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LEUKOTRIENE FORMATION BY HUMAN POLYMORPHONUCLEAR LEUKOCYTES FROM ENDOGENOUS ARACHIDONATE

PHYSIOLOGICAL TRIGGERS AND MODULATION BY PROSTANOIDS

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Abstract—Human polymorphonuclear leukocytes (PMN) were isolated from freshly drawn venous blood by Dextran sedimentation and discontinuous Percoll gradient centrifugation. The effects of several putative triggers of the leukotriene formation such as C_{5a}, PAF, FMLP, C_{3a}, PMA, LTC_4, LTD_4, LTB_4 or arachidonate were studied by RP-HPLC analysis. 280 nM C_{5a}, 100 nM FMLP, 1 μM PAF or 20 μM arachidonate induced a marginal formation of 1.5–18 ng of LTB_4 plus LTB_4 metabolites/2×10^7 PMN. 560 nM C_{3a}, 100 nM PMA, 1 μM LTC_4, 1 μM LTD_4 and 1 μM LTB_4 each failed to induce any formation of 5-lipoxygenase products. Pretreatment of the cells with 40 μM ethylmercurithiosalicylate (merthiolate) enhanced the leukotriene formation by 100 nM FMLP about 40-fold, by 280 nM C_{3a} about 120-fold and by 1 μM PAF about 14-fold. Merthiolate itself induced no leukotriene formation from human PMN and reduced the leukotriene formation by 20 μM arachidonate. The FMLP/merthiolate-induced activation of the PMN was concentration-dependent in respect to both FMLP and merthiolate. 1 μM LTC_4, 1 μM LTD_4 or 1 μM LTB_4 also failed to trigger any LTB_4 formation of methiolate-treated PMN. 560 nM C_{3a} or 100 nM PMA in combination with 40 μM merthiolate induced a slight formation of 28 ng and 10 ng of LTB_4 plus LTB_4 metabolites, respectively. The FMLP/merthiolate-induced leukotriene formation was modulated by prostanoids. PGE_2, PGE_1, PGD_2 and 6-keto-PGE_1 each evoked a concentration-dependent inhibition of the leukotriene formation with IC_{50} values of 0.07 μM, 0.18 μM, 0.27 μM and 6 μM respectively. In addition, significant inhibitory effects by PGI_2, Iloprost (a carbacyclin analogue of prostacyclin), PGF_{2a} or 6-keto-PGF_{1a} were achieved; the corresponding IC_{50} values, however, amounted to 19–59 μM. Thus these compounds were about 500-fold less potent in comparison with PGE_2 in inhibiting LTB_4 formation by human PMN.

LTB_4 is a potent mediator involved in several inflammatory mechanisms, for instance chemotaxis [1–3], oxidative burst [4], degranulation and secretion of hydrolytic enzymes [5]. The human cells primarily forming LTB_4 are polymorphonuclear leukocytes (PMN)* [6–8]. Surprisingly, mainly the Ca^{2+} ionophor A-23187 or raised concentrations of *exogenous* arachidonic acid, both representing non-physiological triggers, have been described to provoke the formation of substantial amounts of LTB_4 by human PMN [6, 8, 9]. To elucidate the mechanisms essential for the LTB_4 formation by human PMN, we investigated the effects of FMLP, PAF, PMA, LTB_4, LTC_4, LTD_4, C_{3a}, C_{5a}, arachidonate and ethylmercurithiosalicylate (merthiolate). Merthiolate has been described in the literature to be an inhibitor of lysophosphatidylcholine–acyltransferase of several cell types [10, 11]. Furthermore we studied the effects of prostaglandins in this *in vitro* model and present data on the modulation of the LTB_4 formation by prostaglandins and prostacyclins.

* Abbreviations used: merthiolate, registered trademark for sodium ethylmercurithiosalicylate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HETE, hydroxyeicosatetraenoic acid; LT, leukotriene; PG, prostaglandin; PMN, polymorphonuclear leukocytes; RBL, rat basophilic leukemia; RP-HPLC, reversed phase high performance liquid chromatography; PMA, phorbol-12-myristate-13-acetate; PAF, platelet activating factor; C, complement factor; TNF, tumor necrosis factor; IFN, interferon; FMLP, *N*-formyl-methionyl-leucyl-phenylalanine; GM-CSF, granulocyte/macrophage colony-stimulating factor. *Enzymes*: lysophatidylcholine-acyltransferase (EC 2.3.1.23); 5-lipoxygenase (EC 1.13.11.34); proteinkinase C (EC 2.7.1.37).

MATERIALS AND METHODS

Materials

Arachidonic acid, prostaglandins, FMLP, merthiolate and PMA were purchased from Sigma (München, F.R.G.). Cytochrome *c* from horse heart was obtained from Serva (Heidelberg, F.R.G.), and dextran T500 and Percoll were obtained from Deutsche Pharmacia (Freiburg, F.R.G.). Solvents and chemicals used were of HPLC or analytical grade and obtained from Merck (Darmstadt, F.R.G.). Leukotrienes, HETEs and HPETEs were from Paesel (Frankfurt/Main, F.R.G.). Pure bovine Cu/Zn-superoxide dismutase was a product from Grünenthal GmbH (Aachen, F.R.G.). Porcine C_{5a} and C_{3a} were kindly provided by Dr Dammerau, MPI (Göttingen, F.R.G.) and Iloprost was a gift from Schering AG (Berlin, F.R.G.). C_{16}-PAF (racemate) was a gift from Natterman GmbH (Cologne, F.R.G.). RBL-1-cells were cultured by Dr Sous, Grünenthal GmbH (Aachen, F.R.G.).

Methods

Preparation of PMN. Fresh venous blood from healthy volunteers was supplemented with 20 mM EDTA. PMN were obtained by Dextran sedimentation and a modified Percoll gradient method as described in [12, 13]. Red blood cells were sedimented after addition of 0.5 vol. of 6% Dextran (w/v) in saline for 60 min at 4°. The resulting supernatant was centrifuged at 350 *g* for 10 min and the cell pellet was resuspended in isotonic sucrose (3 vol. 0.25 M sucrose in water and 1 vol. 3.8% trisodium citrate adjusted with 1 N HCl to pH 7.3) and layered on top of a discontinuous Percoll gradient. The gradient was performed by dilution of buffer A [one part (w/w) of 2.27 M sucrose dissolved with heating in 177 mM triethylamine and pH adjusted with concentrated phosphoric acid to pH 6.8 was mixed with 8.5 parts (w/w) Percoll and finally adjusted to pH 7.3 with 2 N HCl] with isotonic sucrose to obtain 5 ml fractions of 80, 65, 60, 50, 45 and 30% buffer A from bottom to top in 2.7 cm wide centrifugation tubes. Density gradient centrifugation was carried out at 750 *g* for 40 min at 4°. The separated cells banded at the interface of 50 and 60% buffer A were sucked off and washed twice by suspending the cells in buffer B [Hank's balanced salt solution pH 7.4 containing 6 g/l Hepes without Ca^{2+} and Mg^{2+}] and centrifugation at 350 *g* for 10 min. Finally the cells were resuspended in buffer B and adjusted to 10^7 cells/ml. The PMN purity measured by staining with May–Grünwald/Giemsa was >99% and the viability measured by exclusion of Trypan Blue was >99%.

Incubation conditions and sample preparation. Aliquots of 2 ml cell suspension (10^7 cells/ml) were preincubated for 2 min at 37° with 40 μM of merthiolate and in experiments concerning inhibition by prostaglandins for a further 2 min with prostaglandins at concentrations as indicated in the text. $CaCl_2$ (2 mM) and $MgSO_4$ (0.8 mM) were next added followed by addition of agonists as indicated in the text. After incubation for 15 min, 100 ng PGB_2 was added as an internal standard and the incubations were stopped by addition of 4 ml ethanol and 50 μl of acetic acid to adjust pH 3. The supernatants obtained by centrifugation at 2000 *g* for 5 min were diluted with H_2O to 10% ethanol and applied to 0.5 ml ODS-C18 columns (Analytichem International, Harbor City, CA, U.S.A.). After washing the columns with 5 ml of 10% ethanol and 2 ml H_2O, arachidonic acid metabolites were eluted with 1 ml methanol. Experiments were performed in duplicates with 3–9 cell preparations from different donors. Molar concentrations of C_{5a} or C_{3a} were calculated assuming a molecular mass of 9000 for each.

RP–HPLC analysis of arachidonic acid metabolites. HPLC was performed at room temperature on 25 × 0.3 cm columns filled with 5 μm Spherisorb ODS 2 (Bischoff, Leonberg, F.R.G.) using a linear gradient from 100% solvent A [methanol/acetonitrile/water/phosphoric acid/acetic acid/ammonia; 20/20/60/0.3/0.2 to adjust pH 3.9; v/v/v/v/v] to 100% solvent B [methanol/acetonitrile/water/phosphoric acid/acetic acid/ammonia; 35/35/30/0.3/0.2 to adjust pH 3.9; v/v/v/v/v] in 40 min and then in isocratic mode at 100% solvent B for 15 min at a flow rate of 1 ml/min and on-line monitoring at 237 nm and 270 nm. Low pressure gradient former, HPLC pump and variable wavelength UV detector were from Gynkotek (München, F.R.G.). Retention times (min) of synthetic reference standards used were: ω-COOH-LTB_4, 9.3; ω-OH-LTB_4, 10; LTC_4, 23.3; PGB_2, 24.7; LTD_4, 27.9; 5*S*,12*R*-[E,E,E,Z]-LTB_4, 29.2; 5*S*,12*S*-[E,E,E,Z]-LTB_4, 30.2; 5*S*,12*R*-[Z,E,E,Z]-LTB_4, 31.6; 5*S*,12*S*-[E,Z,E,Z]-DiHETE, 32.4; 5*S*,6*S*-DiHETE 39.7; 5*S*6*R*-DiHETE, 40.7; 15-HETE, 45.2; 15-HPETE, 46.4; 12-HETE, 47.2; 12-HPETE, 48.2; 5-HETE, 49.3; 5-HPETE, 50.4.

The chromatograms were calculated by means of a CR-3A integrator (Gynkotek, München, F.R.G.) and standard curves for LTB_4, ω-OH-LTB_4, ω-COOH-LTB_4, 5-HETE and PGB_2. Evaluation of results was performed by summing up the amounts of ω-COOH-LTB_4, ω-OH-LTB_4, LTB_4 and 6-trans-LTB_4 isomers. Results were expressed as mean ± SE and the number of experiments.

Assay of 5-lipoxygenase activity. 5-lipoxygenase activity of 10,000 *g* supernatants from RBL-1 cell homogenates was estimated using a polarographic assay in the presence of 4 mM ATP, 3 mM GSH and 75 μM arachidonic acid as described [14].

Chemotaxis. Chemotaxis of human PMN was measured by the Boyden chamber technique [15] using blind well chambers and polycarbonate chemotaxis membranes with 5 μm pores (Nuclepore, Pleasanton, U.S.A.). Chemotactic indices were expressed by the number of migrated cells in the presence of 10 nM FMLP in the lower chamber compartment divided by the number of spontaneously migrated cells. The numbers of migrated cells were estimated by the lower surface method [16].

Superoxide formation. Superoxide formation of PMN was assayed as described by means of superoxide-dependent cytochrome *c* reduction [17]. Specificity of cytochrome *c* reduction was checked by corresponding controls containing 50 μg/ml bovine superoxide dismutase. An extinction coefficient for reduced cytochrome *c* at 546 nm of 21.1 mM^{-1} cm^{-1} was used.

RESULTS

Leukotriene formation by PMN

The employed PMN isolation provided human PMN free of contamination with other blood cells, as was deduced from May–Grünwald/Giemsa staining and from the absence of any 12-HETE and cysteinoleukotrienes. Various putative physiological agonists such as C_{3a}, PMA, LTC_4, LTD_4 and LTB_4 did not effectively induce any leukotriene formation by PMN. Just slight amounts of LTB_4 plus LTB_4 metabolites were detected by RP–HPLC after stimulation with FMLP, C_{5a}, PAF or arachidonate. The amounts of LTB_4 plus LTB_4 metabolites formed per 2×10^7 PMN amounted to 14 ng at 100 nM FMLP, to 1.5 ng at 1 μM PAF, to 7 ng at 280 nM C_{5a} and to 18 ng at 20 μM arachidonate (Table 1). Considering the specific activities of these compounds, C_{5a} and FMLP were most potent. The main arachidonate metabolite analyzed by RP–HPLC was ω-OH-LTB_4;

Table 1. Effect of nine putative triggers and merthiolate on the PMN leukotriene formation

Merthiolate	FMLP (10^{-7} M)	C_{5a} (2.8×10^{-7} M)	C_{3a} (5.6×10^{-7} M)	PAF (10^{-6} M)	PMA (10^{-7} M)	AA (20 μM)	LTC_4 (10^{-6} M)	LTD_4 (10^{-6} M)	LTB_4 (10^{-6} M)
—	14.3 ± 4.3 N = 4	6.7 ± 1.4 N = 2	— N = 2	1.5 ± 0.7 N = 5	— N = 4	18 ± 7.3 N = 7	— N = 3	— N = 3	— N = 3
	52 ± 20* N = 3								
40 μM	620 ± 90 N = 10	822 ± 180 N = 4	28 ± 13.5 N = 2	21 ± 11.3 N = 4	9.7 ± 4.9 N = 4	2.3 ± 0.5 N = 4	— N = 3	— N = 3	— N = 5

2×10^7 PMN were preincubated with 40 μM merthiolate or vehicle as indicated for 5 min and subsequently stimulated for 15 min with triggers as indicated. Incubation conditions and RP-HPLC were performed as described in Materials and Methods. Data were expressed as ng LTB_4 plus LTB_4 metabolites/2×10^7 PMN. The means and SE with the number of experiments were quoted. * indicates the pretreatment of the PMN with 5 μg/ml cytochalasin B for 5 min.

only minor amounts of LTB_4 and 5-HETE were detectable.

The slight leukotriene formation by 100 nM FMLP was somewhat enhanced by pretreatment of the PMN with 5 μg/ml cytochalasin B, leading to 52 ng/2×10^7 cells of LTB_4 plus LTB_4 metabolites. Treatment of the PMN with 560 nM C_{3a}, 1 μM LTC_4, 1 μM LTD_4 or 100 nM PMA did not induce any formation of 5-lipoxygenase products detectable by RP-HPLC. In addition 1 μM LTB_4 failed to trigger the formation of 5-HETE which represented, in this instance, the sole 5-lipoxygenase product evaluable. In order to exclude any impairment of the PMN used further cellular functions such as LTB_4-induced chemotaxis and PMA-induced oxygen burst were studied. With 25 nM PMA a superoxide formation of 32 ± 1.4 nmoles $O_2^-/2 \times 10^6$ cells/15 min (mean ± SE, N = 4) was measured. The LTB_4-induced chemotaxis revealed optimal chemotactic activity of the PMN at 50 nM LTB_4. The corresponding chemotactic indices amounted to 6.6 ± 0.8 (mean ± SE, N = 6). Thus the failure of LTB_4 and PMA in triggering the PMN leukotriene formation was not due to any general impairment of the PMN.

Treatment of the PMN with 40 μM merthiolate in the absence of further triggers failed to activate any leukotriene formation. Furthermore under this condition the slight leukotriene formation induced by 20 μM arachidonate was inhibited by 90%. In addition merthiolate treatment of the PMN showed no influence on the failure of 1 μM LTD_4, 1 μM LTC_4 or 1 μM LTB_4 in exerting any leukotriene formation (Table 1).

The formation of LTB_4 plus LTB_4 metabolites by FMLP, C_{5a} or PAF was however substantially enhanced when the PMN were pretreated with 40 μM merthiolate. With 100 nM FMLP as a trigger, the leukotriene formation was increased approximately 40-fold corresponding to 620 ng/2×10^7 cells (Table 1). The increase in the leukotriene formation was dependent on the concentration of merthiolate. The maximal effect was obtained at 40 μM merthiolate; but the leukotriene formation decreased by about 20% with concentrations increasing up to 100 μM merthiolate (Fig. 1). The dose–response curve of merthiolate depended on the PMN count per ml, being left-shifted at lower PMN counts. 5-Lipoxygenase activity of a 10,000 *g* supernatant from RBL-1 cell homogenate was also inhibited up to 20% at 100 μM merthiolate in respect to the control (Fig. 2). Thus the decrease in the FMLP/merthiolate-induced leukotriene formation at merthiolate concentrations beyond 60 μM is most likely due to a direct inhibition of 5-lipoxygenase activity. The PMN leukotriene formation by merthiolate and a second triggering signal was abolished when Ca^{2+} was omitted from the incubations.

Expectedly the FMLP/merthiolate-induced leukotriene formation was concentration-dependent on FMLP. At a fixed concentration of 40 μM merthiolate already 1 nM FMLP induced the formation of RP-HPLC detectable amounts of LTB_4 plus LTB_4 metabolites. The maximum leukotriene production was obtained at 0.1–1 μM FMLP (Fig. 3). Thus the formation of 5-lipoxygenase products was enhanced by merthiolate with regard to both the total amount

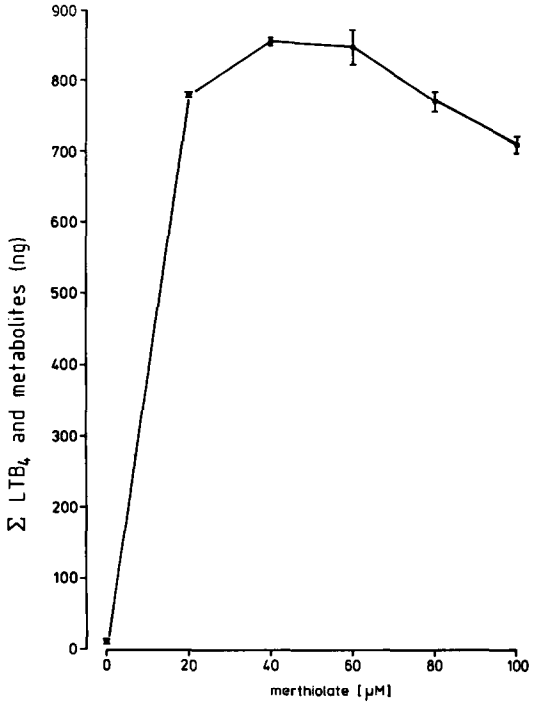

Fig. 1. Effect of merthiolate on the FMLP-induced leukotriene formation. 2×10^7 PMN were preincubated with merthiolate for 5 min at concentrations indicated and subsequently triggered for 15 min with 100 nM FMLP. Incubation conditions and RP-HPLC were performed as described in Materials and Methods. The means and SE from triplicates were plotted.

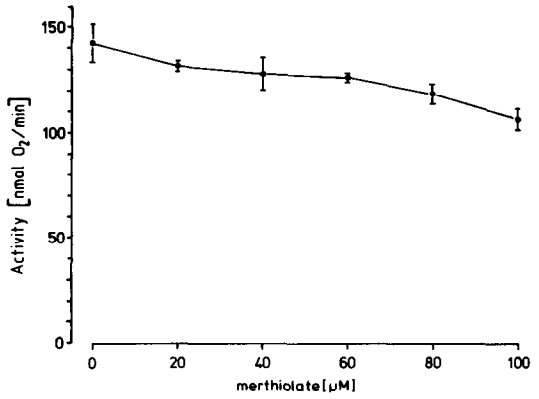

Fig. 2. Effect of merthiolate on 5-lipoxygenase activity. 75 μM arachidonic acid, 4 mM ATP, 3 mM GSH and merthiolate at concentrations indicated were incubated with 10,000 *g* supernatant of RBL-1 cell homogenate (2.1 mg prot./ml). 5-Lipoxygenation of arachidonate was started by addition of 3 mM $CaCl_2$ and the initial velocities of oxygen consumption were determined. The means and SD from triplicates were plotted.

of leukotrienes formed and the FMLP concentrations needed to trigger the effect.

The arachidonate metabolites analysed by RP-HPLC upon stimulation of PMN with merthiolate

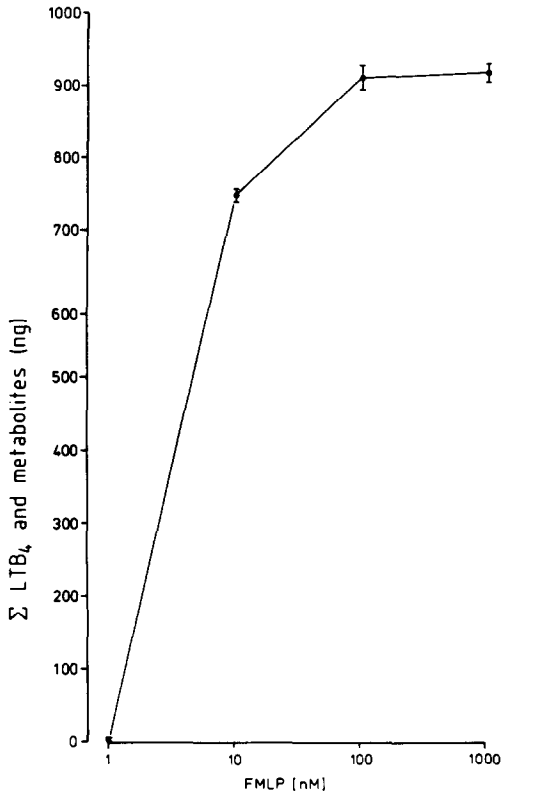

Fig. 3. FMLP-dependence of the leukotriene formation by merthiolate-treated PMN. 2×10^7 PMN were preincubated with 40 μM merthiolate for 5 min and subsequently stimulated for 15 min with FMLP at concentrations indicated. Incubation conditions and RP-HPLC were performed as described in Materials and Methods. The means and SE from triplicates were plotted.

and a second triggering signal were identical regarding the qualitative evaluation. ω-OH-LTB_4, LTB_4 and 5-HETE represented the main products besides smaller amounts of ω-COOH-LTB_4 and the non-enzymatically formed decomposing products of LTA_4: 5,6-DiHETEs and 6-trans-LTB_4 isomers. Only slight amounts of 5*S*,12*S*-DiHETE and no 15-HETE, 12-HETE or cysteino-leukotrienes were detectable (Fig. 4).

The leukotriene formation induced by 280 nM C_{5a} was enhanced by 40 μM merthiolate approximately 120-fold amounting to 822 ng/2×10^7 cells of LTB_4 plus LTB_4 metabolites. Moreover 560 nM C_{3a} induced the formation of 28 ng/2×10^7 cells of LTB_4 plus LTB_4 metabolites under these conditions. The experiments with C_{5a} and C_{3a} as triggers were performed with PMN from the same PMN preparations. Thus the results clearly distinguished C_{5a} from C_{3a} because of a 30-fold enhanced leukotriene formation even at a lower trigger concentration. Forty μM merthiolate also increased the leukotriene formation by 1 μM PAF to 21 ng/2×10^7 cells of LTB_4 plus LTB_4 metabolites and even provoked a weak production of 10 ng/2×10^7 cells of LTB_4 plus LTB_4 metabolites by stimulation with 100 nM PMA. Again, in respect to the specific activity, C_{5a} and

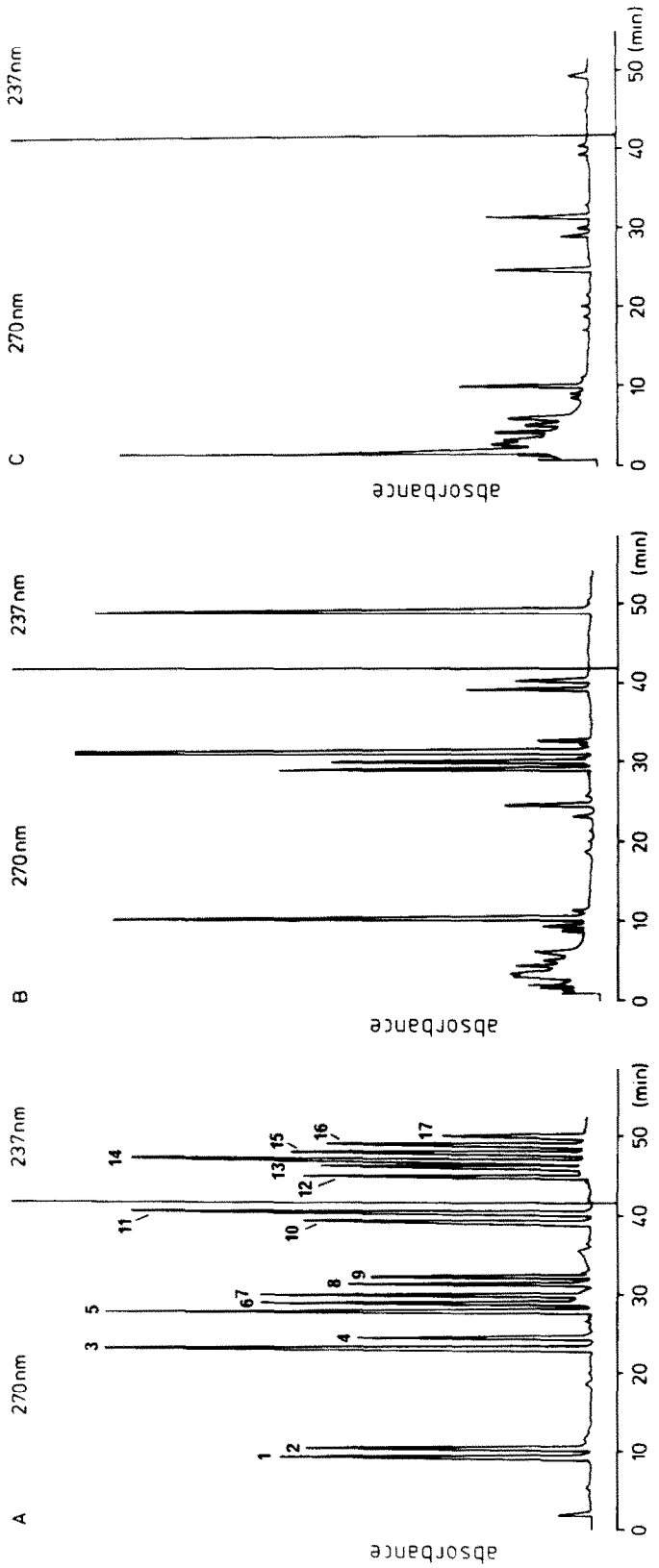

Fig. 4. RP-HPLC analysis of arachidonate metabolites from PMN. (A) Separation of reference compounds. 1: ω-COOH-LTB$_4$; 2: ω-OH-LTB$_4$; 3: LTC$_4$; 4: PGB$_2$; 5: LTD$_4$; 6: 5*S*,12*R*-[*E*,*E*,*E*,*Z*]-LTB$_4$; 7: 5*S*,12*S*-[*E*,*E*,*E*,*Z*]-LTB$_4$; 8: 5*S*,12*R*-[*Z*,*E*,*E*,*Z*]-LTB$_4$; 9: 5*S*,12*S*-[*E*,*Z*,*E*,*Z*]-DiHETE; 10: 5*S*,6*S*-DiHETE; 11: 5*S*6*R*-DiHETE; 12: 15-HETE; 13: 15-HPETE; 14: 12-HETE; 15: 12-HEPTE; 16: 5-HETE; 17: 5-HPETE. (B) 2×10^7 PMN were preincubated with 40 μM merthiolate for 5 min and then triggered for 15 min with 100 nM FMLP. (C) 2×10^7 PMN were preincubated for 5 min with 40 μM merthiolate and subsequently for further 2 min with 2 μM PGE$_2$, followed by activation for 15 min by 100 nM FMLP. Incubation conditions and RP-HPLC were performed as described in Materials and Methods. At a retention time of 42 min the detection wavelength was readjusted to 237 nm.

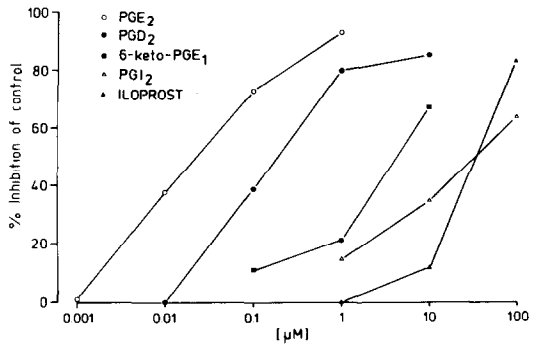

Fig. 5. Inhibition by four prostaglandins and Iloprost of the FMLP/merthiolate-induced leukotriene formation. 2×10^7 PMN were preincubated for 5 min with 40 μM merthiolate and subsequently for 2 min with prostanoids at concentrations indicated, followed by activation for 15 min by 100 nM FMLP. Incubation conditions and RP-HPLC were performed as described in Materials and Methods. The means from duplicates were plotted.

FMLP were the most potent triggers followed by C_{3a} and PAF. This data elucidates that merthiolate is able to unmask the ability of some stimulants like PMA or C_{3a} in inducing the leukotriene formation, but does not alter the relative efficacy of such agonists.

Effects of prostanoids

For these investigations the leukotriene formation by merthiolate-treated human PMN was induced by 100 nM FMLP. The mean formation of 620 ng of LTB_4 plus LTB_4 metabolites permitted a reliable evaluation by RP–HPLC.

PGE_2, PGD_2 and PGE_1 exerted significant and concentration-dependent inhibitory effects at nanomolar concentrations (Fig. 5). PGE_2 was found to be the most potent inhibitor of the FMLP/merthiolate-induced leukotriene formation. It is evident from the HPLC analysis that the inhibition of the leukotriene formation by PGE_2 was paralleled by the inhibition of the 5-HETE formation to nearly the same extent (Fig. 4). However the inhibition of the 5-HETE formation was excluded from the determination of IC_{50} values since 5-HETE can be reacylated to some extent into phospholipids of PMN [18]. The IC_{50} value for PGE_2 amounted to 70 ± 21 nM ($N = 4$) and about 90–100% inhibition was achieved at 2 μM PGE_2. PGE_1 and PGD_2 also were potent inhibitors with IC_{50} values of 178 ± 69 nM ($N = 6$) and 270 ± 161 nM ($N = 5$), respectively (Fig. 6).

PGI_2, Iloprost, 6-keto-PGF_{1a} or PGF_{2a} likewise evoked significant and concentration-dependent inhibitory effects on the leukotriene formation. However, the dose–response curves for these compounds were shifted towards higher concentrations when compared with those for PGE_2, PGD_2 and PGE_1 (Fig. 5). The IC_{50} values amounted to 59 ± 16 μM ($N = 4$) for PGI_2, to 45 ± 11 μM ($N = 6$) for the carbacyclin analogue Iloprost, to 42 ± 11 μM ($N = 3$) for 6-keto-PGF_{1a} and to 19 ± 2 μM ($N = 3$) for PGF_{2a} (Fig. 6). Regarding the IC_{50} values, these compounds were about 500-fold less potent than PGE_2. 6-Keto-PGE_1, a PGI_2 metabolite, proved to be somewhat more potent than prostacyclin or 6-keto-PGF_{1a}. The IC_{50} value for this compound amounted to 6 ± 2.5 μM ($N = 9$). The improved inhibitory potency of 6-keto-PGE_1 as compared to PGI_2 and 6-keto-PGF_{1a} was significant with $P < 0.01$.

DISCUSSION

In this study the evaluation of the PMN leukotriene formation by RP–HPLC was preferred to a radioimmunoassay since LTB_4 is rapidly metabolised by PMN, leading to the formation of substantial amounts of ω-OH-LTB_4 (Fig. 4) which is not detectable by LTB_4 radioimmunoassay. To compensate for the confined sensitivity of RP–HPLC (the detection limits were at 0.5 ng LTB_4) the PMN count was increased to 2×10^7 per incubation.

The data on stimulated leukotriene formation by PMN as presented in this paper reveal that only minor amounts of LTB_4 plus LTB_4 metabolites were formed upon activation by C_{5a}, FMLP, PAF or arachidonate. Moreover, with arachidonate *or* PAF this poor leukotriene formation was only achieved at concentrations which may hardly be considered physiological. The results so far agree with published data [9, 19–22].

Pretreatment of the PMN with 40 μM merthiolate enhanced the leukotriene formation which, however, remained strictly dependent on a second triggering signal. The ranking orders of potencies of the several triggers observed in the absence of merthiolate was not altered by merthiolate, although the amounts of leukotrienes thus formed were increased 120-fold for C_{5a} and 40-fold for FMLP. The ability of human PMN to form leukotrienes upon stimulation by physiological mediators therefore appears to be cryptic, and merthiolate somehow primes the PMN for this cellular function. The PMN priming by merthiolate not only increased the maximum amounts of leukotrienes formed but also substantially lowered the threshold concentration of FMLP (Fig. 3). The slight decrease in the leukotriene formation obtained at high merthiolate concentrations could be attributed to an inhibition of 5-lipoxygenase activity itself by means of control experiments with a cell-free system. PMN priming by merthiolate presumably mimics physiological regulation mechanisms since it solely enhanced the leukotriene formation by distinct triggers and lowered the threshold concentration of FMLP required for PMN activation to physiological levels. Merthiolate has been described to inhibit lysophosphatidylcholine-acyltransferase in several cell types [10, 11]. In our study, however, rather a decrease in the PMN leukotriene formation induced by 20 μM arachidonate was observed at merthiolate concentrations which were optimal for the leukotriene formation triggered by other agonists. This result suggests that for human PMN the inhibition of arachidonate reacylation by merthiolate is of subordinate importance. Therefore other mechanisms of PMN priming, e.g. the modulation of cytosolic Ca^{2+} levels, have to be taken into account [23].

The failure of LTB_4, LTC_4 or LTD_4 to induce any leukotriene formation even in combination with merthiolate excludes that these mediators might rep-

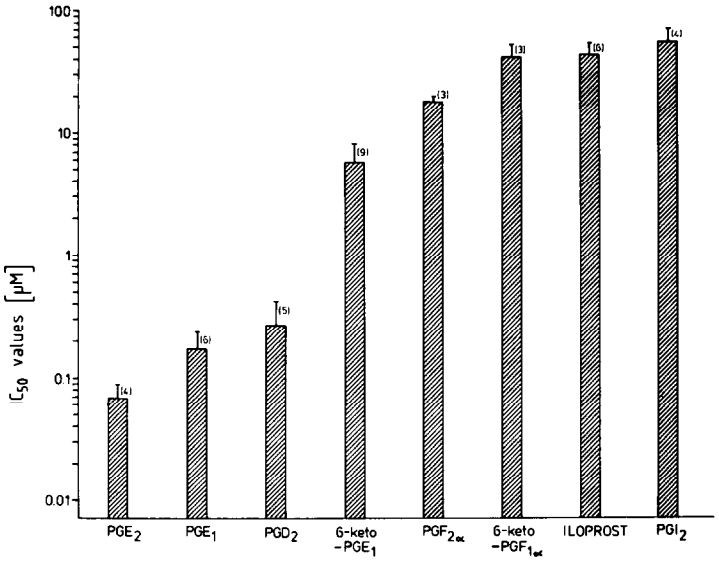

Fig. 6. IC_{50} values of eight prostanoids of the FMLP/merthiolate-induced leukotriene formation by PMN. 2×10^7 PMN were preincubated for 5 min with 40 μM merthiolate and subsequently for 2 min with prostanoids at concentrations from 1 nM to 100 μM, followed by activation for 15 min by 100 nM FMLP. IC_{50} values were estimated from the number of experiments indicated, bars represent SE. Incubation conditions and RP-HPLC were performed as described in Materials and Methods.

resent the physiological triggers of this PMN function. This interpretation is supported by appropriate LTB_4-induced chemotaxis of the PMN preparation investigated. Thus an amplification system as known for thromboxane formation by platelets does not apply to leukotriene formation by PMN.

Likewise PMA even in combination with merthiolate only induced a marginal LTB_4 formation. In contrast, PMA as a direct activator of protein kinase C effectively triggered the respiratory burst in PMN. It is therefore tempting to conclude that activation of protein kinase C is not a prerequisite for leukotriene formation as it is for superoxide production. Respiratory burst and leukotriene formation can obviously be regulated independently. This view is further supported by lack of efficacy of other protein kinase C activators in triggering leukotriene formation by PMN [19].

FMLP, PAF or C_{5a} have been reported to adequately induce LTB_4 synthesis by PMN [24–28]. However, in these studies either non-human PMN were used, or the PMN preparations did not exclude contaminations by monocytes, lymphocytes and platelets. In the latter instance a modulation of leukotriene formation by further mediators or interleukines may be envisaged. The interpretation of published data is further complicated by the methodological approach to estimate total leukotriene biosynthesis by radioimmunoassay which proved unreliable due to both, by its failure to detect LTB_4 metabolites and its crossreactivity with HETEs derived from monocytes or platelets.

Other reports emphasize the requirement of exogenous arachidonic acid for leukotriene formation by PMN in addition to inflammatory mediators as FMLP or C_{5a} [9, 19, 22]. Further arachidonate from platelets has been suggested to be utilized by PMN leukotriene formation [29]. According to our data merthiolate-primed PMN clearly respond to FMLP, C_{5a}, C_{3a} or PAF with formation of LTB_4 in the absence of any exogenous arachidonate. PMN are thus able to generate LTB_4 from their own arachidonate pools, but only if adequately primed. GM-CSF [30], TNF, IFN-γ [31, 32] or other cytokines may be discussed to physiologically prime PMN for optimum responsiveness.

The FMLP merthiolate-induced leukotriene formation is considered a helpful model to study receptor-mediated PMN response and its modulation, as PMN possess FMLP receptors [33, 34]. The inhibition of the leukotriene formation by PGE_2 or PGE_1 with IC_{50} values of 70 nM and 180 nM, respectively, agrees with results published for FMLP/cytochalasin B-induced leukotriene formation by human and rat PMN [35]. In contrast PGD_2 was reported to inhibit leukotriene formation by rat PMN with an IC_{50} value of 70 μM [35] as opposed to 270 nM for human PMN in this study. The 260-fold difference in the IC_{50} values between both species may be due to different specificities of the PGE_2 receptor of human and rat PMN or otherwise may indicate that human PMN, in contrast to rat PMN, possess PGD_2 receptors. Since it has been reported that PGE_1 increases cytosolic c-AMP levels of human PMN [36], this second messenger is likely to be involved in the inhibition of PMN LTB_4 synthesis by PGE_1, as well as by PGE_2 or PGD_2.

The 600–800 fold difference in the IC_{50} values of PGE_2 and prostacyclin or Iloprost questions the physiological importance of prostacyclin receptors in inhibiting leukotriene formation and rather indicates that the effects of the prostacyclins described here were exerted by unspecific actions on the PGE_2 receptor. This suggestion is strengthened by the stronger inhibitory effect of the prostacyclin metabolite 6-keto-PGE_1 which is structurally closer to the

E-type prostaglandins than to prostacyclin.

FMLP-induced respiratory burst of human PMN has also been reported to be modulated by prostaglandins. Again PGE_2, PGE_1 or PGD_2 proved to be much stronger inhibitors than Iloprost or PGI_2 [37]. Yet other prostacyclin analogues and PGI_2 itself were shown to evoke concentration-dependent inhibition of superoxide release [38, 39] and the distance of efficacies between E-type and I-type prostanoids in the suppression of respiratory burst appeared to be less pronounced [39] when compared to the respective effects on the leukotriene formation.

As discussed, leukotriene formation and superoxide release, though mediated by identical receptors, involve in part different activation processes. The similarity in the modulation of the different PMN functions favour the conclusion that common second messengers of the prostaglandins affect common steps of the activation processes. It remains conceivable, however, that differential second messenger responses induced by the different types of prostaglandins characteristically modulate the various PMN responses.

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