LEUKOTRIENES A_4 AND B_4 STIMULATE THE FORMATION OF CYCLIC AMP IN HUMAN LEUKOCYTES

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1. Introduction

Recent studies of arachidonic acid metabolism in polymorphonuclear leukocytes (PMNL) have led to the discovery of leukotrienes [1] (fig.1). These compounds have a wide spectrum of biological effects. The activity of most preparations of slow reacting substance of anaphylaxis (SRS-A) is due to leukotriene (LT) C_4 and its metabolites LTD_4 and LTE_4 [1]. LTB_4 induces release of lysosomal enzymes [2,3] and is a potent chemotactic compound [4,5]. The signal for activation of these processes is unknown.

Inflammatory active agents such as latex particles [6], *N*-formyl-methionyl–leucyl–phenylalanine [7,8], C5a [7], calcium ionophore A23187 [8], immune complex [8], zymosan-treated serum [8] and opsinized zymosan [9] have been shown to cause a small rise in cyclic AMP levels, indicating a relationship between neutrophil activation and this cyclic nucleotide. Therefore, we investigated the effects of various leukotrienes on cyclic AMP formation in human PMNL.

This report shows that both LTB_4 and its precursor LTA_4 cause a small rise in cyclic AMP levels which is greatly amplified by pretreatment with RO 20-1724 (an inhibitor of cyclic 3′:5′-nucleotide phosphodiesterase).

2. Materials and methods

RO 20-1724 was obtained from Hoffman La Roche (Basel); Dextran T-500 from Pharmacia Fine Chemicals (Uppsala) and Hypaque/Ficoll from Nyegaard and Co. (Oslo).

Abbreviations: 5S-HPETE, (5*S*)-hydroperoxy-eicosatetraenoic acid; LTA_4, 5,6-epoxy-7,9,11,14-eicosatetraenoic acid; LTB_4, (5*S*), (12*R*)-dihydroxy-6,8,10,14-eicosatetraenoic acid; LTC_4, (5*S*)-hydroxy-(6*R*)-*S*-glutathionyl-7,9,11,14-eicosatetraenoic acid; RP 20-1724, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone; cyclic AMP, adenosine 3′:5′-monophosphate

2.1. *Separation of leukocytes*

PMNL were either isolated as in [10] or in the following way: Blood from healthy donors was collected from the antecubital vein in tubes containing EDTA (final conc. 6 mM). After centrifugation at 200 × *g* for 15 min, the lower phase was mixed with 2% Dextran T-500 solution containing 0.9% of NaCl. After 30 min, the upper layer was gently removed and centrifugated at 200 × *g* for 15 min at 0°C. Supernatant fluid was aspirated and erythrocytes were removed by hypotonic lysis (0.15 M NH_4Cl, 37°C for 7 min). The cell-rich solution was then fractionated on a Hypaque/Ficoll gradient [11]. After centrifugation at 300 × *g* for 40 min, the pellets were resuspended in phosphate-buffered saline (pH 7.4). Similar results were obtained using either of these preparations of PMNL.

2.2. *Leukotrienes preparation*

Synthetic LTA_4 was kindly given by Dr E. J. Corey. LTB_4, 20-OH-LTB_4 and 20-COOH-LTB_4 were prepared as in [12,13]. LTC_4 was generated biosynthetically [14].

2.3. *Experimental conditions and quantitative analyses of cyclic AMP*

Cell suspensions (10–30 × 10^6 cells/ml) were preincubated for 2 min at 37°C. Compounds to be tested were added in 1–2 μl ethanol to 1 ml cell suspension. Incubations were terminated by adding 1 vol. 10% trichloroacetic acid. Cyclic AMP levels were determined using the protein binding technique in [15]. Prior to analyses the samples were purified [16].

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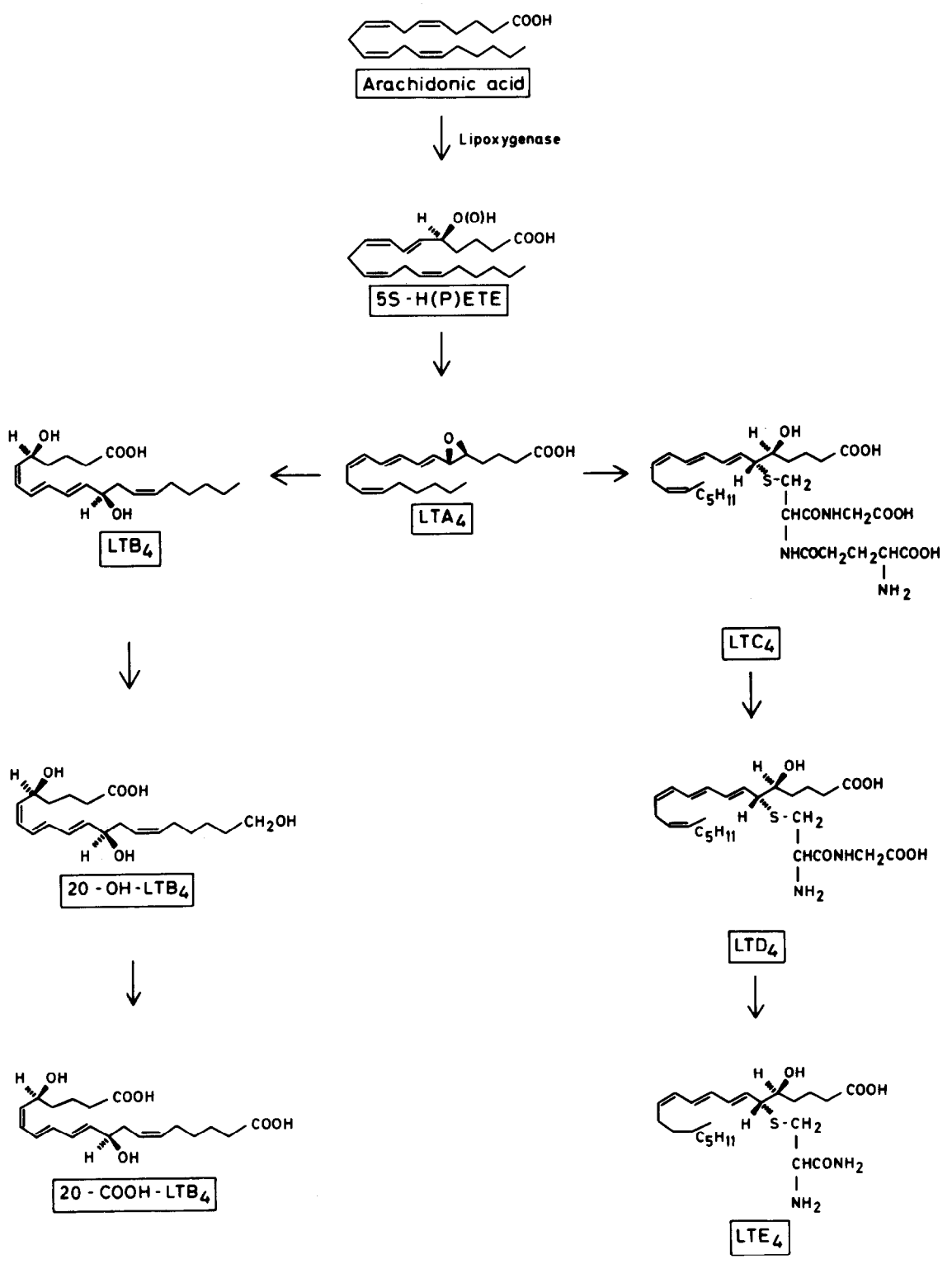


Fig.1. Transformation of arachidonic acid in human PMNL.

3. Results and discussion

A suspension of washed human PMNL was incubated with either LTA_4, LTB_4, or LTC_4. LTA_4 and LTB_4 caused a small rise in cyclic AMP levels (table 1).

Table 1
Effects of LTA_4, LTB_4 and LTC_4 on the levels of cyclic AMP in human PMNL

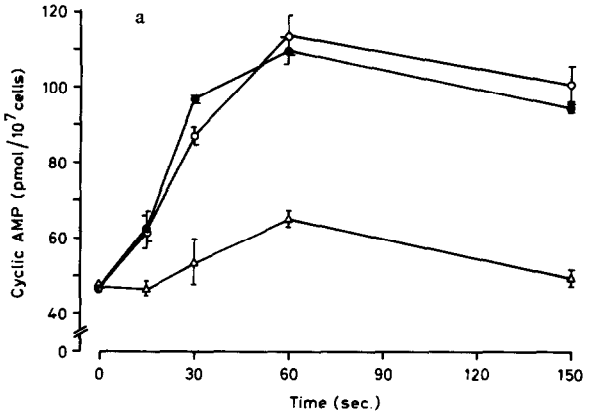
Exp.	Addition (ng/ml)	Cyclic AMP (pmol/10^7 cells)
1	None	22.3 ± 2.4
	LTA_4 (300)	25.6^a ± 1.9
	LTB_4 (100)	28.7^b ± 1.8
	LTC_4 (350)	$21.1^{n.s.}$ ± 1.1
2	None	36.0 ± 3.9
	LTA_4 (100)	71.5^c ± 3.1
	LTB_4 (100)	90.0^c ± 1.8
	LTC_4 (350)	$37.0^{n.s.}$ ± 3.2

Exp. 1: Human PMNL were incubated with either LTA_4, LTB_4 or LTC_4 for 30 s prior to cyclic AMP analyses

Exp. 2: Human PMNL were pretreated 2 min with RO 20-1724 (0.1 mM). Thereafter the cells were incubated with either LTA_4, LTB_4 or LTC_4 for 1 min

Each value represents the mean value ± SD from triplicate determination on each of 6 samples (exp. 1) or 3 samples (exp. 2). Statistical differences were performed using Student's t-test: $^a p < 0.05$; $^b p < 0.01$; $^c p < 0.001$; n.s., not significant

These effects were greatly amplified in the presence of RO 20-1724 (an inhibitor of cyclic 3′:5′-nucleotide phosphodiesterase, EC 3.1.4.17). Similar results were obtained using another phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (not shown). No effect on cyclic AMP synthesis was observed with LTC_4 (table 1). LTA_4 and LTB_4 rapidly elevated the levels of cyclic AMP in human PMNL (fig.2a). A maximal effect was observed after 1 min. Similar time-courses have been reported in PMNL incubated with *N*-formyl-methionyl–leucyl–phenylalanine (fMLP), ionophore A23187, immune complex or zymosan-treated serum after pretreatment with prostaglandin E_1 [8] or a phosphodiesterase inhibitor [17]. Fig.2b shows that LTB_4 and LTA_4 were about equally potent as stimulators of cyclic AMP formation. The effect of LTB_4 on cyclic AMP synthesis is in agreement with the earlier observations that agents which possess the ability to induce superoxide anion (O_2^-) production and secretion of azurophil granules also cause a small increment in cyclic AMP levels [8].



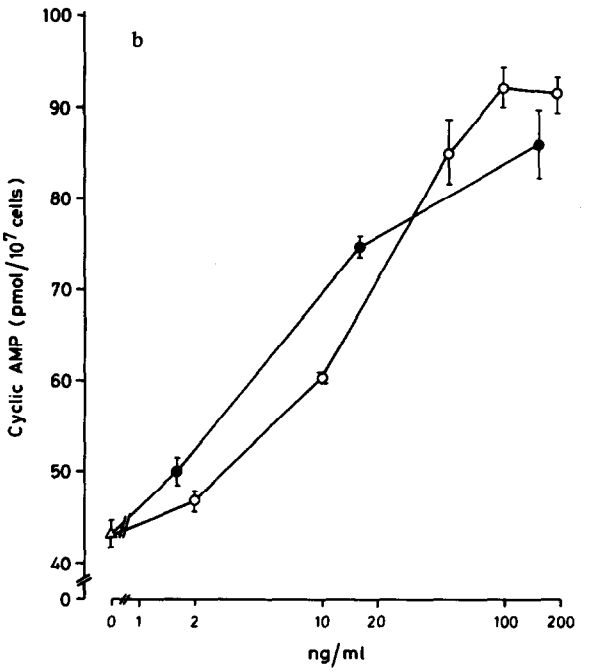


Fig.2. Effects of LTA_4 and LTB_4 on the levels of cyclic AMP in human PMNL: (a) Time-courses of the effects of LTA_4 (100 ng/ml) or LTB_4 (100 ng/ml) on the formation of cyclic AMP in the presence of RO 20-1724 (0.1 mM); (b) Dose–response curves showing the effects of LTA_4 and LTB_4 on cyclic AMP synthesis in the presence of RO 20-1724 (0.1 mM); (△) RO 20-1724; (●) LTA_4 + RO 20-1724; (○) LTB_4 + RO 20-1724. The cells were preincubated for 2 min with RO 20-1724 at 37°C. Thereafter the cells were incubated with LTA_4 or LTB_4 and analyzed for cyclic AMP content after indicated time (a) or 1 min (b). Each value represents the mean value ± SD from triplicate determination on each of 2 samples.

However, elevated cyclic AMP levels obtained after stimulation with various prostaglandins or phosphodiesterase inhibitors lead to inhibition in [10] of LTB_4 synthesis and in [8,18] of enzyme release. Thus, an increment of cyclic AMP levels seems to be associated both with stimulation and inhibition of granulocyte function. A possible explanation for this is that agents which activate neutrophils increase the levels of cyclic AMP in certain compartments [9].

LTB_4 has been shown to release prostaglandins from guinea pig lung [19]. Therefore, the effect of indomethacin and aspirin on the stimulatory action of LTB_4 on cyclic AMP formation was investigated. Human PMNL were incubated with indomethacin (1 μM) or aspirin (22 μM) in combination with RO 20-1724 (0.1 mM) for 2 min prior to the addition of LTB_4. In the absence of prostaglandin synthesis inhibitors, LTB_4 (100 ng/ml) increased the levels of cyclic AMP from 29.0 ± 0.04 to 56.2 ± 2.9 pmol/10^7 cells after 1 min. In the presence of indomethacin or aspirin, LTB_4 increased the cyclic AMP content to 50.7 ± 1.5 and 55.2 ± 3.1 pmol/10^7 cells, respectively, indicating that this effect on cyclic AMP was not mediated by prostaglandins.

Omega oxidation of LTB_4 leads to formation of 20-OH-LTB_4 and 20-COOH-LTB_4 [13] (fig.1). These compounds were less effective than LTB_4 in stimulating cyclic AMP formation. In the presence of RO 20-1724 (0.1 mM), LTB_4 (100 ng/ml) elevated the basal level of cyclic AMP, 31.3 ± 2.5 pmol/10^7 cells, to 70.3 ± 6.4 pmol/10^7 cells whereas 20-OH-LTB_4 (100 ng/ml) and 20-COOH-LTB_4 (100 ng/ml) increased the levels to 51.1 ± 1.7 and 45.5 ± 4.5 pmol/10^7 cells, respectively, after 1 min incubation.

This study suggests that cyclic AMP is associated with the biological effects of LTB_4 in human polymorphonuclear leukocytes. We are further investigating the relationship between cyclic AMP levels and degranulation in LTB_4-stimulated leukocytes.

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References

[1] Samuelsson, B., Hammarström, S., Murphy, R. C. and Borgeat, P. (1980) Allergy 35, 375–381.
[2] Goetzl, E. J. and Pickett, W. C. (1980) J. Immunol. 125, 1789–1791.
[3] Feinmark, S. J., Lindgren, J. Å., Claesson, H.-E., Malmsten, C. and Samuelsson, B. (1981) FEBS Lett. 136, 141–144.
[4] Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E. and Smith, M. J. H. (1980) Nature 286, 264–265.
[5] Malmsten, C., Palmblad, J., Udén, A.-M., Rådmark, O., Engstedt, L. and Samuelsson, B. (1980) Acta Physiol. Scand. 110, 449–451.
[6] Herlin, T., Petersen, C. S. and Esmann, V. (1978) Biochim. Biophys. Acta 542, 63–76.
[7] Simchowitz, L., Fischbein, L. C., Spilberg, I. and Atkinson, J. P. (1980) J. Immunol. 124, 1482–1491.
[8] Smolen, J. E. and Weissmann, G. (1981) Biochim. Biophys. Acta 672, 197–206.
[9] Pryzwansky, K. B., Steiner, A. L., Spitznagel, J. K. and Kapoor, C. L. (1981) Science 211, 407–410.
[10] Claesson, H.-E., Lundberg, U. and Malmsten, C. (1981) Biochem. Biophys. Res. Commun. 99, 1230–1237.
[11] Boyum, A. (1968) Scand. J. Clin. Lab. Invest. 21, 77–89.
[12] Lindgren, J. Å., Hansson, G. and Samuelsson, B. (1981) FEBS Lett. 128, 329–335.
[13] Hansson, G., Lindgren, J. Å., Dahlén, S.-E., Hedqvist, P. and Samuelsson, B. (1981) FEBS Lett. 130, 107–112.
[14] Hansson, G. and Rådmark, O. (1980) FEBS Lett. 122, 87–90.
[15] Gilman, A. G. (1970) Proc. Natl. Acad. Sci. USA 67, 305–312.
[16] Claesson, H.-E. (1980) Biochim. Biophys. Acta 618, 399–406.
[17] Jackowski, S. and Sha'afi, R. I. (1979) Mol. Pharm. 16, 473–481.
[18] Smolen, J. E., Korchak, H. M. and Weissmann, G. J. (1980) J. Clin. Invest. 65, 1077–1085.
[19] Sirois, P., Borgeat, P., Jeanson, A., Roy, S. and Girard, G. (1980) Prostagland. Med. 5, 429–444.