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GENERATION OF LEUKOTRIENE B_4, ITS ALL *trans* ISOMERS AND 5-HYDROXYEICOSATETRAENOIC ACID BY RAT BASOPHILIC LEUKAEMIA CELLS

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1 When rat basophilic leukaemia (RBL-1) cells were incubated for 4 min, in the presence of arachidonic acid (AA) and calcium ionophore A23187, three isomers of leukotriene (LT) B_4 and 5-hydroxyeicosatetraenoic acid (5-HETE) were generated.

2 The isomers of LTB_4 separated by reverse phase high pressure liquid chromatography (h.p.l.c.) were found to be 5(**S**),12(**S**)-dihydroxy-6,14-*trans*-8,10-*trans*-eicosatetraenoic acid; 5(**S**), 12(**R**)-dihydroxy-6,14-*trans*-8,10-*trans*-eicosatetraenoic acid and 5(**S**),12(**R**)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid or LTB_4.

3 5(**S**), 12(**R**)-*cis*, *trans*, *trans*-LTB_4 produced by RBL-1 cells caused aggregation of rat polymorphonuclear leucocytes (PMNs). Its other two isomers (5(**S**), 12(**S**)-all *trans*-LTB_4; 5(**S**),12(**R**)-all *trans*-LTB_4) were much less active and 5-HETE was found to be inactive.

4 5(**S**),12(**R**)-*cis*, *trans*, *trans*-LTB_4 formed from RBL-1 cells contracted guinea-pig parenchymal strips (GPPs) and was indistinguishable from synthetic 5(**S**),12(**R**)-*cis*, *trans*, *trans*-LTB_4. The other two isomers of LTB_4, i.e. 5(**S**),12(**S**)-all *trans*-LTB_4 and 5(**S**),12(**R**)-all *trans*-LTB_4 as well as 5-HETE were inactive on GPP.

5 RBL-1 LTB_4 was equipotent with LTD_4, in most experiments, in contracting GPP but had no activity on guinea-pig ileum smooth muscle (GPISM).

6 RBL-1 cells contain the enzyme systems necessary for generating LTB_4 as well as the leukotriene(s) with amino acid residues at C-6.

Introduction

When stimulated with calcium ionophore A23187, rat basophilic leukaemia (RBL-1) cells have been shown to be a rich source of slow-reacting substance (SRS) (Jakschik, Kulczycki, Macdonald & Parker, 1977). The yield of SRS is further increased by inclusion of arachidonic acid (AA) in the incubation medium (Jakschik, Falkenhein & Parker, 1977). SRS from RBL-1 cells has been characterized and the major biological activity, as measured by contraction of the guinea-pig ileum, shown to be leukotriene (LT) D_4 (Morris, Taylor, Piper, Samhoun, Tippins, 1980). However, RBL-1 SRS also contained substantial amounts of another u.v.-absorbing material λ_{max}^{MeOH} 268 nM (compound III, Morris *et al.*, 1980) but which had no activity on strips of guinea-pig ileum smooth muscle (GPISM). This material was subsequently shown to be a less active isomer of LTB_4. These findings suggested that RBL-1 cells might possess the necessary enzyme systems to generate LTB_4 as well as the peptidolipid leukotriene(s). Little evidence of LTC_4 was found in SRS from RBL-1 cells, probably because these cells contain sufficient γ-glutamyl transferase to convert tens of nanomoles/min LTC_4 to LTD_4 (Morris, Taylor, Jones, Piper, Tippins & Samhoun, 1981). The release of an LTB_4-like material from RBL-1 cells has previously been shown (Morris, Piper, Samhoun & Taylor, 1981). The experiments described in this paper show that LTB_4, its all *trans* isomers and 5-hydroxyeicosatetraenoic acid (5-HETE) were produced by RBL-1 cells.

Part of this work has been communicated to the British Pharmacological Society.

Methods

RBL-1 cells were grown in spinner cultures in Eagle's minimum essential medium, supplemented with a

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penicillin streptomycin solution (80 units/ml), 10% foetal calf serum, 10% newborn calf serum and an atmosphere of 5% CO_2 in air.

Cells were harvested, centrifuged at 1900 *g* for 10 min, at room temperature and were then washed twice with incubation buffer (composition (mM): NaCl 150, KCl 3.7, Na_2HPO_4 3.0, KH_2PO_4 3.5, $CaCl_2$ 0.9 and D-glucose 5.6 with 0.1% (w/v) bovine serum albumin (essentially fatty acid-free) and heparin 10 units/ml) and the final pH adjusted to 7 with NaOH. They were then resuspended at 1×10^7 cells/ml in buffer and 25–50 ml aliquots were incubated with AA (25 μg/ml) and calcium ionophore A23187 (10 μg/ml) while stirring for 4 min at 37°C. Control incubations were carried out by dividing cells harvested, as described above, from the same spinner into three equal parts. The first sample was treated with AA and A23187, the second sample was incubated with AA alone and the third sample was treated with A23187, in the absence of AA. The incubation was terminated by adjusting the pH to 3 with 0.1N HCl and the cells centrifuged at 1900 *g* for 10 min. The supernatant was collected, the cell pellets washed with absolute MeOH (less than 10% of the supernatant volume), the cells centrifuged for a further 5 min and this supernatant added to that previously collected.

Separation of leukotrienes and mono-hydroxyeicosatetraenoic acids

The combined supernatant and the methanol wash were applied to a 1 g C_{18} SEP-PAK cartridge (Waters' Associates, Inc., Northwich, Cheshire) which had previously been treated with 20 ml of ethanol followed by 20 ml of water. After sample application, the C_{18} cartridge was washed with 20 ml of H_2O and 20 ml of 15% v/v ethanol. The leukotrienes and mono-HETEs were then eluted with 10 ml of methyl formate. The methyl formate was removed under reduced pressure and the residue redissolved in 50 μl of methanol for application to the high pressure liquid chromatograph.

The sample was separated on Waters' radial compression separation system using a 5 mm i.d. C_{18} cartridge eluted with 75:25:0:01 methanol-water:acetic acid (v:v:v) at a flow rate of 3 ml/min. The eluate was monitored initially at 280 nM and after the elution of the leukotrienes at 232 nM. The column was calibrated with synthetic LTB_4 and naturally occurring LTB_4, the two all *trans* isomers of LTB_4 and 5-HETE derived biologically from rat polymorphonuclear leukocytes (PMNs) treated with ionophore A23187 (Ford-Hutchinson, Bray, Cunningham, Davidson & Smith, 1981). Leukotriene B_4, LTB_4 isomers and 5-HETE were collected in separate fractions, the fractions dried down under reduced pressure and the ultraviolet spectra recorded in methanol. Leukotrienes were quantitated using $\varepsilon^{MeOH}_{281\,nM}$ of 39,500 and 5-HETE using $\varepsilon^{MeOH}_{232\,nM}$ of 30,500 (Borgeat & Samuelsson, 1979).

Neutrophil aggregation assay

Neutrophil cell suspensions (>85% PMNs) were prepared from peritoneal exudates obtained 24 h after the injection of 12% (w/v) sodium caseinate into 250–400 g male Wistar rats (Cunningham, Smith, Ford-Hutchinson & Walker, 1979). The cells were washed and resuspended at a concentration of 1×10^7 cell/ml in Eagle's minimum essential medium buffered to pH 7.4 with 30 mM N'-2-hydroxyethylpiperazine-N'-2-ethane sulphonic acid. Neutrophil aggregation was assessed by nephelometry in a Payton aggregometer (Cunningham, Shipley & Smith, 1980). The results are expressed as the increase in light transmission measured in mm on the recorder. The settings on the aggregometer were as follows: range, 5; level, 0.3; zero, 40; output, 80; temperature, 37°C and stir speed, 800 rev/min. The recorder amplifier was set for 20 mV. Leukotrienes and 5-HETE were tested over the concentration range 100 pg/ml to 100 ng/ml and were compared with a dose-response curve obtained with synthetic LTB_4.

Preparation of assay tissues

Male guinea-pigs, Dunkin Hartley strain (500–700 g) were used and their lungs removed after cervical dislocation. Lungs were inflated via the trachea and perfused via the pulmonary artery with Tyrode solution gassed with oxygen (5 ml/min) until free from blood. Strips of parenchymal tissue (30 × 3 × 3 mm) (GPP) were cut from the major lobes, distal to the main airways (using a modification of the method of Lulich, Mitchell & Sparrow, 1976). The GPPs and strips of GPISM were superfused in series with Tyrode solution (5 ml/min) containing a mixture of antagonists to histamine, acetylcholine, 5-hydroxytryptamine (5-HT), α- and β-adrenoceptors.

Preparation and administration of drugs

Stock solutions of AA and A23187 were prepared in ethanol and dimethyl sulphoxide respectively and the final concentration of either solvent in the cell suspensions was always less than 0.25%. U-44069 and synthetic LTD_4 were stored in ethanol, synthetic LTB_4 in methanol and for bioassay purposes, care was taken to prepare alcohol-free dilutions in Tyrode solution. Agonists were administered to the assay tissues as bolus injections into the superfusing fluid

and U-44069, a stable thromboxane A_2 analogue, was used to test and to ensure the sensitivity of the GPPs.

Materials

The following drugs were used: arachidonic acid (Grade I, Sigma), calcium ionophore A23187 (Calbiochem). Synthetic LTB_4 (5(**S**), 12(**R**)-dihydroxy-6, 14-*cis*-8,10-*trans*-eicosatetraenoic acid) and synthetic LTD_4 (5(**S**)-hydroxy-6(**R**)-cysteinylglycinyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid) were gifts from Dr J. Rokach (Merck Frosst Laboratories) and U-44069 from Dr J.E. Pike (the Upjohn Company).

Results

Separation of isomers of leukotriene B_4 and 5-hydroxyeicosatetraenoic acid

Figure 1 shows the separation by reverse phase high pressure liquid chromatography (h.p.l.c.) of lipoxygenase products of AA metabolism released by RBL-1 cells exposed to ionophore A23187 and AA. Similar patterns were obtained in five separate occasions. Peaks 1, 2 and 3 all have u.v. spectra with characteristic triplets indicating the presence of conjugated triene structures. The retention times for these peaks are identical to those observed for 5(**S**), 12(**S**)-all *trans*-LTB_4, 5(**S**), 12(**R**)-all *trans*-LTB_4 and LTB_4 respectively. There is a small shoulder to the LTB_4 peak which may be a δ-lactone. A large polar u.v. absorbant peak is observed eluting at 3 min. This has a similar retention to metabolites of LTB_4 such as 20-hydroxy-LTB_4. When this peak was examined by u.v. spectroscopy, no evidence for triene structures was obtained suggesting that LTB_4 metabolites are not formed in any significant amounts under these incubation conditions. Peak 4 has an identical retention time and u.v. spectrum (maximal absorbance at 232 nM) to 5-HETE. Immediately prior to the 5-HETE peak are four other small peaks which probably represent small amounts of other mono-HETEs such as 12- and 15-HETE. These other mono-HETEs account for $<5\%$ of the total mono-HETE produced. Immediately after peak 4 is another peak which is probably an isomer of 5-HETE in which the 8—9 bond is *trans* rather than *cis* (Borgeat, personal communication). This has also been observed following exposure of rat macrophages to ionophore A23187 (Doig & Ford-Hutchinson, 1980).

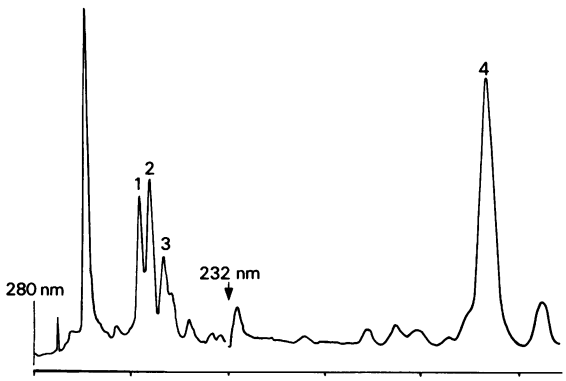

Figure 1 Reverse phase high pressure liquid chromatography separation of 5(**S**), 12(**S**)-all *trans*-leukotriene B_4 (1), 5(**S**), 12(**R**)-all *trans*-LTB_4 (2), LTB_4 (3) and 5-hydroxyeicasatetraenoic acid (5-HETE)(4) derived from RBL-1 cells exposed to ionophore A23187 and arachidonic acid (preparation 3, see Table 1). Column conditions were: flow rate, 3 ml/min; pressure, 15,000 kPa; injection volume, 25 μl; absorbance setting, 0.5. The effluent was initially monitored at 280 nM and after 10 min at 232 nM.

The amounts of LTB_4, LTB_4 isomers and 5-HETE released were quantitated by u.v. spectroscopy using known extinction coefficients and are shown in Table 1. In all preparations, 5-HETE was the dominant product and the amount of LTB_4 produced was less than that of its non-enzymic all *trans* isomers. The cells used for preparations 2, 4 and 5 were split into

Table 1 Production of leukotriene B_4 (LTB_4), LTB_4 isomers and 5-hydroxyeicaratetraenoic acid (5-HETE) by RBL-1 cells exposed to ionophore A23187 (10 μg/ml) and arachidonic acid (25 μg/ml)

	5(**S**), 12(**S**)-all *trans*-LTB_4	5(**S**), 12(**R**)-all *trans*-LTB_4	LTB_4	5-HETE
Preparation 1	0.5	0.7	0.3	3.5
Preparation 2	0.6	0.6	0.5	1.3
Preparation 3	0.5	0.6	0.4	2.6
Preparation 4	0.7	1.1	0.4	2.5
Preparation 5	0.2	0.4	0.3	1.9

Results are expressed as amount of leukotriene or 5-HETE released (μg) per 10^8 cells as determined by u.v. absorbance.

three identical aliquots which were exposed to ionophore A23187 and AA, ionophore A23187 alone and AA alone. In the presence of ionophore A23187 alone, no peaks were visible on the h.p.l.c. trace in either the leukotriene or mono-HETE regions demonstrating that exogenous AA is needed to produce measurable amounts of lipoxygenase products. In the presence of AA alone, small peaks were observed in the same region as LTB_4 and its all *trans* isomers. The amounts formed were too small to quantitate by u.v. spectroscopy and are $< 1\%$ of that produced in the presence of ionophore A23187 and AA. Significant amounts of 5-HETE were produced, the quantities being 0.4, 0.9 and 0.3 $\mu g/10^8$ cells respectively, compared with 1.3, 2.5 and 1.2 $\mu g/10^8$ cells in the presence of A23187 and AA. In addition the four peaks (probably other mono-HETEs) eluting immediately prior to 5-HETE were greatly enhanced in the absence of ionophore A23187 and the areas of the peaks were comparable to those of 5-HETE.

Effects of isomers of leukotriene B_4 and 5-hydroxyeicosatetraenoic acid on the aggregation of rat PMNs

Figure 2 shows the effects of synthetic LTB_4 as well as LTB_4 and its two all *trans* isomers derived from RBL-1 cells in the aggregation of rat PMNs. Leukotriene B_4 from RBL-1 cells was slightly less active

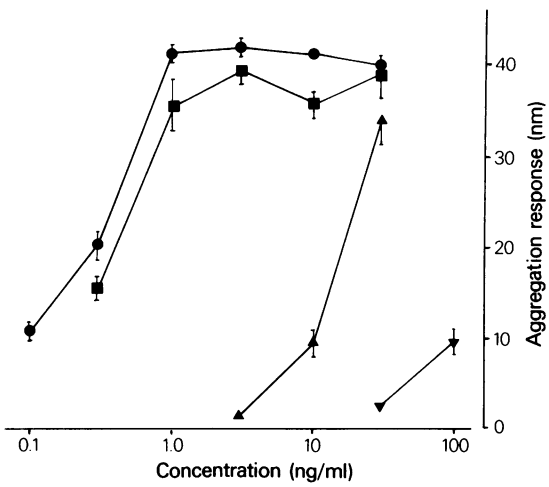

Figure 2 The effects of leukotrienes on the aggregation of rat neutrophils. Symbols represent synthetic leukotriene B_4 (LTB_4)(●); compound 3 (mainly LTB_4) (■); compound 2 (mainly 5(**S**), 12(**R**)-all *trans*-LTB_4) (▲); compound 1 (mainly 5(**S**), 12(**S**)-all *trans*-LTB_4) (▼). Results are expressed as the height of the aggregation response measured in mm. Bars represent mean ± s.e.mean. $n = 5-37$.

than synthetic LTB_4 whereas the two all *trans* isomers were considerably less active, as has previously been reported (Ford-Hutchinson *et al.*, 1981). At the highest concentration tested (100 ng/ml), 5-HETE did not cause PMN aggregation.

Effects of isomers of leukotriene B_4 and 5-hydroxyecosatetraenoic acid on guinea-pig parenchyma and ileum

Samples purified on h.p.l.c., corresponding to all three LTB_4 isomers and 5-HETE (as described above and shown in Figure 1) and quantitated by u.v. absorbance were assayed on GPP and GPISM against synthetic LTD_4 and LTB_4. Successive administrations of any of these samples and of synthetic LTB_4 were avoided (to prevent tachyphylaxis) and these substances were assayed alternatively with other antagonists, namely LTD_4 and U-44069. U-44069 (3×10^{-9} mol), synthetic LTB_4 ($1 \times 10^{-11} - 1 \times 10^{-10}$ mol) and samples containing 5(**S**), 12(**R**)-*cis*, *trans*, *trans*-LTB_4 ($1 \times 10^{-11} - 1 \times 10^{-10}$ mol) obtained from RBL-1 cells, contracted the GPPs in a dose-related manner, but had no effect on GPISM. LTD_4 ($1 \times 10^{-11} - 1 \times 10^{-10}$ mol) induced dose-related contractions of both tissues. Synthetic LTD_4, LTB_4 and, on most occasions, 5(**S**), 12(**R**)-*cis*, *trans*, *trans*-LTB_4 from RBL-1 cells were found to be approximately equipotent on GPPs. Administrations of 5(**S**), 12(**S**)-all *trans*-LTB_4 ($1 \times 10^{-11} - 2.5 \times 10^{-10}$ mol); 5(**S**), 12(**R**)-all *trans*-LTB_4 ($1 \times 10^{-11} - 2.5 \times 10^{-10}$ mol) and 5-HETE ($1 \times 10^{-11} - 5 \times 10^{-10}$ mol) obtained from RBL-1 cells failed to produce a response on both tissue preparations (see Figure 3).

Discussion

The RBL-1 cells used in this series of experiments generated 5(**S**), 12(**R**)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid or LTB_4, its two all *trans* isomers (5(**S**), 12(**S**)-all *trans*-LTB_4; 5(**S**), 12(**R**)-all *trans*-LTB_4) and 5-HETE when incubated with A23187 in the presence of AA. LTB_4 and its isomers were identified chemically and biologically. The trienes were identified by their characteristic retention times on reverse phase h.p.l.c. compared with synthetic LTB_4; LTB_4 and its two all *trans* isomers and 5-HETE obtained from rat PMNs treated with A23187 and by their characteristic u.v. spectra. LTB_4 and its all *trans* isomers obtained from RBL-1 cells were also assayed biologically by means of PMN aggregation and by their differential effects on GPP and GPISM (Piper & Samhoun, unpublished). The results in Figure 2 show that material eluting in peak 1 is almost equi-active with synthetic LTB_4 while

material eluting in peaks 2 and 3 has much less aggregatory activity. This variation in potency of the isomers of LTB_4 has been demonstrated on materials derived from PMNs (Ford-Hutchinson *et al.*, 1981). Synthetic LTB_4 was equipotent with LTD_4 in contracting GPP but lacked contractile activity on GPISM. LTB_4 from RBL-1 cells (peak 1) was almost equi-active with synthetic LTB_4 and showed the same pharmacological profile; peaks 2 and 3 had no activity on GPP or GPISM. Peak 4 was identified as 5-HETE by its retention time on reverse phase h.p.l.c. and its u.v. spectrum (λ max 232 nM). 5-Hydroxyeicosatetraenoic was inactive on either GPP or GPISM.

The most active isomer of LTB_4, i.e. 5(**S**), 12(**R**)-6,14-*cis*-8,10-*trans*-LTB_4, is formed enzymatically (Ford-Hutchinson *et al.*, 1981) and in the RBL-1 cell preparations used, was almost always formed in lower concentrations than the two all *trans*-LTB_4 isomers. This is in contrast with the formation of lipoxygenase products in PMN leukocytes where 5(**S**), 12(**R**)-6,14-*cis*-8, 10-*trans*-LTB_4 is formed in greater amounts than its isomers. However, the total amounts of LTB_4 and its isomers formed by RBL-1 cells is similar to that released by other leucocyte preparations (Ford-Hutchinson *et al.*, 1981).

In addition to LTB_4 and its two isomers, some mono-hydroxy fatty acids were generated by the RBL-1 cells in the presence of AA and A23187. The predominant mono-hydroxy fatty acid was always 5-HETE, other mono-HETEs accounting for less than 5% of the total. When stimulated with A23187, RBL-1 cells require the presence of exogenous AA for the generation of LTB_4 since stimulation with A23187 alone did not generate detectable amounts of lipoxygenase products. This differs from the production of SRS (LTD_4) by these cells which is greatly increased by exogenous AA but can occur in its absence (Jakschik *et al.*, 1977). No measurable amount of LTD_4 was generated during the 4 min incubation period used in these experiments. The requirement of exogenous AA by RBL-1 cells contrasts with PMN leucocytes which generate substantial amounts of LTB_4, its non-enzymic all *trans* isomers and 5-HETE when stimulated with A23187 alone (Ford-Hutchinson, Bray, Doig, Shipley &

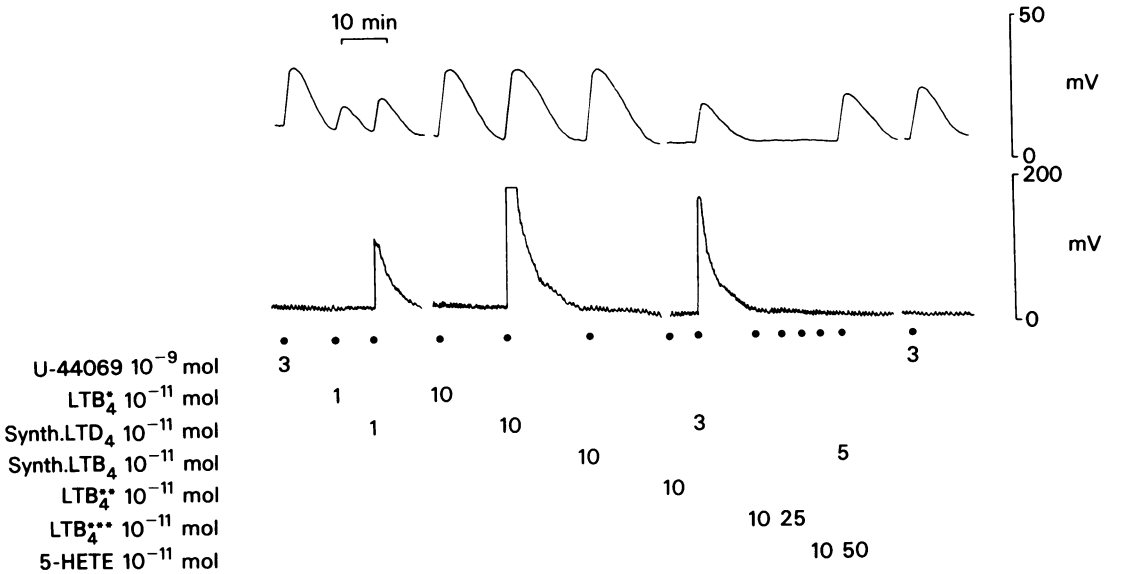

Figure 3 Effects of synthetic leukotriene B_4 (LTB_4), synthetic LTD_4 and LTB_4, its isomers and 5-hydroxyeicosatetraenoic acid (5-HETE), obtained from RBL-1 cells on strips of guinea-pig parenchyma (GPP) and ileum (GPISM). GPP and GPISM were superfused in series and U-44069 (3×10^{-9} mol) contracted the GPP only. 5(**S**), 12(**R**)-*cis*, *trans*, *trans*-LTB_4 (LTB_4^*) from RBL-1 cells, given at 1×10^{-11} mol and 1×10^{-10} mol contracted GPP only while LTD_4 (1×10^{-11}, 3×10^{-11} and 1×10^{-10} mol) contracted both GPP and GPISM. Synthetic LTB_4 (1×10^{-10} mol) produced a response on GPP equal to that elicited on this tissue by LTD_4 and LTB_4 from RBL-1 cells both administered at 1×10^{-10} mol. When 5(**S**), 12(**R**)-all *trans*-LTB_4 (LTB_4^{**}) (1×10^{-10} mol), 5(**S**), 12(**S**)-all *trans*-LTB_4 (LTB_4^{***}) (1×10^{-10} mol and 2.5×10^{-10} mol) and 5-HETE (1×10^{-10} and 5×10^{-10} mol), all produced from RBL-1 cells, were administered, the assay tissues did not contract, while the GPPs were still responsive and contracted following injections of synthetic LTB_4 (5×10^{-11} mol) and U-44069 (3×10^{-9} mol).

Smith, 1980). When RBL-1 cells were incubated with AA alone, only mono-HETEs were produced, the relative proportions differing from those seen during AA and A23187 stimulation. These results suggest that the strain of RBL-1 cells used cannot really utilize endogenous AA for leukotriene synthesis during the 4 min incubation period used in these experiments. In these cells, A23187 stimulates the calcium-dependent 5-lipoxygenase (Jakschik, Sun, Lee & Steinhoff, 1980), as shown by the production of 5-HETE, and also stimulates the enzyme(s) involved in leukotriene synthesis (leukotriene A_4 synthetase).

The formation of LTA_4, the common precursor of the leukotrienes, by RBL-1 cells has been described by Jakschik *et al.* (1980). The experiments described in this paper show that RBL-1 cells contain a complete 5-lipoxygenase system which leads to the formation of 5-HETE, LTB_4, its all *trans* isomers and the leukotriene(s) containing amino acid residues at C-6. LTD_4 is the peptidolopid leukotriene formed by RBL-1 cells (Morris *et al.*, 1980) but in the presence of an inhibitor of γ-glutamyl transpeptidase, LTC_4 is formed by these cells as an intermediate in the biosynthesis of LTD_4 (Örning & Hammarström, 1980). The 5-lipoxygenase pathway present in RBL-1 cells provides a suitable system for testing inhibitors of total leukotriene formation. In addition the RBL-1 cell system may be used to test specific inhibitors of the formation of the chemotactic agent LTB_4 and of the peptidolipid leukotrienes which have potent smooth muscle contracting properties.

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References

BORGEAT, P. & SAMUELSSON, B. (1979). Metabolism of arachidonic acid in polymorphonuclear leukocytes. Structural analysis of novel hydroxylated compounds. *J. biol. Chem.*, **254,** 7865–7869.

CUNNINGHAM, F.M., SHIPLEY, M.E. & SMITH, M.J.H. (1980). Aggregation of rat polymorphonuclear leucocytes *in vitro*. *J. Pharm. Pharmac.*, **32,** 377–380.

CUNNINGHAM, F.M., SMITH, M.J.H., FORD-HUTCHINSON, A.W. & WALKER, J.R. (1979). Migration of peritoneal polymorphonuclear leucocytes in the rat. *J. Path.*, **128,** 15–20.

DOIG, M.V. & FORD-HUTCHINSON, A.W. (1980). The production and characterisation of products of the lipoxygenase enzyme system released by rat peritoneal macrophages. *Prostaglandins*, **20,** 1007–1019.

FORD-HUTCHINSON, A.W., BRAY, M.A., CUNNINGHAM, F.M., DAVIDSON, E.M. & SMITH, M.J.H. (1981). Isomers of leukotriene B_4 possess differing biological potencies. *Prostaglandins*, **21,** 143–152.

FORD-HUTCHINSON, A.W., BRAY, M.A., DOIG, M.V., SHIPLEY, M.E. & SMITH, M.J.H. (1980). Leukotriene B_4: a potent chemokinetic and aggregating substance released from polymorphonuclear leucocytes. *Nature*, **286,** 264–265.

JAKSCHIK, B.A., FALKENHEIN, S. & PARKER, C.W. (1977). Precursor role of arachidonic acid in release of slow reacting substance from rat basophilic leukemia cells. *Proc. natn. Acad. Sci. U.S.A.*, **74,** 4577–4581.

JAKSCHIK, B.A., KULCZYCKI, A. JR., MACDONALD, H.H. & PARKER, C.W. (1977). Release of slow reacting substance (SRS) from rat basophilic leukemia (RBL-1) cells. *J. Immunol.*, **119,** 618–622.

JAKSCHIK, B.A., SUN, F.F., LEE, L. & STEINHOFF, M.M. (1980). Calcium stimulation of a novel lipoxygenase. *Biochem. biophys. Res. Commun.*, **95,** 103–110.

LULICH, K.M., MITCHELL, H.W. & SPARROW, M.P. (1976). The cat lung strip as an *in vitro* preparation of peripheral airways: a comparison of β-adrenoceptor agonists, autacoid and anaphylactic challenge on the lung strip and trachea. *Br. J. Pharmac.*, **58,** 71–79.

MORRIS, H.R., PIPER, P.J., SAMHOUN, M.N. & TAYLOR, G.W. (1981). Generation of a leukotriene (LT) B_4-like material from rat basophilic leukaemia (RBL-1) cells and its actions in guinea-pig lung *in vitro*. *Br. J. Pharmac.*, **74,** 922–923P.

MORRIS, H.R., TAYLOR, G.W., JONES, C.M., PIPER, P.J., TIPPINS, J.R. & SAMHOUN, M.N. (1981). Structure elucidation, biosynthesis and biodegradation of SRS-A from lung. In *SRS-A and Leukotrienes.* ed. Piper, P.J. pp. 19–44. Chichester, New York, Brisbane, Toronto: Research Studies Press, John Wiley.

MORRIS, H.R., TAYLOR, G.W., PIPER, P.J., SAMHOUN, M.N. & TIPPINS, J.R. (1980). Slow reacting substances (SRSs): the structure identification of SRSs from rat basophil leukaemia (RBL-1) cells. *Prostaglandins*, **19,** 185–201.

ÖRNING, L. & HAMMARSTRÖM, S. (1980). Inhibition of leukotriene C and leukotriene D biosynthesis. *J. biol. Chem.*, **255,** 8023–8026.

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