leukocytes (8,9). In brief, 50 x 10^6 cells were incubated (37°C, 4 min) in the presence of ionophore A23187 (10ug/ml) and arachidonic acid (50ug/ml). The reaction was stopped with methanol and the acidified (pH 3) supernatant was extracted with ether. The extract was fractionnated by silicic acid chromatography (Silicar CC-4, Mallinckrodt, 3g) and the fraction containing LTB_4 was further purified by high performance liquid chromatography (HPCL) on Lichroprep Si 60 (Merck) using a gradient of isopropranol in hexane (0% to 10%) and a 50 x 1 cm column. The elution was monitored at 280 nm. The final purification was performed by reversed-phase HPCL on a C18-column (8 x 100 mm) using methanol/water, 70/30, V/V (0.01% acetic acid) as mobile phase (this solvent was used to stock LTB_4). Gas chromatographic analysis of the purified compound showed no important contaminant other than the trans-trans-trans isomers of LTB_4 (less than 5%) (1). The identity of LTB_4 was ascertained by mass spectrometry and its concentration established by ultra-violet spectrophotometry ($\varepsilon \approx 51000$ at 270 nm)

Synthetic 5(S), 12(R)-dihydroxy-6,8,10,14 eicosatetraenoic acids

Synthetic LTB_4 and the various geometric isomers ($\Delta^{6,8,10}$) trans-trans-cis, trans-cis-trans and trans-trans-trans were prepared as described previously (10, 11).

Preparation and superfusion of lung parenchymal strips

Strips of lung parenchyma (3 x 3 x 30 mm) were cut from the edges of the lobes of adult guinea-pig lungs (8,9) superfused in cascade and the contraction to bolus injections of LTB_4 were recorded as described previously (12).

Perfusion of the guinea-pig lung

The lungs of healthy adult guinea-pig were dissected and perfused via the pulmonary artery as described previously (8,13). The effluent was directed to superfuse over a strip of rabbit aorta to detect thromboxane A_2, and over strips of guinea-pig ileum and rat stomach to detect prostaglandins and posibly SRS-A. The stability and specificity of the assay organs for the metabolites of arachidonic acid were insured by the perfusion of a mixture of antagonists containing (ug/ml): methysergide (0.2) Sandoz Ltd; propranolol (3.0) Ayerst Lab.; phenoxybenzamine (0.1) S.K.F.; atropine (0.1) Sigma Chem.; diphenhydramine (0.1) Parke, Davis & Co.

RESULTS

In the first series of experiments, dose-response curves to natural LTB_4 and to four synthetic isomers were otained on lung parenchymal

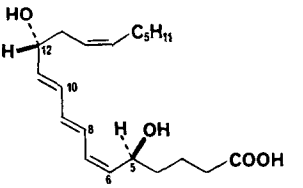

Fig. 1. Structure of leukotriene B_4.

strips. As shown on Fig. 2, the natural LTB_4 (opened circles) contracted in a dose-dependant manner the tissues at doses ranging from 3×10^{-11} to 1.5×10^{9} mole. Synthetic LTB_4 (Fig. 1) (closed circles) had the same potency as natural LTB_4. The other three synthetic isomers of LTB_4, trans-cis-trans, trans-trans-cis and trans-trans-trans were almost inactive in contraction of lung strips. The maximal dose used (3×10^{9} mole, 1 ug) only induced very little myotropic activity as compared to the trans-trans-cis molecule.

In a second series of experiments, we have compared the effects of synthetic LTB_4 and the three isomers with natural LTB_4 in release of

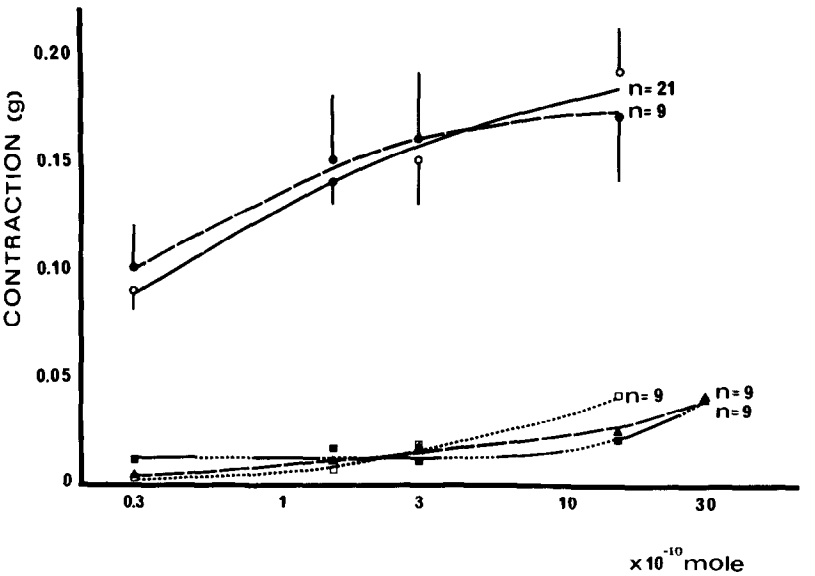

Fig. 2. Dose-response curves of the guinea-pig lung parenchymal strip to increasing doses (from 3×10^{-11} to 3×10^{-9} mole; 10 ng to 1 ug) of natural leukotriene B_4 (LTB_4; opened circles), of four synthetic 5(S),12 (R)-dihydroxy-6,8,10,14-eicosatetraenoic acids: synthetic LTB_4, trans-cis-trans, trans-trans-cis and trans-trans-trans at $\Delta^{6,8,10}$ and cis at Δ^{14} in each isomer (closed circles, closed squares, closed triangles and opened squares respectively). Each point is a mean ± S.E.M. Figures in parentheses represent the number of observations. Ordonate: contraction in grams. Abscissa: log dose (mole).

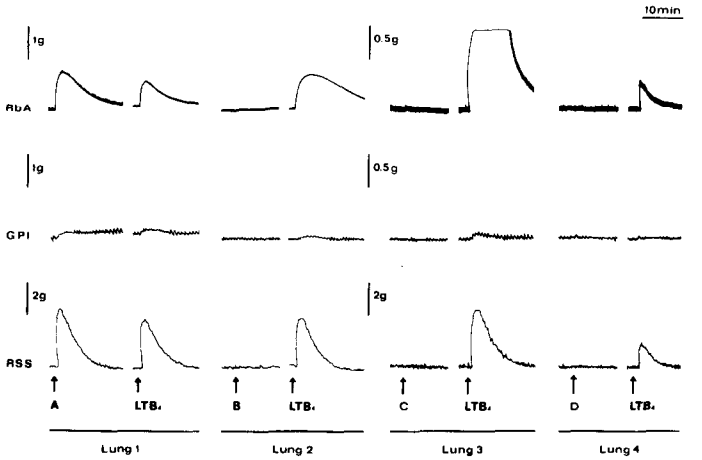

Fig. 3. Release of prostaglandins and thromboxanes from the guinea-pig lung by natural LTB_4 (3×10^{-10} mole; 100 ng) and by the four synthetic dihydroxy acids (3×10^{-10} mole; 100 ng). The rabbit aorta (RbA), the guinea-pig ileum (GPI) and the rat stomach strips (RSS) were superfused with the effluent of the perfused lungs. All substances were injected in the pulmonary artery as bolus. Compounds A, B, C, and D are respectively synthetic LTB_4, the trans-cis-trans, the trans-trans-cis and the trans-trans-trans isomers.

prostaglandins and thromboxanes (8) from the perfused guinea-pig lung. Since some lung preparations appeared to be desensitized by repeated injections of LTB_4, only two injections were done in each lung: first the synthetic isomer was injected, followed by a control injection of LTB_4. As shown on Fig. 3, natural LTB_4 as well as the synthetic LTB_4 (A) injected in the lung at the dose of 3×10^{-10} mole (100 ng) caused the release of prostaglandins and thromboxanes as detected by the contraction of the rabbit aorta and of the rat stomach strip. As in the previous series of experiments, the trans-cis-trans (B), the trans-trans-cis (C) and the trans-trans-trans (D)isomers (3×10^{-10} mole; 100 ng) were inactive.

DISCUSSION

These results confirmed our previous observations showing that LTB_4 is a powerful stimulant of the lung parenchymal strip and can release prostaglandins and thromboxanes from the perfused lung (8,9). They further show that the synthetic LTB_4 is as potent as the natural compound but that the trans-cis-trans, the trans-trans-cis and the trans-trans-trans geometric isomers were almost completely inactive in both system used. Thus, the action of LTB_4 which was shown previously to

be unaffected by antagonists such as methysergide, propranolol, phenoxybenzamine, atropine, diphenhydramine and FPL-55712, appears to require an exact conformation of the LTB_4 molecule (9). Although the presence of LTB_4 receptor sites in the lung seems likely, the physiological role of this molecule in the lung is still speculative and the assessment of such a role for LTB_4 must await studies of the compound in lungs and blood in health and disease.

In conclusion, we have shown that the synthetic LTB_4 (Fig. 1) is equipotent with natural LTB_4 in producing contractions of the lung parenchymal strip and the release of prostaglandins and thromboxanes from the perfused guinea-pig lung. It is also clear that the stereoisomeric dihydroxy acids (trans-cis-trans, trans-trans-cis and trans-trans-trans) were almost inactive in our preparations which suggest that the geometry of the conjugated double bonds of LTB_4 is critical for the myotropic activity of LTB_4 on the lung.

In addition, our data brings further evidence on the identity of synthetic and native LTB_4. Although it was shown previously that LTB_4 is 5(S),12(R)-dihydroxy-6, 8, 10, 14-eicosatetraenoic acid and that the conjugated triene contained two trans and one cis double bonds, the relative position of the trans and cis double bonds was still unknown. The present study which shows that only one of these synthetic dihydroxy acids (each carrying one of three possible combinations of cis and trans conjugated double bonds at ($\Delta^{6,8,10}$) share with natural LTB_4 some biological activities on the guinea-pig lung, confirms the conclusion (10, 11) that LTB_4 is (5S),12(R)-dihydroxy-6(Z), 8(E), 10(E), 14(Z)-eicosatetraenoic acid (Fig. 1).

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