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2-NOR-LEUKOTRIENE ANALOGS: ANTAGONISTS OF THE AIRWAY AND VASCULAR SMOOTH MUSCLE EFFECTS OF LEUKOTRIENE C_4, D_4 AND E_4

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SUMMARY: A structural analog of LTD_4, 4R-hydroxy-5S-cysteinylglycyl-6Z-nonadecenoic acid (4R, 5S, 6Z-2-nor-LTD_1) has been synthesized and pharmacologically characterized. It significantly antagonized the contractile action of LTD_4, LTC_4 and LTE_4 in guinea pig airways. In addition, this compound antagonized the in vitro vasoconstrictive effects of LTD_4 in the guinea pig pulmonary artery. The study of a series of structural analogs of 4R, 5S, 6Z-2-nor-LTD_1 suggests that the spatial separation of the C-1 (eicosanoid) carboxyl relative to the hydroxyl is a critical determinant in LTD_4 agonist/antagonist activity.

Slow reacting substance of anaphylaxis (SRS-A) is comprised of three structurally related biologically active lipoxygenase metabolites of arachidonic acid: leukotrienes C_4 (LTC_4), D_4 (LTD_4) and E_4 (LTE_4) (1,2,3). Release of these peptidoleukotrienes has been demonstrated following antigen provocation of sensitized human and animal lung tissue (2,4). In man, they are potent bronchoconstrictive agents both in vitro (5) and in vivo (6,7); in animal models, they exhibit potent contractile effects on airway and vascular smooth muscle (8,9,10), induce tracheal edema (11) and stimulate mucous hypersecretion (12,13). Thus, the peptidoleukotrienes may function as mediators of anaphylactic reactions and could contribute to the pathophysiology of allergic asthma.

Structural requirements for leukotriene agonist activity on guinea pig lung parenchymal strips have been reported by several groups (14,15,16). A high preference for the 5S, 6R absolute stereochemistry and the requirement

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for the C-5 hydroxyl and C-1 carboxyl, and a fourteen carbon lipid chain, all suggest structural specificity expected of a receptor-mediated effect. If so, it should be feasible to design agents which act at such putative leukotriene receptors to block the effects of the peptidoleukotrienes. This communication describes a novel series of leukotriene analogs which, as a consequence of changes in the polar C-1 to C-6 region of the eicosanoid structure, possess significant leukotriene antagonist activity in several pharmacologically relevant models.

MATERIALS AND METHODS

Chemistry. The leukotriene analogs 2, 3 and 4 (Tables 1,3) were prepared from the appropriate LTA_4 methyl ester analog and methyl N-trifluoroacetyl cysteinylglycinate (MeOH, Et_3N). The resulting adducts were lactonized (TsOH, CH_2Cl_2), separated by HPLC and hydrolyzed (K_2CO_3, aq. MeOH) to afford 2a, 2b, 3a, 3b, 4a, and 4b.

The absolute stereochemistry of 4a and 4b was determined by Raney nickel desulfurization of the chirally pure lactones to give the γ-lactones of 4R($[\alpha]_D$+17 , 1%, MeOH) and 4S ($[\alpha]_D$-17 , 1%, MeOH) -4-hydroxynonadecanoic acid, respectively, from 4a and 4b (17). Assuming stereochemical inversion at C-5 upon opening of the trans epoxide, the 4R, 5S and 4S, 5R absolute stereochemistry for 4b and 4a, respectively, was established. The absolute stereochemistry of the isomers of 2, 3 and 6 were inferred from comparison of CD spectra with those of 4a and 4b.

The 6E-2-nor-LTD_4 isomers 5a and 5b were prepared by photoisomerization (PhSSPh, MeOH/toluene) of the protected lactone precursors for 4a and 4b, HPLC purification and hydrolysis. Compounds 6a, 6b, and diastereoisomeric 7, were prepared from methyl 6Z-2-nor-LTA_1 and methyl N-trifluoroacetylcysteinate and glutathione respectively.

The requisite 2-nor-LTA_1 ester was prepared by condensation of methyl 4-oxobutyrate and formylmethylenetriphenylphosphorane, epoxidation of the resultant unsaturated aldehydic ester (H_2O_2, pH 9.5) and subsequent Wittig reaction with tri-n-decylidenetriphenylphosphorane. The stereochemistry of the epoxide (trans) and olefin (cis) of methyl 6Z-2-nor-LTA_1 was established by analysis of nmr coupling constants and by Nuclear Overhauser experiments. The C_{21} analogs 2a and 2b were prepared from 8Z-2-homo-LTA_1 which was obtained in an analogous fashion from methyl 6-oxohexanoate. LTD_4 diastereomeric LTC_4 and LTE_4 (18, 8) and analogs 3a and 3b (15) were prepared as previously described.

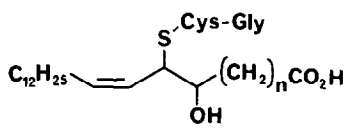
Pharmacological Evaluation. Changes in isometric tension elicited by synthetic LTC_4, LTD_4 and LTE_4 on isolated guinea pig tracheal spirals, lung parenchymal strips and pulmonary arterial rings were quantitated as previously described (10,19). To prevent the compensatory release of dilator prostaglandins (10), tracheal spirals were pretreated with 1×10^{-6}M meclofenamic acid. To assess antagonist activity, antagonist or vehicle (20 mM sodium carbonate on the trachea, DMSO on the parenchyma, or saline on the pulmonary artery) was added and incubated for 30 min.; then cumulative concentration-response curves for LTC_4, LTD_4 or LTE_4 were generated for each tissue. In order to minimize the effects of inter-tissue variability, contractile responses were normalized by expressing them as a percentage of the maximum response obtained to reference standards: carbachol (10^{-5}M) on

the trachea, histamine (10^{-3}M) on the lung parenchymal strips, norepinephrine (10^{-4}M) on the arterial rings. None of the test compounds significantly affected the contractions elicited by these reference agonists. The K_B, an estimate of the potency of the antagonists on the trachea and pulmonary artery was calculated from the equation: K_B=Agonist concentration/(X-1), where X is the dose ratio obtained by comparing the concentration of LTD_4 needed to elicit an equal contraction in the presence and absence of the test compound. Since the antagonism observed on lung parenchymal strips was not characterized by parallel shifts in the dose response curves, no K_B was calculated.

RESULTS

The initial series of hexahydroleukotriene analogs assessed the effect of altering the distance between the apparently critical C-1 carboxyl and C-5 hydroxyl groups of the natural product. The hexahydro derivatives were selected to increase chemical stability of the leukotriene analogs; however, some loss in agonist potency (e.g., 2a *vs* LTD_4; Table 1) was observed. Lengthening the C-1 to C-5 chain by one methylene residue, e.g. 3a, 3b had little effect on the agonist activity profiles on guinea pig lung parenchyma (Table 1). In contrast, deletion of a methylene group between C-1 and C-4, particularly in the "unnatural" 4(R), 5(S) diastereomer 4b, (4R, 5S, 6Z-2-nor-LTD_1; Fig. 1), afforded a compound which only weakly contracted the lung parenchymal tissue, achieving a maximum contraction only 10% that of LTD_4.

TABLE 1
BIOLOGICAL COMPARISON OF LTD_4 ANALOGS



Compound	n	Stereochemistry	G.P. Lung Parenchyma Agonist Activity EC_{50} (nM)	Relative Contractile Activity*
1a	LTD_4	5(S), 6(R)	1.4	1
1b	5,6-epi-LTD_4	5(R), 6(S)	120	1
2a	4	6(S), 7(R)	6.5	1
2b	4	6(R), 7(S)	4.5	1
3a	3	5(S), 6(R)	15	1
3b	3	5(R), 6(S)	610	1
4a	2	4(S), 5(R)	630	0.5
4b	2	4(R), 5(S)	500	0.1

*Contractile activity is expressed as the maximal contraction achieved relative to natural LTD_4.

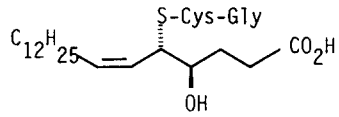


Fig. 1. 4R,5S,6Z-2-nor-LTD_1, a leukotriene antagonist.

However, pretreatment of the lung parenchyma with 10^{-4}M 4b significantly antagonized the contractile action elicited by LTD_4 (Fig. 2A). That the antagonist properties of 4b are selective for a putative LT receptor was demonstrated by the lack of effect by 4b against contractions elicited by histamine, carbachol, and KCl (data not shown).

The ability of 4b to antagonize the leukotriene induced contraction of guinea pig tracheal strips was also explored. In the presence of 1 μM meclofenamic acid which inhibits the compensatory release of dilating prostaglandins produced in response to tissue contraction (10), 4b significantly inhibited (Fig. 2B) the LTD_4-induced contractions with a K_B=7.0 μM. A similar degree of antagonism could be demonstrated in the absence of meclofenamic acid. Comparable antagonism of LTC_4 and LTE_4 was

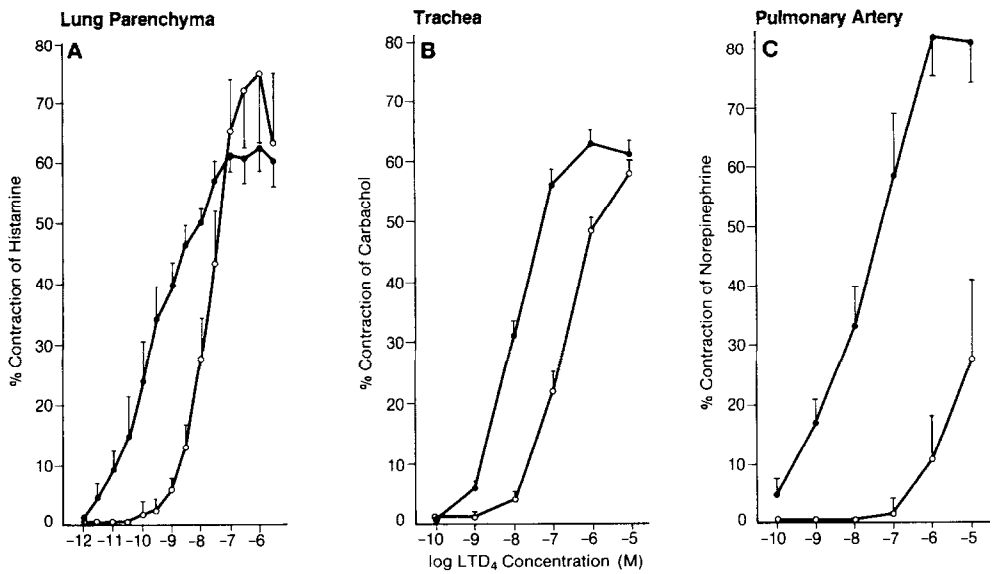


Figure 2. Antagonism of the LTD_4-induced contraction of guinea pig a) lung parenchyma, b) trachea, and c) pulmonary arterial tissues by 4b (10^{-4}M). Tissues were incubated with 4b (O) or vehicle (●) for 30 minutes prior to construction of cumulative LTD_4 concentration response curves. Each point represent the mean percentage of contraction ± S.E.M.

TABLE 2

ANTAGONISM OF LEUKOTRIENE-INDUCED CONTRACTION OF GUINEA PIG TRACHEA

	K_B (μM)	
LEUKOTRIENE	4b	6b
LTC_4	8.9	ND
LTD_4	7.0	14
LTE_4	11.8	7.5

also observed (Table 2). No agonist activity was noted on the trachea employing 4b at concentrations as high as 10^{-4}M.

The 4S, 5R-diastereomer 4a, which stereochemically more closely resemble LTD_4, differed considerably in pharmacological profile. On the lung parenchymal tissue, 4a exhibited significant contractile activity which precluded the observation of antagonist properties. On the trachea, although 4a still possessed weak, but significant agonist activity, its intrinsic activity was small enough to enable the demonstration of antagonist activity.

LTD_4 is a potent vasoconstrictive agent on the guinea pig pulmonary artery proximal to the lung, with an EC_{50} of 20 nM. The ability of the leukotriene antagonist 4b to inhibit this vascular effect is demonstrated in Fig. 2C. In this vascular model, the antagonist activity of 4b is considerably more pronounced (K_B=23 nM) than that observed in the trachea.

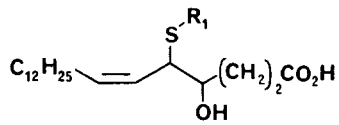
To further explore the structure-activity requirements for antagonism of leukotriene-mediated contractions, and to improve antagonist potency, a series of structural analogs of 4b was prepared. Antagonism was assessed in vitro on the guinea pig trachea, and the results are summarized in Table 3.

DISCUSSION

The present study indicates that the spatial separation (or orientation) of the C-1 carboxyl relative to the rest of the peptidoleukotriene is a critical determinant of both affinity and intrinsic activity at the leukotriene receptor. Except for the LTC analog, 7, shortening the carbon

TABLE 3

COMPARATIVE ANTAGONIST ACTIVITY OF NOR-LEUKOTRIENE ANALOGS ON GUINEA PIG TRACHEAL SPIRAL STRIPS



	R_1	Stereochemistry	Antagonist Activity* K_B (μM)
4a	Cys-Gly	4S, 5R, 6Z	10.0[b]
4b	Cys-Gly	4R, 5S, 6Z	7.0
5a	Cys-Gly	4S, 5R, 6E	20.4[b]
5b	Cys-Gly	4R, 5S, 6E	25.6[b]
6a	Cys	4S, 5R, 6Z	3.1
6b	Cys	4R, 5S, 6Z	14
7	Cys(γ-Glu)Gly	4RS, 5SR, 6Z	Agonist

*in comparison, the K_B for FPL 55712 is 0.1 μM.
partial agonist

chain by one methylene residue results in antagonist properties. The absolute stereochemistry of the hydroxyl and thioether groups also is important; however, the relationship is complex. The unnatural 4R, 5S configuration is preferred for pure antagonist activity in the LTD class of analogs (i.e. 4b), while an apparent preference for the natural configuration may exist in the LTE series.

The nature of the sulfur ligand has considerable impact on intrinsic activity. While the cysteine and cysteinyl-glycine derivatives appear equipotent as leukotriene antagonists, the nor-LTC_1 analog 7 is a full agonist. Further studies are underway to explore the structural requirements for leukotriene antagonist activity.

That the pharmacological profile of these nor-leukotriene analogs is fully consistent with that expected of a leukotriene antagonist is best exemplified by compound 4b. This compound significantly inhibited the contractions induced by LTD_4 on guinea pig lung parenchymal strips. The weak and variable contractile activity observed with 4b may reflect the contribution of an indirect thromboxane-mediated component of LTD_4 previously characterized (10). Indeed, 4b increased basal thromboxane B_2 concentration

in the tissue bath by 4-fold, but suppressed the ability of LTD_4 to subsequently elicit thromboxane synthesis (unpublished results). In tracheal spiral strips, a model in which the thromboxane component is absent and only direct leukotriene effects are observed, this compound antagonized the contractile activity of LTC_4, LTD_4 and LTE_4. No inhibition of histamine, carbachol or KCl induced contractions was observed. In the guinea pig pulmonary artery, a model of the vascular effects of leukotrienes, 4b selectively antagonized an LTD_4 induced vasoconstriction.

These results demonstrate that 4R, 5S, 6Z-2-nor-LTD_1 (4b) and related structural analogs possess significant leukotriene antagonist activity. While the precise role of leukotrienes in the pathophysiology of asthma remains to be determined, a leukotriene antagonist of this type, with adequate potency and duration of effect, may offer new and useful therapeutic opportunities in asthma and other immediate hypersensitivity diseases, as well as provide tools for the exploration of the role of leukotrienes in mammalian physiology and pathophysiology.

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