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Studies on the uptake, binding and metabolism of leukotriene B_4 by human neutrophils

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SUMMARY

Human polymorphonuclear granulocytes (PMN) generate the inflammatory mediator leukotriene B_4 (LTB_4) as a response to cell activation. In addition, PMN inactivate LTB_4 by ω-oxidation resulting in the formation of 20-OH- and 20-COOH-LTB_4. The transport of exogenous LTB_4 to the metabolizing enzymes is mediated via high- and low-affinity receptor subsets. Uptake of [^{3}H]LTB_4 by the cells was carried out in a time-dependent fashion, reaching maximal values after 5 min of incubation. No additional uptake of [^{3}H]LTB_4 then occurred. Prestimulation of PMN with phorbol myristate acetate or sodium fluoride resulted in the loss of high- and low-affinity receptors. Deactivating concentrations of LTB_4 specifically reduced the high-affinity receptor subset. Prestimulation of PMN with cytochalasin B or with the membrane fluidizer butanol shifted the low-affinity receptors to the high-affinity state. The polyene antibiotic amphotericin B shifted high-affinity receptors to the low-affinity subset. The changes in the receptor expression pattern correlated with the respective conversion rate of exogenously added LTB_4. Our results suggest that the distribution of high- and low-affinity receptors is regulated by GTP-binding proteins, the activation of protein kinase *C* and the organization of the membrane bilayer. In this way, human neutrophils control the respective level of the lipid mediator LTB_4.

INTRODUCTION

Polymorphonuclear granulocytes (PMN) provide a major role during host defence against bacterial infections. In this way, human PMN generate and release the lipid mediator leukotriene B_4 (LTB_4) as a consequence of cell activation (Borgeat & Samuelsson, 1979). LTB_4 reveals pronounced biological actions such as vasoactive effects of chemotactic activities for neutrophils and eosinophils. Therefore, LTB_4 was described as a fundamental mediator for the pathophysiology of inflammation, ischemia and shock (Bray, 1983; Lefer, 1986). The level of LTB_4 is regulated by metabolization via the ω-oxidation pathway, resulting in the formation of 20-OH-LTB_4 and 20-COOH-LTB_4. These metabolites show reduced biological activities compared to LTB_4, or are totally inactive (Hansson *et al.*, 1981).

Abbreviations: AmB, amphotericin B; HPLC, high-pressure liquid chromatography; LTB_4, leukotriene B_4; NaF, sodium fluoride; PBS, phosphate-buffered saline; 4α-PDD, 4α-phorbol-12,13-dideconate; PMA, phorbol 12-myristate 13-acetate; PMN, polymorphonuclear granulocytes.

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Neutrophils respond to LTB_4 or chemotactic peptides such as f-met-leu-phe via specific receptors localized on the cell surface (Goldman & Goetzl, 1982; Kreisle & Parker, 1983; Aswanikumar *et al.*, 1977; Williams *et al.*, 1977). Previously, the existence of two receptor subclasses were described mediating different biological responses of the cell (Goldman & Goetzl, 1984; Koo, Lefkowitz & Snyderman, 1982; Mackin, Huang & Becker, 1982). The high-affinity receptor subset transmits the adherence of the neutrophils to endothelial walls and their chemotactic migration. Ligand interaction with the low-affinity subset results in the degranulation response. The receptor affinity state is regulated by guanine nucleotide-binding proteins (G-proteins). In this way, the receptor subsets of isolated membranes are interconvertible by guanine nucleotides (Koo, Lefkowitz & Snyderman, 1983; Snyderman *et al.*, 1984). Therefore, a portion of the high affinity LTB_4 receptor was converted to the low-affinity state after the addition of GTP or GTP-analogues (Sherman, Goetzl & Koo, 1988). Furthermore, an experimental approach for receptor affinity shifting was described for the oligopeptide binding site on human neutrophils. Aliphatic alcohols transformed the low-affinity receptors to the high-affinity class; the vice versa effect was observed using the polyene antibiotic amphotericin B (Liao & Freer, 1980; Yuli, Tomonaga & Snyderman, 1982; Lohr & Snyderman, 1982).

Recently, we demonstrated that LTB_4 transport to the metabolizing enzymes was mediated via specific receptors

(Brom, Schönfeld & König, 1988b). It was the purpose of the study to analyse the uptake of LTB_4, the conversion of LTB_4 by PMN as well as changes in the distribution of receptor expression induced by defined compounds.

MATERIAL AND METHODS

Materials

Leukotriene standards (20-COOH-LTB_4, 20-OH-LTB_4, LTB_4) were generously provided by Dr Rokach (Merck Frosst, Pointe Claire-Dorval, Canada). [14,15-^{3}H]LTB_4, specific activity 1·11–2·22 TBq/mmol, was supplied by New England Nuclear, Dreieich. Ficoll 400 was from Pharmacia, Uppsala, Sweden; Macrodex (6%) was from Schiwa, Glandorf; metrizoate (75% w/v) was from Nycomed, Oslo, Norway. Phorbol-12-myristate 13-acetate, 4α-phorbol-12,13-dideconate, sodium fluoride, amphotericin B, cytochalasin B, NADH, pyruvate and heparin were purchased from Sigma, Deisenhofen. Bovine serum albumin was obtained from Boehringer, Mannheim. Rotiszint 2211 was from Roth, Karlsruhe. n-butanol and the organic solvents were supplied by J. T. Baker Chemicals, Deventer, The Netherlands. All other chemicals were from Merck, Darmstadt.

Purification of human neutrophils

Human neutrophils were prepared from heparinized peripheral blood of healthy donors using a Ficoll-metrizoate gradient and dextran sedimentation (Böyum, 1968). Contaminating erythrocytes were removed by hypotonic lysis, platelets by repeated centrifugation at 300 ***g*** (Minifuge T, Heraeus-Sepatech, Osterode). The purified cell fraction contained more than 95% PMN. All experiments were carried out in Dulbecco's phosphate-buffered saline (PBS) without calcium and magnesium. The pH was adjusted to a value of 7·4.

Uptake of [^{3}H]LTB_4

Uptake of [^{3}H]LTB_4 by neutrophils was determined using 1×10^7 cells (in a final volume of 0·5 ml PBS-buffer) and 5 nM [^{3}H]LTB_4. The incubations were performed over the indicated time periods in the presence of physiological concentrations of Ca^{2+} and Mg^{2+} at 37°. The reactions were stopped by the addition of one volume of ice-cold buffer containing 0·5 μM non-labelled LTB_4 in order to displace the dissociable amount of LTB_4 binding from PMN. Associated [^{3}H]LTB_4 was separated from non-associated [^{3}H]LTB_4 by short-time centrifugation at 9600 ***g*** (Biofuge A, Heraeus-Sepatech, Osterode), or alternatively by vacuum filtration as described for the binding assay. However, both separation methods led to similar results.

Incubation conditions

Purified PMN were prestimulated with the following agents: LTB_4 (1 nM) for 10 min, phorbol-12-myristate-13-acetate (PMA; 0·23 μM) for 10 min, sodium fluoride (NaF; 20 mM) for 10 min, *n*-butanol (0·25%) for 15 min, amphotericin B (AmB; 2 μM) for 30 min and cytochalasin B (15 μM) for 60 min. Preincubation was carried out in the presence of calcium (0·9 mM) and magnesium (0·5 mM) at 37° with the exception of butanol-prestimulation, which was performed at room temperature. After the prestimulation period the cells were washed twice by rapid centrifugation and resuspended in PBS buffer. The cell suspension was adjusted to a cell concentration of 2×10^7 PMN/ml. To measure the metabolization of exogenous LTB_4, prestimulated PMN (1×10^7 cells) were incubated with LTB_4 in the presence of Ca^{2+} (0·9 mM) and Mg^{2+} (0·5 mM) at 37°. The respective substrate concentrations were 0·8 nM [^{3}H]LTB_4 in a final incubation volume of 1400 μl (incubation time proceeded over 30 min) or 1·6 nM [^{3}H]LTB_4 in the presence of 0·42 μM LTB_4 in a volume of 700 μl using a reduced incubation period of 15 min. Reactions were terminated by the addition of two volumes of methanol/acetonitrile (1:1, v/v) and the samples were stored under argon at −20°.

Specific binding of LTB_4

The binding studies were carried out as was previously described (Brom *et al.*, 1988a). Neutrophils at a concentration of 2×10^7 cells/ml were resuspended in PBS-buffer containing 0·2 mg/ml of bovine serum albumin. 4×10^6 cells were incubated with various concentrations of LTB_4 for 45 min at 4°. The concentration ranged between 0·2 and 71·6 nM for [^{3}H]LTB_4 (0·2, 0·4, 0·8, 1·6 nM) and increasing amounts of unlabelled LTB_4 (up to 70 nM). The reactions were terminated by rapid filtration using 96-well filtration plates (5-μm pore size polyvinylidene fluoride membranes) in combination with the Millititer vacuum holder (Millipore, Eschborn). The filters were transferred to scintillation vials, methanol (0·5 ml) and Rotiszint 2211 (4 ml) were added and the radioactivity was measured by liquid scintillation counting (Rack beta 1209; LKB, Turku, Finland). Specific binding was expressed as total binding minus non-specific binding. The non-specific binding component was determined in the presence of 0·5 μM of non-radioactive LTB_4. The binding parameters were calculated by Scatchard plot analysis according to Rosenthal (1967) and Melikhova *et al.* (1988).

Analysis of LTB_4 conversion

To study the conversion of exogenous [^{3}H]LTB_4, the samples were centrifuged at 3000 ***g*** for 15 min, followed by extraction with 7 ml of chloroform. The extracts were dried under a stream of nitrogen, resuspended in 200 μl of methanol/water (30:70, v/v) and applied to reverse-phase high-pressure liquid chromatography (HPLC) analysis. The HPLC-system consisted of a CM3000 pump (Laboratory Data Control, Milton Roy, Hasselroth) and a radioactivity continuous-flow monitor (Raytest, Straubenhardt) equipped with a flow cell mixing the column eluate and scintillator (Rotiszint 2211) in the ratio 1:2. Sample injection was carried out using a Rheodyne valve (Cotati, CA). The column was packed with Nucleosil C_{18} (pore size 5 μm; Macherey-Nagel, Düren). Elution was carried out using solvent systems consisting of methanol/acetonitrile/water (solvent system A: 20:20:60, v/v; solvent system B: 30:30:40, v/v). The mobile phases contained 0·5 g of EDTA/1 water and 0·75 g of K_2HPO_4/l water. The pH value was adjusted to 5·0 by the addition of phosphoric acid. The samples were eluted using a linear gradient between 100% A and 100% B within the first 30 min of the analysis. The elution was carried out for additional 5 min at 100% of solvent system B. The flow rate was maintained at 0·9 ml/min.

Generation of leukotrienes was determined using reverse-phase HPLC as was described recently (Knöller *et al.*, 1988). Amounts as little as 0·5 ng of LTB_4 or the ω-oxidized metabolites were detectable. The recovery values were in the range of 91 ± 2% (mean ± SD, $n = 4$).

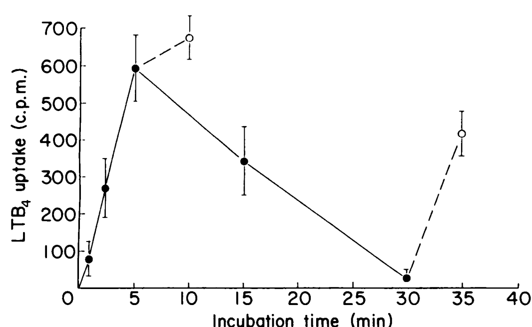

Figure 1. Uptake of [^{3}H]LTB$_4$ by human neutrophils. Purified PMN (1×10^7 cells) were incubated with [^{3}H]LTB$_4$ (5 nM) for the indicated time periods at 37°. The associated [^{3}H]LTB$_4$ was separated from free [^{3}H]LTB$_4$ and was measured by liquid scintillation counting (●). The dashed lines and the open circles (○) indicate uptake experiments for 5 min or 30 min, respectively, an extensive cell washing procedure and an incubation with [^{3}H]LTB$_4$ (5 nM) for additional 5 min. The presented data were mean values $\pm$ SD ($n=4$).

Viability

Cell viability was assayed microscopically by trypan blue dye exclusion as well as by release of cytoplasmic lactate dehydrogenase. Under the incubation conditions used no cell toxicity could be observed.

Statistics

All data were presented as mean $\pm$ SD. Significance was calculated using the paired Student's *t*-test.

RESULTS

In the initial studies the time–course of LTB$_4$-uptake by human PMN was determined. Purified neutrophils (1×10^7 cells in a volume of 0·5 ml) were incubated with [^{3}H]LTB$_4$ (5 nM) in the presence of Ca^{2+} (0·9 mM) and Mg^{2+} (0·5 mM), for the indicated time periods, at 37°. The amount of associated [^{3}H]LTB$_4$ was separated from non-associated [^{3}H]LTB$_4$ by rapid centrifugation or vacuum filtration. [^{3}H]LTB$_4$ was taken up by the cells in a time-dependent manner and reached maximal values after 5 min of incubation. The amount of [^{3}H]LTB$_4$-uptake decreased with prolonged incubation periods (Fig. 1). Cells after 5 min of incubation were then washed extensively and incubated with [^{3}H]LTB$_4$ for additional 5 min. It is apparent that the amount of associated radioactivity increased slightly. Spiking the cells after 30 min of incubation with [^{3}H]LTB$_4$ when no LTB$_4$ was present on the cell surface, led to a normal rate of LTB$_4$ uptake. The results indicate that no additional uptake of LTB$_4$ was achieved when maximal binding was obtained.

Next, we analysed the distribution pattern of LTB$_4$ and its ω-oxidized products within the separated fractions, the supernatant and the cell pellet, by reversed phase-HPLC (Table 1). The data demonstrate (i) that resting human PMN converted LTB$_4$ into 20-carboxy-LTB$_4$ via the hydroxy-metabolite and (ii) that all products were mainly localized within the supernatant, indicating an effective mechanism of extrusion. The main product within the cells was 20-COOH-LTB$_4$, which was released after a lag phase.

The specific binding of LTB$_4$ by human PMN (4×10^6 cells) was determined using various ligand concentrations (0·2 nM–71·2 nM). Analysis of the data reveals that the LTB$_4$ receptors exist as two subsets with different ligand affinities. The respective binding parameters suggested $2{\cdot}2 \pm 0{\cdot}9 \times 10^3$ receptors per cell and a Kd of $2{\cdot}6 \pm 1{\cdot}4$ nM for the high affinity and $8{\cdot}2 \pm 5{\cdot}3 \times 10^4$ receptors per cell and a Kd of $15{\cdot}3 \pm 8{\cdot}7$ nM for the low affinity subset ($n=6$).

As was described, the affinity states were interconvertible and controlled by guanine nucleotide regulatory proteins. Recently, we demonstrated that the LTB$_4$ transport into the cell interior was mediated via specific binding sites. It was also shown that the receptor expression may be controlled by protein kinase *C*. In order to analyse the relationship between the distribution pattern of the receptors and conversion of LTB$_4$, the following experimental approaches were chosen: the neutrophils were prestimulated with various compounds in the presence of Ca^{2+} (0·9 mM) and Mg^{2+} (0·5 mM) and the receptor expression was determined. In parallel, the conversion of LTB$_4$ was measured using two substrate concentrations; at low ligand concentrations of 0·8 nM of [^{3}H]LTB$_4$ only the high-affinity receptors were occupied, higher LTB$_4$ concentrations (0·43 μM) interacted with both receptor subsets.

Prestimulation of the cells with LTB$_4$ (1 nM) resulted in a specific deactivation and in a down-regulation of the high-affinity receptors; the low-affinity receptors were not involved. PMA (0·23 μM), an activator of protein kinase *C*, and sodium fluoride (NaF; 20 mM), a direct activator of G-proteins, reduced the high affinity receptors totally and the low affinity receptors partially. The polyene antibiotic amphotericin B (2 μM) shifted high-affinity receptors to the low-affinity subset, whereas the membrane fluidizer n-butanol (0·25%) shifted low-affinity receptors to the high-affinity state. A similar pattern was observed using cytochalasin B (15 μM) as the priming stimulus. Preincubation of PMN with the indicated concentration of cytochalasin B for 60 min resulted in inhibition of actin-filament polymerization (Al-Mohanna & Hallett, 1987). The respective results are presented in Fig. 2.

At very low LTB$_4$ concentrations it was demonstrated that high-affinity receptors are able to transfer the substrate LTB$_4$ to the metabolizing enzymes. All stimuli, which reduced the expression of high-affinity receptors (PMA, NaF, LTB$_4$), also reduced the conversion of low amounts of LTB$_4$. However, the slight increase of the high-affinity receptor expression induced by cytochalasin B and butanol did not significantly correlate with LTB$_4$ conversion (Fig. 3a). Similar results were obtained at higher LTB$_4$ concentrations (Fig. 3b). PMA and NaF showed inhibitory effects on the LTB$_4$ catabolism in correlation with the receptor expression. In contrast, cytochalasin B or butanol, which also reduced the low-affinity receptors, and amphotericin B prestimulation, which increased the amount of low-affinity receptors, did not show a different conversion rate compared to the control incubations.

In addition, the respective distribution pattern of the LTB$_4$ metabolites, 20-OH-LTB$_4$ and 20-COOH-LTB$_4$, was analysed. It was shown that the inhibition of LTB$_4$ conversion was mainly a reduced formation of 20-carboxy-LTB$_4$ (Fig. 4).

To consider the effects of PMA on protein kinase *C*, the biologically inactive phorbol ester 4α-phorbol 12,13-dideconate (4α-PDD) was included in the study. Pretreatment of neutrophils with PMA (0·23 μM) decreased the amounts of high- and low-affinity binding sites by 96% and 68%, compared with unstimulated control cells. Preincubation with equimolar con-

Table 1. Distribution of LTB_4, 20-OH-LTB_4 and 20-COOH-LTB_4 within the cell and the cellular supernatant

Incubation time (min)	% cell fraction			% surrounding medium		
	20-COOH-LTB_4	20-OH-LTB_4	LTB_4	20-COOH-LTB_4	20-OH-LTB_4	LTB_4
2	15·5	2·5	—	6·1	13·0	62·8
5	20·1	2·8	—	20·0	32·4	24·6
10	15·4	5·1	—	33·7	40·7	5·1
15	15·0	10·7	—	36·6	33·6	4·0
30	11·1	3·4	—	59·6	24·9	1·0
60	8·4	—	—	66·3	25·2	—

Purified PMN (1×10^7 cells) were incubated with [^{3}H]LTB_4 (5 nM) at 37°. After the indicated time periods the samples were separated by centrifugation. The extracted cell pellet and the cell supernatant were analysed for the distribution of leukotrienes by HPLC-technique. The data are presented as percentages of the total recovered radioactivity. Two experiments were carried out that showed the same pattern.

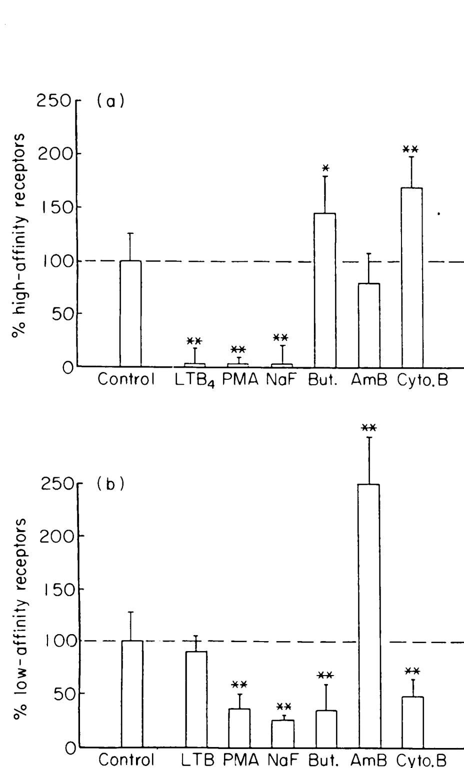

Figure 2. High-affinity (a) and low-affinity (b) LTB_4 receptor subsets in prestimulated human neutrophils. Purified cells were prestimulated with the following compounds: LTB_4 (1 nM) for 10 min, PMA (0·23 μM) for 10 min, NaF (20 mM) for 10 min, butanol (But.) (0·25%) for 15 min, amphotericin B (AmB) (2 μM) for 30 min, cytochalasin B (Cyto. B) (15 μM) for 60 min. Control incubations were performed by prestimulation of cells with PBS-buffer for the indicated time periods. The cells were washed by centrifugation and resuspension in PBS-buffer at the end of the prestimulation period. Determination of the binding parameters was carried out by Scatchard plot analysis. The amount of specific binding sites for the high- and low-affinity receptor subsets of the control incubations were expressed as 100%. All values were the mean ± SD of four individual experiments. Significance was assessed by paired t-test; * = $P \leq 0·05$, ** = $P \leq 0·01$ compared with the controls.

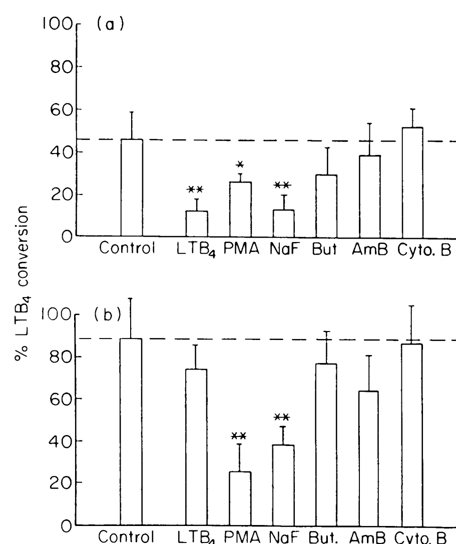

Figure 3. Conversion of [^{3}H]LTB_4 by prestimulated human neutrophils. Purified PMN (1×10^7 cells) were prestimulated as described in Fig. 2. The cells were subsequently incubated with exogenously added [^{3}H]LTB_4 (0·8 nM) in a final volume of 1400 μl for 30 min at 37° (a) or with a mixture of [^{3}H]LTB_4 (1·6 nM) and LTB_4 (0·42 μM) in a volume of 700 μl for 20 min at 37° (b). All samples were analysed by HPLC technique. The ordinate shows the percentage of LTB_4 conversion into the ω-oxidized products. The values were mean ± SD ($n = 4$). Significance was assessed by paired t-test; * = $P \leq 0·05$, ** = $P \leq 0·01$ compared with the controls.

centrations of 4α-PDD reduced the receptor expression only by 34% and 45%, respectively. In contrast, the rate of LTB_4 conversion was not influenced after prestimulation with 4α-PDD, whereas the addition of PMA resulted in a marked inhibition of LTB_4 catabolism (data not shown). Therefore, we concluded that the efficacy of PMA was in part the result of direct protein kinase *C* activation, but an indirect effect of the phorbol esters, e.g. membrane pertubation, cannot be excluded.

Endogenous generation of leukotrienes may interfere with the binding and metabolism of exogenously added LTB_4.

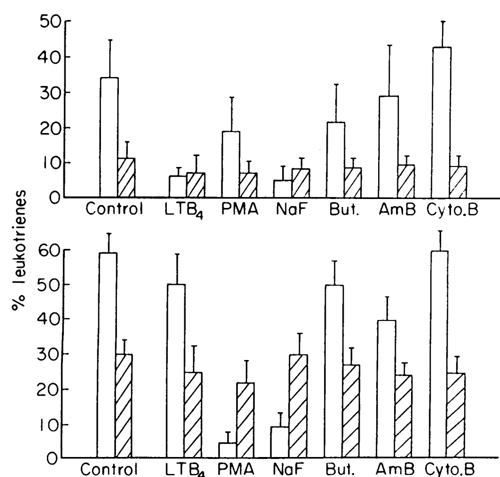

Figure 4. Distribution of 20-COOH-LTB$_4$ and 20-OH-LTB$_4$ in prestimulated human neutrophils. The neutrophils were prestimulated, incubated with exogenously added substrate and analysed as described for Figs 2 and 3. The percentages of the generated 20-COOH-LTB$_4$ (□) and 20-OH-LTB$_4$ (■) were shown for the conversion of 0·8 nM [^{3}H]LTB$_4$ (top) or for the conversion of 0·43 μM [^{3}H]LTB$_4$/LTB$_4$ (bottom). The results were expressed as mean ± SD ($n = 4$).

Therefore, the amounts of LTB$_4$ or its ω-oxidized products were determined at the end of the preincubation period and after the washing procedure. The described HPLC technique is capable of detecting as little as 0·5 ng of LTB$_4$. In addition, the washing procedure reduced the amounts of endogenously synthesized leukotrienes by a factor of 95%. Within the detection limits the described preincubation conditions did not result in the generation of leukotrienes. As an exception, formation of leukotrienes was observed following stimulation with NaF; the respective amounts at the end of the preincubation were below 1 ng. Therefore, we excluded that endogenous leukotrienes interfered with the exogenously added LTB$_4$ for receptor binding or metabolization.

DISCUSSION

Receptors are crucially involved with regard to the transformation of external signals into the biological responses of the cell. Studies on the subcellular localization of the LTB$_4$-converting enzymes (Shak & Goldstein, 1985; Sumimoto, Takeshige & Minakami, 1985; Brom *et al.*, 1987) and the use of non-penetrating agents interacting with membraneous proteins facing the cell exterior (such as the diazonium salt of sulphanilic acid and the protease thermolysin) revealed that LTB$_4$ metabolization was restricted to the cellular interior. The substrates LTB$_4$ and 20-OH-LTB$_4$ enter the cell via interaction with specific binding sites (Brom *et al.*, 1988b). These results were supported by uptake experiments. At every time-point of incubation the main bulk of radioactivity was obtained within the supernatant, confirming a rapid mechanism of uptake and release of LTB$_4$ and the respective metabolites. The radioactivity within the cells was detected as 20-COOH-LTB$_4$ as a result of rapid conversion of LTB$_4$ and the hydroxy-metabolite by the metabolizing pathway. At the maximal point of [^{3}H]LTB$_4$ association, an additional uptake of the ligand was reduced. The short exposure of cells with LTB$_4$ seemed to result in an altered affinity state, whereas the amount of receptors was only slightly decreased (Winkler *et al.*, 1988). It is possible that the receptors were laterally segregated by the control of cytoskeleton elements (Painter, Zahler-Bentz & Dukes, 1987; Jesaitis *et al.*, 1988). This could limit or delay a further subsequent uptake of LTB$_4$. In contrast, the chemotactic peptide f-met-leu-phe was taken up by human PMN in a saturable fashion, reflecting the poor releasability of the oligopeptide or its degradation products (Perez, Elfman & Lobo, 1987).

Our data demonstrate that LTB$_4$ conversion was mediated by high- and low-affinity receptors, respectively. To ascertain whether changes of the LTB$_4$ receptor subset distribution affected the conversion of LTB$_4$, neutrophils were modulated by prestimulation with defined agents. As is shown, preincubation with LTB$_4$ acted as the most physiological stimulus. LTB$_4$ at the indicated concentration specifically deactivated the cell and down-regulated all high-affinity receptors without affecting the low-affinity subset (as it was also shown by Goldman & Goetzl, 1984). However, deactivated cells did not catabolize low concentrations of exogenously added [^{3}H]LTB$_4$, which interacted only with the high-affinity subset. PMA activates the protein kinase *C*, which apparently regulates the expression of various receptor systems, also that of the neutrophil LTB$_4$ receptor (O'Flaherty, Redman & Jacobson, 1986; Brom *et al.*, 1988b). However, an unspecific effect of PMA, e.g. modulation of membrane bilayer properties, cannot be excluded. NaF was reported to activate the signal transmitting G-proteins simulating a receptor-mediated event without receptor occupation (Curnutte, Babior & Karnovsky, 1979). Consequently, NaF also increases the internal Ca^{2+} level and activates the protein kinase *C* (Della Bianca *et al.*, 1988). The loss of the high-affinity receptor subpopulation after prestimulation with LTB$_4$, PMA or NaF strictly correlated with the subsequent conversion of low LTB$_4$ concentrations.

The membrane bilayer is composed of phospholipids and provides a fluid matrix for protein organization and movement. Furthermore, protein orientation and fluidity are regulated by cytochalasin B-sensitive microfilament structures. The effects of cytoskeleton modifiers on cell surface structures have been described (Van Obberghen, De Meyts & Roth, 1976). In addition, the final response of target cells seems to be the result of diffusion-controlled processes such as the accessibility of receptors, coupling of G-proteins and activation of the effector enzymes that were dependent on lipid fluidity. It may be possible that each receptor system expects an optimal bilayer environment for maximal activation (Heron *et al.*, 1980). We suggested that membrane perturbation by fluidizers, by binding of amphotericin B to sterols or by cytoskeleton modification, affected the interactions between the LTB$_4$ receptors and the respective G-proteins. In this way, treatment of neutrophils with butanol or cytochalasin B resulted in the shift of the receptors to a higher affinity state. In addition, small but not significant differences were obtained between butanol and cytochalasin B-pretreated cells with regard to LTB$_4$-metabolization. In this way the effects of cytochalasin B seems to be heterogenous. It may be possible that inhibition of the sequestration of occupied receptors or the alteration of the levels of intracellular second messengers improve the metabolization rate (Jesaitis, Tolley & Allen, 1986). PMA and NaF pretreatment also resulted in reduced amounts of the low-affinity receptor subset. The loss of available binding sites could reflect a real down-regulation, a

shift to a higher affinity state or a change in the oligomeric topology of the binding region. However, reduction of the low-affinity subpopulation was also paralleled by a decreased conversion of high concentrations of exogenous LTB_4. Amphotericin B, a membrane perturbating agent that alters the lipid environment of the receptors, increased the low-affinity receptor subset. However, there was no increase in LTB_4 conversion, suggesting that the limitation was at the level of metabolization.

Our data clearly show a correlation between receptor expression and the respective uptake and conversion of LTB_4. The expression is regulated by homologous deactivation mechanisms and the activation state of the cell, especially by the control of protein kinase *C*. Furthermore, the receptor affinity for the ligand seems to be under the strict control of components from the cytoskeleton and by a co-ordinated lipid bilayer structure.

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