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EVIDENCE OF LEUKOTRIENE B_4 BIOSYNTHESIS IN EPITHELIAL LENS CELLS

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ABSTRACT

Metabolism of sodium (^{14}C)-arachidonate by bovine epithelial lens cells was studied in culture. The cells converted arachidonic acid into a major product whose formation was not inhibited by aspirin, a cyclo-oxygenase inhibitor, but was suppressed by nordihydroguaiaretic acid, a lipoxygenase inhibitor and by dexamethasone. This metabolite co-migrated with leukotriene B_4 in thin layer chromatography and high pressure liquid chromatography. These data represent the first evidence for a lipoxygenase product in the lens. LTB_4 could play an important role in the physiopathology of this organ.

INTRODUCTION

Eicosanoids : prostaglandins (PGs), thromboxanes (TXs), HETEs and leukotrienes (LTs) have received much attention for their regulatory role in various organs (1, 2) including ocular tissues (3) concerned in the present study. However, nothing has been reported about these mediators in the lens. The aim of this work was to investigate arachidonic acid (AA) metabolism in epithelial lens cells.

10 µl aliquotes were separated by reverse phase HPLC as previously described (5) using a µ-Bondapak C_{18} column (Waters). The solvent used was a mixture of methanol : water (75 : 25 v/v) plus acetic acid to 0.01 %. The analyses were performed at a solvent flow rate of 1 ml x min^{-1} at 25°C. The effluent was monitored by spectrophotometry at 280 nm during 20 min.

RESULTS

(^{14}C)-AA metabolism by BEL Cells (Fig. 1)

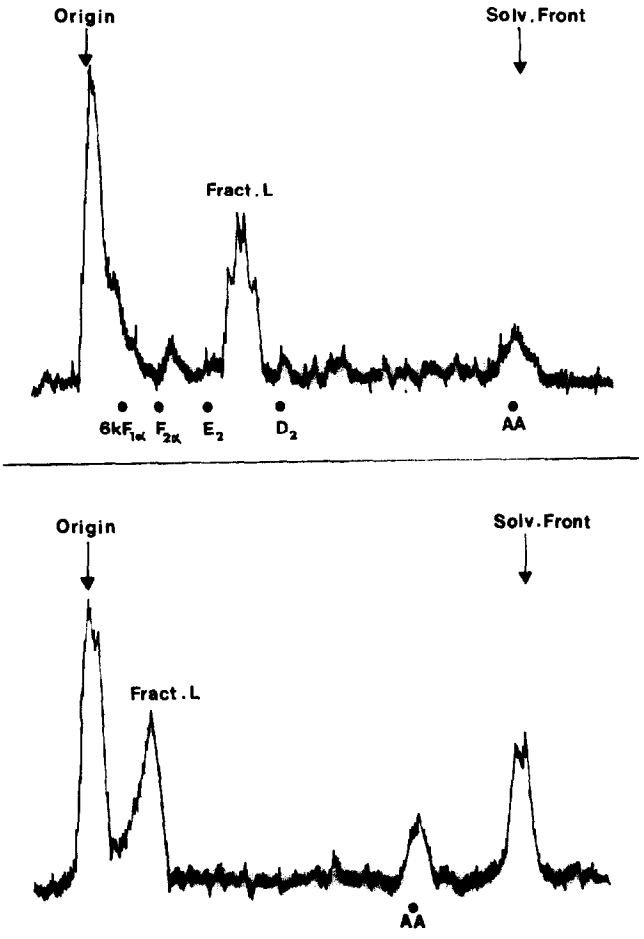

Fig. 1 : TLC profiles of (^{14}C)-AA metabolism by BEL cells (13th subculture)

up : solvent system I (one migration)
down : solvent system II (double migration)

MATERIALS AND METHODS

Bovine Epithelial Lens (BEL) cells

Cultures of BEL cells were performed as previously described (4). Briefly, sterile anterior capsules were placed in a 3 cm diameter petri dish kept in place by a sterile glass coverlip and covered by minimum essential medium (MEM - Seromed) with 6 % calf serum (Seromed) penicillin, streptomycin 100 U x ml^{-1} (Eurobio), mycostatine 500 U x ml^{-1} (Eurobio), fungizone 2.5 µg x ml^{-1} (Eurobio), glutamine 10^{-3} mol x l^{-1} (Seromed) in a 95 % air-CO_2 5 % mixture. The medium was changed every third day. When the cells were confluent, they were subcultured and plated at a density of 10^4 cells x cm^{-2}. The cells were maintained for up to several months.

AA metabolism by BEL cells

Confluent cells ($\sim 8 \times 10^5$ cells per dish) were washed three times with saline (154×10^{-3} mol x l^{-1}) and preincubated for 5 hours with 10^{-5} mol x l^{-1} (^{14}C)-AA, specific activity : 55 mCi x $mmol^{-1}$ (New England Nuclear) or unlabelled AA (Sigma). Cells were washed again three times and incubated for 16 hours in the presence or absence of tested compounds i.e. calcium ionophore A 23187, dexamethasone, nordihydroguaiaretic acid (NDGA) and aspirin (ASA) (Sigma). The incubation medium was then acidified to pH 3 and extracted twice with three volumes of ethyl acetate. The organic phase was evaporated to dryness under nitrogen.

Thin Layer Chromatography (TLC) of (^{14}C)-AA metabolites

The ethyl acetate extract was dissolved in chloroform : methanol (2 : 1 v/v). This extract and authentic PG standards 6-keto-$PGF_{1\alpha}$, $PFG_{2\alpha}$, PGE_2 and PGD_2 (Sigma) as well as AA were separated by TLC on silica-gel 60F.254 plates (Merck). Development was carried out in solvent system I (isooctane : ethyl acetate : acetic acid : water ; 25 : 55 : 10 : 50 v/v) or in the solvent system II (diethylether : petroleum ether : acetic acid ; 50 : 50 : 1 v/v). All the solvents, analytical grade, were purchased from Merck. Radioactive zones were located by radio-scanning and unlabelled standards by spraying 10 % phosphomolybdic acid in ethanol.

High Pressure Liquid Chromatography (HPLC) of unlabelled AA metabolites

Extracts of culture medium from A 23187 stimulated cells were dissolved in methanol or standard LTB_4 (Merck Frosst).

TLC analysis of the radioactive ethyl acetate extract of culture medium using solvent system I showed that half the radioactivity was detected at the origin, 15 % co-migrated with AA (Rf$\sim$1) and the remaining fraction migrated with a Rf = 0.32 (L peak). No metabolite co-migrated with PG standards. Using solvent system II (double migration), one peak of radioactivity, co-migrating with triacylglycerols (Rf = 0.98), appeared and the fraction L migrated as LTB_4 (Rf = 0.12).

Effects of pharmacological agents (Table I)

Pre-incubation of BEL cells for 5 hours in MEM containing dexamethasone (5×10^{-6} mol x l^{-1}) resulted in total inhibition of the L peak seen on TLC. When cells were treated with ASA (2×10^{-4} mol x l^{-1}), TLC profiles were not modified. On the contrary, NDGA (5×10^{-5} mol x l^{-1}) totally suppressed L peak formation.

TABLE I

PERCENT L PEAK RADIOACTIVITY IN TLC ANALYSIS

Exp. N°	control	Dexamethasone ($5x10^{-6}$molxl^{-1})	ASA ($2x10^{-4}$molxl^{-1})	NDGA ($5x10^{-5}$molxl^{-1})
1	30	0		
	31	0		
	18	0		
2	12		15	0
	14		22	0
	10		19	0
	12		15	0
3	18		18	0
	21		26	0
	11		16	0

Experiment N° 1 was carried out from 3 different 13^{th} subculture dishes, N° 2 and N° 3 from 4 and 3 different 16^{th} subculture dishes.

HPLC analysis of culture medium extract

HPLC analysis performed with detection at 280 nm separated several peaks with retention times between 0 and 20 min. One of them presented the same retention time as authentic LTB_4, 8.35 min (Fig. 2). This isolated corresponding fraction co-migrated with an internal LTB_4 sample.

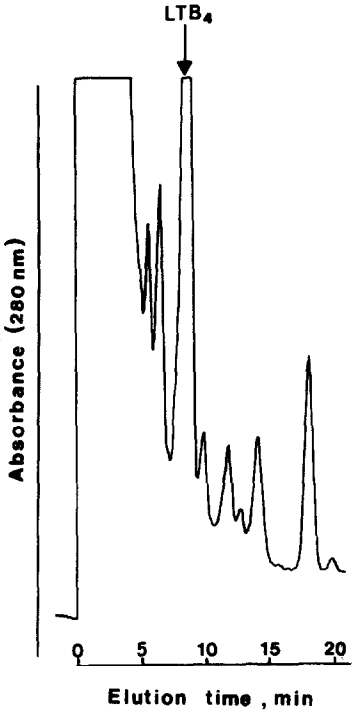

Fig. 2 : HPLC elution profiles of AA metabolism by A 23187-stimulated BEL cells (3rd subculture).

DISCUSSION

We demonstrated that BEL cells, incubated with AA, released a metabolite the production of which was inhibited by dexamethasone and NDGA but not by ASA. Since ASA is a selective inhibitor of cyclo-oxygenase (6) while NDGA is a dual cyclo-oxygenase-lipoxygenase inhibitor (7), this metabolite must

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be formed through the lipoxygenase pathway. Moreover, this compound presented TLC and HPLC characteristics of LTB_4, a 5-lipoxygenase product. This metabolite was produced in relatively large amounts with respect to the low number of incubated cells.

LTB_4 has been previously identified in various cell lines (8-11) and recognized as an important cell regulator (12). Our data represent the first evidence of a 5-lipoxygenase activity in the lens. LTB_4 and possibly other leukotrienes could be implicated in the physiopathology of this organ and this discovery might open fertile areas for future investigation.

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