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DELTA FOUR ACETYLENIC ACIDS - SELECTIVE INHIBITORS OF THE FORMATION OF SLOW REACTING SUBSTANCE

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SUMMARY: The delta four acetylenic fatty acids, 4,7,10-hexadecynoic acid, 4,7,10,13-nonadecatetraynoic acid and 4,7,10,13-henicosatetraynoic acid were investigated for their effect on cyclooxygenase and 5-lipoxygenase activity as well as formation of slow reacting substance by homogenates of rat basophilic leukemia (RBL-1) cells. None of these compounds tested altered arachidonic acid oxygenation by the cyclooxygenase or 5-lipoxygenase. However, all four compounds markedly suppressed SRS synthesis with 4,7,10,13-henicosatetraynoic acid being the most potent one. These compounds are therefore valuable tools in elucidating the contribution of slow reacting substance (leukotriene C_4 and D_4) in biological functions.

INTRODUCTION

SRS[1] is a potent biologically active factor which causes bronchoconstriction and plasma exudation (1,2). SRS has been described as an arachidonic acid metabolite (3,4) and a member of the leukotriene (LT) family (5-7). It is composed of LTC_4 and/or LTD_4. LTC_4 is formed from arachidonic acid via two labile intermediates, the 5-hydroperoxyicosatetraenoic acid (5-HPETE) and the 5,6-oxido-7,9,11,14-icosatetraenoic acid (LTA_4) (5). A glutathione transferase acts upon LTA_4 and synthesizes a thioether with glutathione (8,9) which is LTC_4. LTD_4 is formed by the removal of glutamic acid from LTC_4 (10). 5-HPETE can also be converted to 5-HETE and LTA_4 to various diHETEs including LTB_4 (11).

[1]Abbrevations used: SRS, slow reaction substance; RBL-1 cells, rat basophilic leukemia cells; LT, leukotrienes; LTA_4, leukotriene A_4; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; HETE, hydroxyicosatetraenoic acid; HPETE, hydroperoxyicosatetraenoic acid; PG, prostaglandin.

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Arachidonic acid, therefore, can be oxygenated by various enzymatic pathways. The cyclooxygenase will generate prostaglandins and thromboxanes and lipoxygenases polyunsaturated hydroxy fatty acids. In many tissues and cells two distinct pathways for the oxygenation of arachidonic acid can be found. In rat basophilic leukemia (RBL-1) cells as well as in normal mast cells arachidonic acid is converted to prostaglandin D_2 via the cyclooxygenase (12,13) and to leukotrienes (predominantly LTD_4) via the 5-lipoxygenase (6,7,10,14). In order to study the function and biological significance of the different arachidonic acid metabolites, it would be desirable to have specific blockers for each pathway. In this paper we report on a group of Δ4 acetylenic polyenoic acids which preferentially block SRS (LTC_4 and LTD_4) formation and do not inhibit the cyclooxygenase or 5-lipoxygenase.

MATERIALS AND METHODS

The acetylenic fatty acids were prepared by total organic synthesis as described previously (15,16). Arachidonic acid was obtained from Nu-Check Prep and [^{14}C]arachidonic acid (55 Ci/mole) from New England Nuclear. Leukotriene A_4 was kindly supplied by Dr. J. Rokach (Frosst Merck, Canada) and prostaglandin standards by Dr. J. Pike (Upjohn Co., Kalamazoo, MI).

RBL-1 cells were grown in suspension cultures as described previously (12). The cells were washed with 50 mM phosphate buffer, 1 mM EDTA, and homogenized in 35 mM phosphate buffer, 1 mM EDTA, as described previously (17).

Determination of 5-lipoxygenase activity: For these experiments the 10,000 x g supernatant of the RBL-1 homogenate was used. The acetylenic acids (0-50 μM) were preincubated with 0.5 ml of the 10,000 x g supernatant, 1.5 mM Ca^{++}, for 10 min on ice, followed by incubation with [^{14}C]arachidonic acid (220,000 cpm, 4 μM). Preincubation at 37°C was not possible since the enzyme activity was lost under these conditions. The protein was precipitated with 2 volumes of acetone and the supernatant extracted with chloroform (pH 3.4). Chromatography was performed in solvent system A9: ethyl acetate:2,2,4-trimethylpentane:acetic acid:water (110:50:20:100). This system separates prostaglandins (18) as well as 5-hydroxyeicosatetraenoate (5-HETE) and 5,12-diHETE (19). Radioautography was performed with Kodak X-Omat RAX-5 film (3 days). Quantitation was achieved by scraping of the appropriate bands and liquid scintillation counting. The sum of the cpm in the 5-HETE and 5,12-diHETE bands was designated as the 5-lipoxygenase products and the sum of the cpm of the bands comigrating with $PGF_{2\alpha}$, PGE_2 and PGD_2 as cyclooxygenase products. All experiments were performed in duplicate.

Determination of SRS activity: For the generation of SRS, a mixture of LTC_4 and LTD_4 (20), the whole homogenate was used (20). The homogenate, 1.5 mM Ca^{++}, was preincubated with the acetylenic acid (0-50 μM) on ice, 10 min. Then GSH, 1 mM, and arachidonic acid (4 μM) were added and the

TABLE 1

COMPARISON OF THE EFFECT OF Δ4 ACETYLENIC ACIDS

Compound	IC_{50} (μM)* Cyclooxygenase	5-lipoxygenase	SRS Activity
16:3 4a,7a,10a	>60	>60	31±8
19:4 4a,7a,10a,13a	>55	>55	26±2
20:4 4a,7a,10a,13a	>50	>50	14±2
21:4 4a,7a,10a,13a	>50	>50	7±0

*IC_{50} is the concentration of the acetylenic acid required to cause 50% reduction in activity as compared to the control.

mixture incubated at 37°C for 15 min. The SRS activity was monitored on the superfused guinea pig ileum in the presence of various inhibitors including pyrilamine maleate, 8 μg/ml, and indomethacin, 1 μg/ml (12). Quantitation was obtained by determining the area under the curve produced by the contraction.

Evaluation of data: Dose reponse curves expressed as per cent of control were obtained for each acetylenic acid.

RESULTS AND DISCUSSION

When the 10,000 x g supernatant of RBL-1 cell homogenates was incubated with [^{14}C]arachidonic acid in the presence and absence of the Δ4 acetylenic acids no significant alteration in the formation of prostaglandins as well as the two 5-lipoxygenase products, 5-HETE and 5,12-diHETE, was observed (Table 1 and Fig. 1). However, when we tested the effect of these acetylenic acids on the formation of SRS (LTC_4 and LTD_4) a marked dose dependent inhibition was observed (Fig. 1 and 2). No inhibition was observed when no acetylenic acid was present during the preincubation (10 min, 4°C) and incubation (15 min, 37°C) but added just prior to the application of the sample to the guinea pig ileum. These results suggested that these compounds act upon the glutathione transferase, the enzyme that synthesizes LTC_4 from LTA_4 and glutathione. The inhibition of SRS activity increased with carbon chain length with 21:4 (4a,7a,10a,13a) as the most potent inhibitor of the compounds tested.

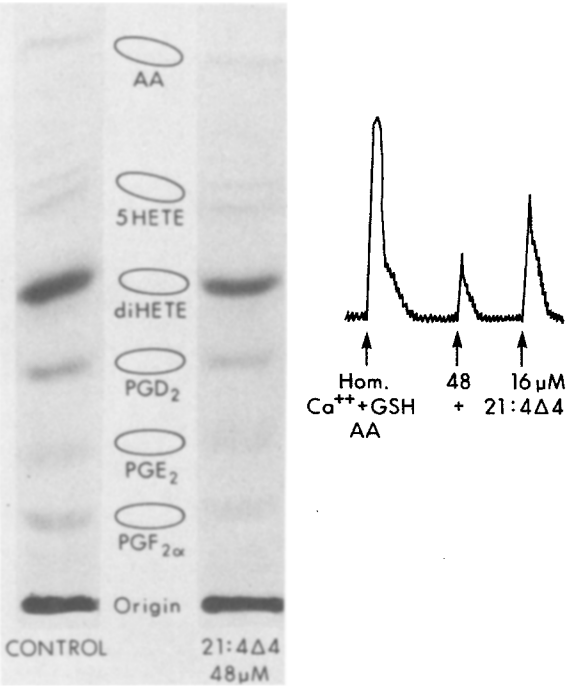

Figure 1: Inhibition of SRS formation from arachidonic acid by 4,7,10,13-henicosatetraynoic acid. Left: 10,000 x g supernatant of RBL-1 cell homogenate was preincubated with and without (control) the acetylenic acid at the concentration indicated followed by incubation with arachidonic acid, extraction, thin layer chromatography and radioautography as indicated in Methods. Right: RBL-1 cell homogenate was preincubated with and without (control) the acetylenic acid at the concentrations indicated followed by incubation with arachidonic acid (AA) as described in Methods. The samples were applied to the superfused strips of guinea pig ileum.

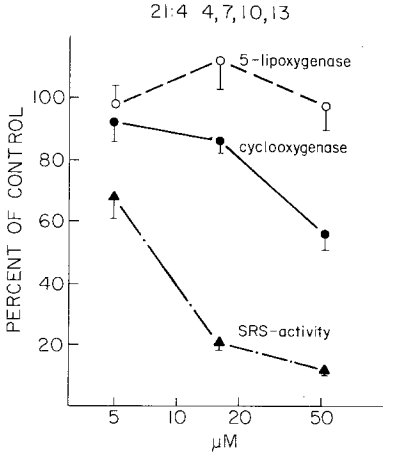

Figure 2: Differential action of 4,7,10,13-henicosatetraynoic acid. The experiments are described in Methods. The bars indicate SEM (mean of 3-8 experiments).

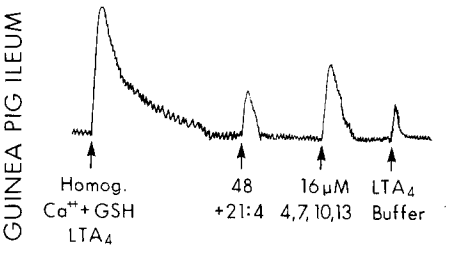

Figure 3: Inhibition of SRS formation from LTA_4 by 4,7,10,13-henicosatetraynoic acid. RBL-1 homogenate was treated as in the legend of Figure 1, except LTA_4 was used as substrate instead of arachidonic acid.

Figure 2 illustrates the differential action of this acetylenic acid on the cyclooxygenase, 5-lipoxygenase and the formation of SRS.

In order to further substantiate the observation that the Δ4 acetylenic acids inhibit the glutathione transferase, RBL-1 homogenate was preincubated with the acetylenic acid followed by incubation with LTA_4, the immediate percursor of LTC_4. Again a dose dependent inhibition of SRS activity was observed. A representative experiment is illustrated in Fig. 3 (n=3).

From these data it can therefore be concluded that the Δ4 acetylenic acid specifically inhibit SRS (LTD_4 and LTC_4) formation, with 21:4 (4a,7a,10,13a) the most potent compound of that group. These compounds will be valuable tools in the study of the function of LTC_4 and LTD_4 in cellular mechanisms. It has been reported that SRS (LTC_4 and/or LTD_4) is released from macrophages during phagocytosis (21). It is also known that SRS is released by macrophages (22), neutrophils (23), mast cells (14) and basophils (3) when stimulated with A23187. However, the role of SRS in the mast cell and basophil release reaction and in phagocytosis is not understood. Therefore, the availability of specific inhibitors of LTC_4 and LTD_4 formation may aid in the elucidation of their role in these processes.

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