Biochemical Pharmacology, Vol. 34, No. 9, pp. 1485–1490, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00
© 1985 Pergamon Press Ltd.

COMPARISON OF THE EFFECTS OF SOME COMPOUNDS ON HUMAN NEUTROPHIL DEGRANULATION AND LEUKOTRIENE B_4 AND THROMBOXANE B_2 SYNTHESIS

RICHARD M. J. PALMER* and JOHN A. SALMON

Departments of Pharmacology and Prostaglandin Research, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, U.K.

(*Received* 10 *September* 1984; *accepted* 13 *November* 1984)

Abstract—The effects of aspirin, indomethacin, phenylbutazone, eicosatetraynoic acid (ETYA), nordihydroguaiaretic acid (NDGA) and 3-amino-1-[3-(trifluoromethyl)-phenyl]-2-pyrazoline (BW755C) on human neutrophil degranulation induced by A23187 and F-Met-Leu-Phe (FMLP) have been studied. These effects have been compared with those on A23187 induced leukotriene B_4 (LTB_4) and thromboxane B_2 (TXB_2) synthesis by these cells to elucidate the relationship between LTB_4 formation and degranulation. All compounds inhibited TXB_2 synthesis by 50% at concentrations between 0.0016 and 50 μM. The synthesis of LTB_4 was inhibited by 50% by ETYA (1.9 μM) and by NDGA (0.52 μM). Degranulation induced by A23187 and FMLP was inhibited by 50% by ETYA (16 and 11 μM respectively) and by NDGA (1.5 and 6.5 μM respectively). In the case of ETYA the concentrations required to inhibit degranulation were significantly higher than those required to inhibit LTB_4 synthesis. In contrast, BW755C inhibited LTB_4 synthesis by 50% at 2.8 μM but did not affect A23187-induced degranulation and was only a weak inhibitor of FMLP-induced degranulation (50% inhibition at 89 μM). The effects of the above compounds on the ω-oxidation of LTB_4 by human neutrophils has also been studied to investigate the mechanism of action of these compounds. None of the above compounds affected the metabolism of LTB_4 by these cells suggesting that their actions are not as non-specific anti-oxidants. These data indicate that human neutrophil degranulation induced by FMLP and A23187 is independent of LTB_4 synthesis.

Human neutrophils (PMN)† aggregate, secrete lysosomal enzymes, generate superoxide and show chemotactic locomotion when exposed to stimuli such as F-Met-Leu-Phe (FMLP [1–3]). These cells also convert arachidonic acid (AA) to prostaglandins and thromboxanes via the cyclooxygenase pathway and to monohydroxy-eicosatetraenoic acids and leukotrienes via the lipoxygenase enzyme system [4–7]. Compounds which inhibit both pathways of AA metabolism such as eicosatetraynoic acid (ETYA) and nordihydroguaiaretic acid (NDGA) also inhibit the above responses of PMN [8–12]. Selective cyclooxygenase inhibitors such as aspirin do not affect PMN responses.

Leukotriene B_4 (LTB_4) induces PMN chemotaxis, aggregation, degranulation and superoxide production, comparable both quantitatively and qualitatively, with responses to the chemoattractants FMLP and C5a [13–17]. These findings suggest that the mechanism whereby PMN respond to chemoattractants may involve products of the lipoxygenase pathway, and in particular LTB_4. However, determination of LTB_4 and β-glucuronidase release from PMN in response to A23187, FMLP and serum treated zymosan led us to suggest that LTB_4 does not mediate PMN responses to these stimuli as the two processes were neither quantitatively nor qualitatively related [18].

In the present investigation we have further evaluated the possible involvement of LTB_4 in the response of PMN to stimulation by studying the effects of various inhibitors of arachidonic acid metabolism on degranulation induced by either A23187 or FMLP and compared them with their effects on the synthesis of LTB_4 and thromboxane B_2 (TXB_2) by these cells. Since some of the compounds under investigation could inhibit lipoxygenase product synthesis as non-specific anti-oxidants, we have also investigated their effects on the metabolism of LTB_4 in these cells which is reported to be by ω-oxidation [19, 20].

MATERIALS AND METHODS

Indomethacin, phenylbutazone, NDGA, aspirin, heparin, *N*-2-hydroxyethyl piperazine-*N*-2-ethane sulphonic acid (HEPES), dimethyl sulphoxide (DMSO), phenolphthalein-β-D-glucuronic acid, FMLP and cytochalasin B (Cyt B) were obtained from Sigma Chemical Co., Poole, Dorset. Ficoll-Paque (Pharmacia Fine Chemicals, Upsaala,

* To whom correspondence should be addressed.

† Abbreviations used: ETYA, eicosatetraynoic acid; NDGA, nordihydroguaiaretic acid; BW755C, 3-amino-1-[3-(trifluoromethyl)-phenyl]-2-pyrazoline; FMLP, *N*-formyl-methionyl-leucyl-phenylalanine; LTB_4, leukotriene B_4; LTA_4, leukotriene A_4; TXB_2, thromboxane B_2; PMN, polymorphonuclear neutrophils; HEPES, N-2-hydroxyethylpiperazine-N-2-ethane-sulphonic acid; CytB, cytochalasin B; HBSS, Hank's balanced salts solution; DMSO, dimethyl sulphoxide; RIA, radioimmunoassay.

Sweden), A23187 (Calbiochem-Behring Corp., La Jolla, CA), indicator-free Hanks' balanced salts solution (Wellcome Diagnostics Ltd., Dartford, Kent) and [^{3}H]TXB_2 and [^{3}H]LTB_4 (Amersham International, Amersham, Bucks) were obtained as indicated. ETYA was a generous gift from Dr. J. M. Osbond (Roche Products, Welwyn, Herts). All other chemicals were of reagent or superior grade.

Human PMN were purified from heparinized (20 u/ml) venous blood from healthy volunteers on Ficoll-Paque gradients followed by ammonium chloride lysis of erythrocytes as described [14] giving a purity of >95%. The final PMN concentration was 5×10^6 cells/ml in indicator-free Hanks' balanced salts solution buffered to pH 7.4 with 30 mM HEPES (HBSS). The cell suspension was equilibrated at 37° for at least 30 min prior to addition of test compounds.

The test compounds and FMLP were dissolved in DMSO and diluted in HBSS to give 10× concentrated solutions in 2.5% DMSO:HBSS. A stock solution of Cyt B (50 μg/ml in 0.5% DMSO:HBSS) was prepared in the same way. A stock solution of A23187 (10 mM) in ethanol was diluted in HBSS to give a final concentration of 2 μM in 0.2% ethanol in contact with the cells. The final concentration of DMSO in the incubation mixture in experiments with FMLP was 0.55%. These concentrations of solvents did not affect cell viability as shown by negligible release of lactate dehydrogenase.

Incubations with A23187 as stimulus were initiated by addition of the appropriate concentration of test compound or vehicle control followed 15 min later by 2 μM A23187. After a further 5 min incubation the cells were pelleted by centrifugation at 12000 *g* for 30 sec in a microcentrifuge and the supernatant removed for determination of LTB_4, TXB_2 and β-glucuronidase release. Incubations with FMLP as stimulus were initiated by addition of test compound or vehicle control followed 1 min later by Cyt B (5 μg/ml) and incubation continued for a further 14 min. FMLP (0.1 μM) was then added and incubation continued for a further 5 min before pelleting the cells and removing the supernatant as above. All incubations were carried out at 37° in duplicate. The concentrations of LTB_4 and TXB_2 were determined by specific radioimmunoassay (RIA [21, 22]). The concentrations of β-glucuronidase and lactate dehydrogenase were determined colourimetrically [23, 24]. The absolute amount of β-glucuronidase released by A23187 and FMLP was expressed as units/10^6 cells where 1 unit of enzyme liberated 1 μg phenolphthalein from phenolphthalein-β-glucuronic acid in 18 hr at 37° and pH 4.5

The metabolism of LTB_4 by human PMN was determined in incubations containing 5×10^6 cells/ml and 20 ng/ml LTB_4. After incubation at 37° for the appropriate time supernatants were obtained by

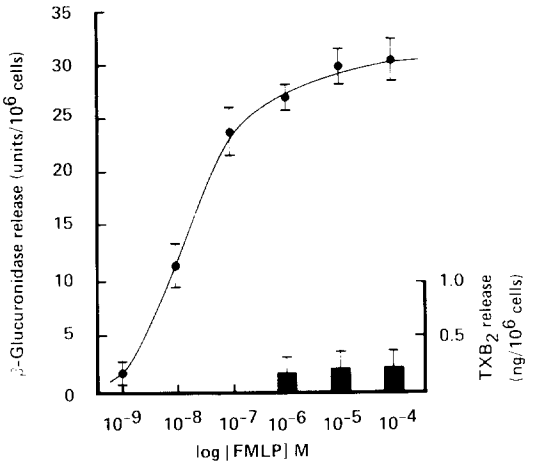

Fig. 1. FMLP induced release of β-glucuronidase and TXB_2. Human PMN were treated with Cyt B (5 μg/ml) for 14 min prior to addition of FMLP and incubation continued for 5 min. The amounts of β-glucuronidase and TXB_2 were determined as described in Materials and Methods. Each value is the mean ± S.E.M. of three experiments performed in duplicate. Release of TXB_2 by FMLP concentrations below 1 μM was not detectable (<0.1 ng/10^6 cells) as was the release of LTB_4 by all concentrations of FMLP tested (<0.02 ng/10^6 cells).

Table 1. Inhibition of A23187 induced release of LTB_4, TXB_2 and β-glucuronidase and FMLP induced β-glucuronidase release from human PMN

Compound	No. of Expts N	Approximate IC_{50} (μM) A23187 LTB_4	A23187 TXB_2	A23187 β-glucuronidase	FMLP β-glucuronidase
Aspirin	2	Potn (100–1000)	50	N.E. at 1000	N.E. at 1000
Indomethacin	3	27	0.0016	*	59
Phenylbutazone	3	85	5.4	78	9.4
ETYA	3	1.9	0.63	16	11
NDGA	3	0.52	7.9	1.5	6.5
BW755C	3	2.8	2.2	N.E. at 100	89

N.E. = no effect; Potn = potentiation.

* 30% inhibition at 100 μM.

The compounds, together with Cyt B (5 μg/ml) in experiments with FMLP, were preincubated with the cells for 15 min prior to stimulation with A23187 (2 μM) or FMLP (0.1 μm). Incubation was continued for a further 5 min and the concentrations of LTB_4, TXB_2 and β-glucuronidase determined as described in Materials and Methods. The effects of the compounds are expressed as the mean IC_{50} from (N) experiments performed in duplicate.

pelleting the cells and were assayed for LTB_4 by RIA as above.

RESULTS

Human PMN released the lysosomal marker enzyme β-glucuronidase when exposed to FMLP in the presence of Cyt B with a half maximal effect at approximately 0.02 μM. The release of this enzyme was maximal (30.8 ± 1.8 units/10^6 cells; mean ± S.E.M., N = 3), with concentrations of FMLP above 10 μM. The formation of TXB_2 (0.2 ± 0.15 ng/10^6 cells; mean ± S.E.M., N = 3) was only detectable at concentrations of FMLP above 1 μM (Fig. 1) and LTB_4 synthesis was not detectable (<0.02 ng/10^6 cells) under these conditions. In contrast A23187 (2 μM) induced the synthesis of both LTB_4 (5.8 ± 1.2 ng/10^6 cells; mean ± S.E.M., N = 4) and TXB_2 (0.25 ± 0.08 ng/10^6 cells; mean ± S.E.M., N = 4). This stimulus caused the release of β-glucuronidase (8.6 ± 1.7 units/10^6 cells; mean ± S.E.M., N = 4), but was less effective than FMLP.

The effects of aspirin, indomethacin, phenylbutazone, ETYA, NDGA and BW755C on A23187-induced production of LTB_4 and TXB_2 and on degranulation induced by either A23187 or FMLP is summarized in Table 1. Both ETYA and NDGA caused dose-dependent inhibition of the synthesis of LTB_4, TXB_2 and degranulation induced by A23187 at concentrations between 0.032 and 32 μM (Fig. 2). However, each compound showed a different potency against the individual responses (IC_{50} values for ETYA of 1.9, 0.63 and 16 μM respectively and for NDGA of 0.52, 7.9 and 1.5 μM respectively). Thus ETYA was a more potent inhibitor of cyclooxygenase and NDGA was more active against lipoxygenase. Both compounds inhibited FMLP-induced degranulation at concentrations similar to those that inhibited A23187-induced degranulation (IC_{50} values of 11 and 6.5 μM respectively). BW755C inhibited both LTB_4 and TXB_2 synthesis at approximately the same concentrations (50% inhibition at 2.8 and 2.2 μM respectively) but unlike the other dual-inhibitors was only a weak inhibitor of FMLP-induced degranulation (50% inhibition at 89 μM) and did not affect A23187-induced degranulation (Fig. 3).

Aspirin inhibited TXB_2 formation (IC_{50} 50 μM) and simultaneously potentiated LTB_4 synthesis (190% of control at 100 μM) without affecting degranulation induced by either stimulus. Indomethacin was a potent inhibitor of TXB_2 synthesis (IC_{50} 0.0016 μM) but was only a weak inhibitor of LTB_4 synthesis (IC_{50} 27 μM) and A23187-induced degranulation (30% inhibition at 100 μM; Fig. 3). In contrast, phenylbutazone was a weak inhibitor of TXB_2 synthesis (IC_{50} 5.4 μM) and also inhibited both degranulation and LTB_4 synthesis by 50% at 78 and 85 μM respectively. Both indomethacin (IC_{50} 59 μM) and phenylbutazone (IC_{50} 9.4 μM) were more effective inhibitors of degranulation induced by FMLP than by A23187. However, at these concentrations

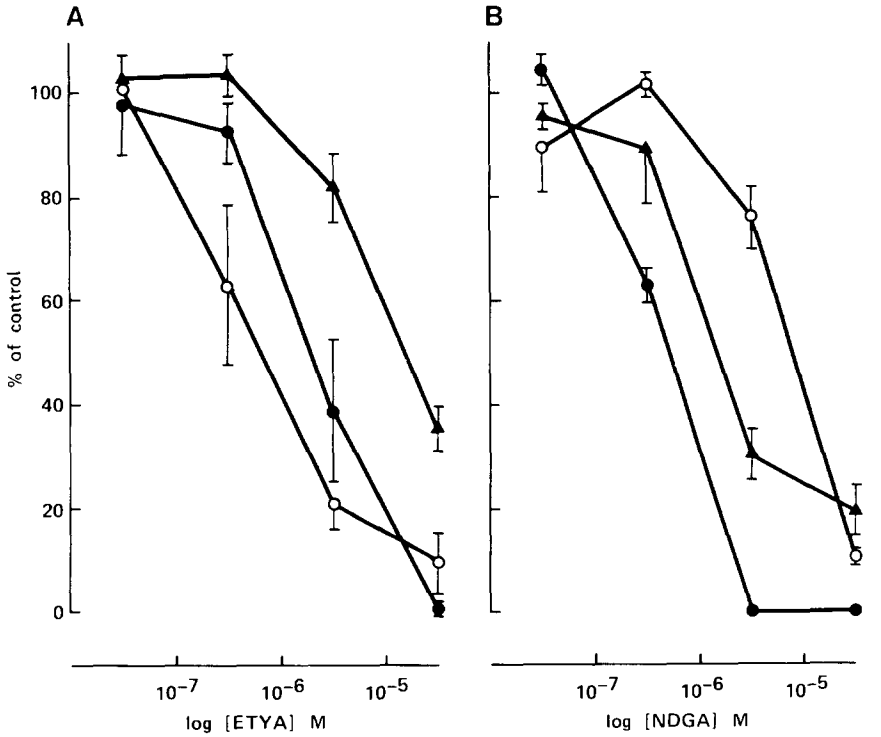

Fig. 2. Effect of ETYA and NDGA on A23187-induced LTB_4, TXB_2 and β-glucuronidase release. The compounds were incubated with the cells for 15 min prior to the addition of A23187 (2 μM) and incubation continued for a further 5 min. The concentrations of LTB_4 (●), TXB_2 (○) and β-glucuronidase (▲) were determined as described in Materials and Methods. Each point is the mean ± S.E.M. of three experiments performed in duplicate.

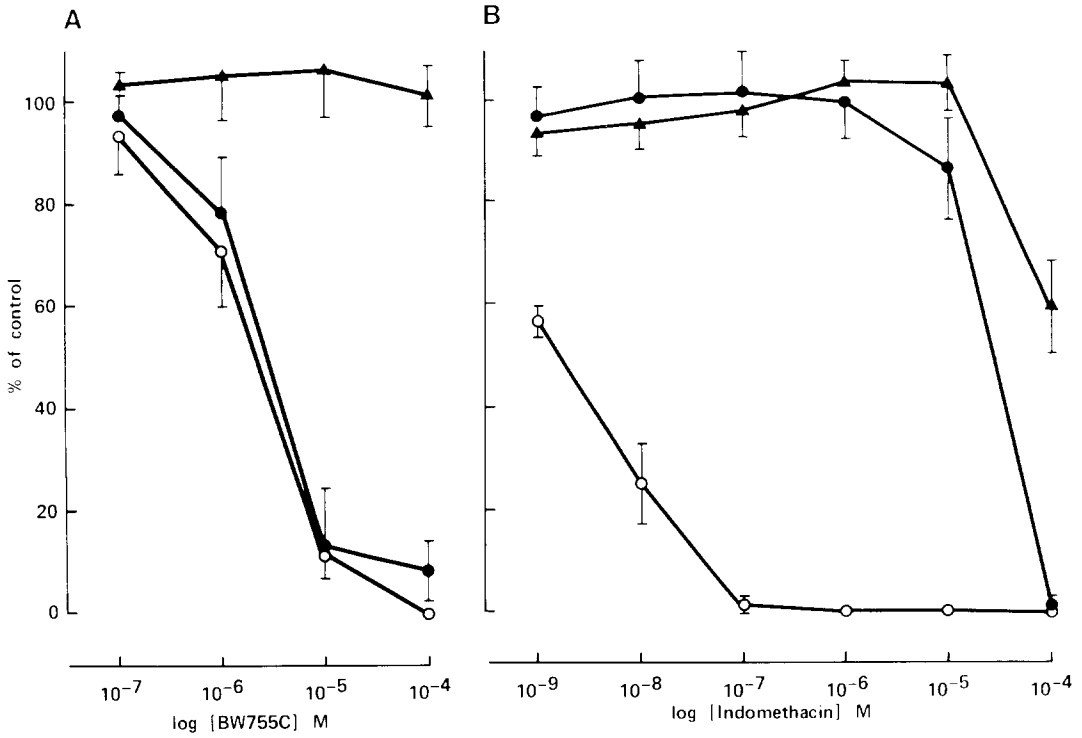

Fig. 3. Effect of BW755C (A) and indomethacin (B) on A23187-induced LTB_4, TXB_2 and β-glucuronidase release. The effects of each compound were studied as described in the legend of Fig. 2. Each point is the mean ± S.E.M. of three experiments performed in duplicate. Key as in Fig. 2.

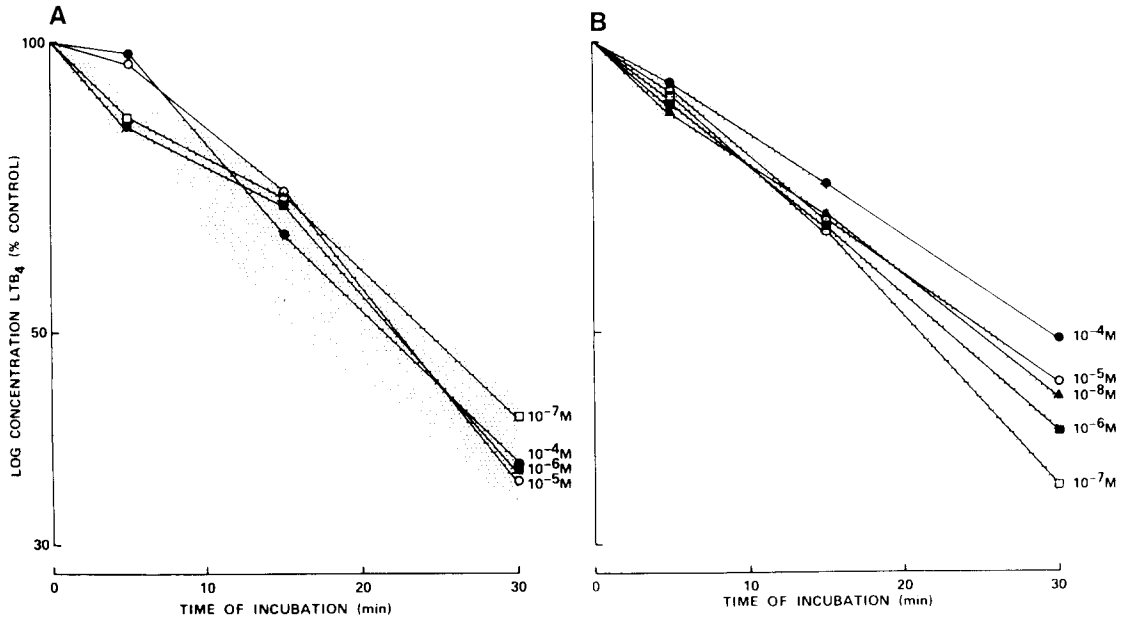

Fig. 4. Effect of BW755C (A) and indomethacin (B) on the metabolism of LTB_4 by human PMN. The cells were incubated with 20 ng LTB_4 for the appropriate time and the disappearance of LTB_4 was determined as described in Materials and Methods. Each point is the mean of duplicate determinations in a single experiment which is representative of two others and the hatched area represents the absolute range in three control experiments.

both compounds inhibit the binding of F-Met peptides to PMN [25, 26].

The potency difference between inhibition of A23187 induced LTB_4 synthesis and degranulation was most marked for BW755C, but was also statistically significant ($P < 0.01$) for ETYA, but not for NDGA, when the dose–response curves were fitted [27] and compared by Student's *t*-test.

Neither indomethacin nor BW755C inhibited the metabolism of immunoreactive LTB_4 by human PMN at concentrations up to 100 μM (Fig. 4), the other compounds tested in this system also did not reduce the rate of metabolism of LTB_4. We have previously shown that the products of LTB_4 metabolism by these cells do not cross react in the RIA [28].

DISCUSSION

The systematic determination of the effects of aspirin, indomethacin, phenylbutazone, ETYA, NDGA and BW755C on the synthesis of LTB_4 and TXB_2 measured by specific RIA and on degranulation induced by A23187 and FMLP has enabled us to evaluate further our previous suggestion that LTB_4 does not mediate PMN degranulation induced by these stimuli [18].

The synthesis of LTB_4 and TXB_2 was inhibited by similar concentrations of ETYA but higher concentrations of this compound were required to abrogate degranulation induced by either A23187 or FMLP. The effectiveness of ETYA as an inhibitor of eicosanoid synthesis differs slightly from that reported by some authors [12, 29] but this may reflect the difference between measuring the products of endogenous AA metabolism determined in the present study as opposed to exogenous AA metabolism measured by others. Similarly BW755C inhibited LTB_4 and TXB_2 synthesis equally but was only a weak inhibitor of FMLP-induced degranulation and did not affect degranulation induced by A23187. These data confirm previous reports of dual inhibition of cyclooxygenase and lipoxygenase by this compound [30–34] and also show that both ETYA and BW755C are significantly more potent inhibitors (5- and 40-fold respectively) of LTB_4 synthesis than of degranulation. These data suggest that LTB_4 synthesis is unrelated to the degranulation response of human PMN exposed to A23187 and FMLP. Since similar concentrations of ETYA to those which reduce degranulation also inhibit PMN aggregation and superoxide production in response to stimulation [8, 9, 11] it is likely that these responses are also not mediated by LTB_4.

Although the synthesis of both eicosanoids was inhibited by NDGA, this compound was a more potent inhibitor of LTB_4 synthesis and also abrogated degranulation induced by both A23187 and FMLP confirming previous reports [32, 33, 35]. Systematic examination of these actions showed that NDGA inhibited both degranulation and LTB_4 synthesis with equal potency. However, since approximately 12-fold higher concentrations were required to block degranulation induced by FMLP, it is reasonable to propose that this effect of NDGA is also not mediated by LTB_4.

The present data emphasises that inhibition of a PMN response by ETYA or another lipoxygenase inhibitor does not necessarily indicate the involvement of lipoxygenase products as mediators of that response. These compounds may have other activities; for example ETYA inhibits the binding of [^{3}H] FMLP to PMN at concentrations similar to those that inhibit responses of these cells to FMLP [36]. Since LTB_4 was measured specifically in the present study, the possibility that another lipoxygenase product may mediate PMN responses cannot be excluded, particularly as these inhibitors show some selectivity for individual reactions. For example ETYA is more potent as an inhibitor of the 11-, 12- and 15-lipoxygenases than of the 5-lipoxygenase [37]; the inhibition of LTB_4 synthesis observed with ETYA in this and other studies has been attributed to inhibition of LTA_4 formation rather than to an effect on 5-lipoxygenase [38]. Furthermore, BW755C is a much weaker inhibitor of the synthesis of 15-hydroperoxy-eicosatetraenoic acid than of 5-, 11- or 12-hydroperoxy-eicosatetraenoic acids [39].

Indomethacin inhibits cyclooxygenase at concentrations several orders of magnitude below those required to inhibit LTB_4 synthesis and degranulation. Phenylbutazone showed a similar profile of activity albeit with considerably reduced potency against cyclooxygenase when compared to indomethacin. Although both compounds inhibited A23187-induced LTB_4 synthesis and degranulation at similar concentrations, these concentrations are high and in the case of indomethacin are known to affect Ca^{2+} fluxes [40] phospholipase A_2 [32, 41] and the binding of F-Met peptides to PMN [5]. Since LTB_4 synthesis is a reflection of both AA mobilization and activation of the LTB_4 synthetic system, and since degranulation is Ca^{2+} dependent [40–44], inhibition of degranulation by these concentrations of indomethacin and phenylbutazone cannot be attributed exclusively to a single mechanism.

Aspirin was a weak inhibitor of cyclooxygenase and potentiated LTB_4 synthesis without affecting degranulation induced by either FMLP or A23187. Therefore there was no quantitative relationship between LTB_4 synthesis and degranulation as the latter was not potentiated by this compound, further emphasizing that LTB_4 does not mediate degranulation. The simultaneous inhibition of cyclooxygenase and potentiation of LTB_4 synthesis by this compound may indicate diversion of substrate into the lipoxygenase pathway or reduced formation of inhibitory prostaglandins [45–47].

Human PMN metabolize LTB_4 by ω-oxidation [19, 20] to products that do not cross react in the RIA used in the present studies [28]. This has enabled us to study the effect of the above compounds on the disappearance of LTB_4. None of the compounds studied at concentrations up to 100 μm, or 1000 μm for aspirin, affected the rate of disappearance of LTB_4. Since one enzymic oxidation of AA (ω-oxidation) is not impaired by BW775C and the other compounds, this data suggests that their action on LTB_4 synthesis is not as non-specific anti-oxidants. This suggestion is not consistent with the observation that BW755C and ETYA inhibit non-enzymic lipid peroxidation [48], since the latter data indicate a non-

specific mechanism. However, the effects of drugs in the latter system are not quantitatively related to their effects on enzymic oxidation.

In conclusion, indomethacin, phenylbutazone, ETYA, NDGA and BW755C inhibit TXB_2 and LTB_4 synthesis and lysosomal enzyme release from human PMN in response to A23187 and/or FMLP in a manner suggesting that neither cyclooxygenase products nor LTB_4 are involved in mediating the degranulation response of these cells. Therefore the mechanism whereby these compounds affect PMN responses remains to be elucidated. The present studies do not contradict the suggestion that LTB_4 may mediate PMN accumulation *in vivo*.

Acknowledgements—The authors are indebted to Miss L. C. Tilling for excellent technical assistance and to Drs S. Moncada, L. Garland and G. A. Higgs for helpful discussion.

REFERENCES

1. H. J. Showell, R. J. Freer, S. M. Zigmond, E. Schiffmann, S. Aswanikumar, B. Corcoran and E. L. Becker, *J. exp. Med.* **143**, 1154 (1976).
2. J. T. O'Flaherty, D. L. Kreutzer and P. A. Ward, *J. Immunol.* **119**, 232 (1977).
3. E. L. Becker, M. Sigman and J. M. Oliver, *Am. J. Pathol.* **95**, 81 (1979).
4. P. Borgeat, M. Hamberg and B. Samuelsson, *J. biol. Chem.* **251**, 7816 (1976).
5. E. J. Goetzl and F. F. Sun, *J. exp. Med.* **150**, 406 (1979).
6. P. Borgeat and B. Samuelsson, *J. biol. Chem.* **254**, 2643 (1979).
7. P. Borgeat and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **76**, 2148 (1979).
8. J. T. O'Flaherty, H. J. Showell, P. A. Ward and E. L. Becker, *Am. J. Pathol.* **96**, 799 (1979).
9. J. E. Smolen and G. Weissmann, *Biochem. Pharmac.* **29**, 533 (1980).
10. H. J. Showell, P. H. Naccache, R. I. Sha'afi and E. L. Becker, *Life Sci.* **27**, 421 (1980).
11. G. M. Bokoch and P. W. Reed, *Biochem. Biophys. Res. Commun.* **90**, 481 (1979).
12. R. J. Smith, F. F. Sun, S. S. Iden, B. J. Bowman, H. Sprecher and J. C. McGuire, *Clin. Immunol. Immunopathol.* **20**, 157 (1981).
13. A. W. Ford-Hutchinson, M. A. Bray, M. V. Doig, M. E. Shipley and M. J. H. Smith, *Nature, Lond.* **286**, 264 (1980).
14. R. M. J. Palmer, R. J. Stepney, G. A. Higgs and K. E. Eakins, *Prostaglandins* **20**, 411 (1980).
15. E. J. Goetzl and W. C. Pickett, *J. Immunol.* **125**, 1789 (1980).
16. D. F. Chenoweth and T. E. Hugli, *Mol. Immunol.* **17**, 151 (1980).
17. C. N. Serhan, A. Radin, J. E. Smolen, H. Korchak, B. Samuelsson and G. Weissman, *Biochem. Biophys. Res. Commun.* **107**, 1006 (1982).
18. R. M. J. Palmer and J. A. Salmon, *Immunology* **50**, 65 (1983).
19. J. A. Lindgren, G. Hansson and B. Samuelsson, *FEBS Lett.* **128**, 329 (1981).
20. G. Hansson, J. A. Lindgren, S. E. Dahlen, P. Hedqvist and B. Samuelsson, *FEBS Lett.* **130**, 107 (1981).
21. J. A. Salmon, P. M. Simmons and R. M. J. Palmer, *Prostaglandins* **24**, 255 (1982).
22. J. A. Salmon, *Prostaglandins* **15**, 383 (1978).
23. P. S. Ringrose, M. A. Parr and M. McLaren, *Biochem. Pharmac.* **24**, 607 (1975).
24. R. Wroblewski and J. S. La Due, *Proc. Soc. exp. Biol. Med.* **90**, 210 (1955).
25. R. D. Nelson, J. M. Grayk, V. D. Feigel, M. J. Herron and D. E. Chenoweth, *Blood* **58**, 752 (1981).
26. R. M. J. Palmer, Ph.D. Thesis, London University (1983).
27. M. J. D. Powell, in *Numerical Methods for Non-Linear Algebraic Equations* (Ed. P. Rabinowitz), pp. 87–161. Gordon & Breach, London (1970).
28. J. A. Salmon, P. M. Simmons and R. M. J. Palmer, *FEBS Lett.* **146**, 18 (1982).
29. H. B. Kaplan, H. S. Edelson, H. M. Korchak, W. P. Given, S. Abramson and G. Weissmann, *Biochem. Pharmac.* **33**, 371 (1984).
30. R. W. Randall, K. E. Eakins, G. A. Higgs, J. A. Salmon and J. E. Tateson, *Agents and Actions* **10**, 553 (1980).
31. J. A. Salmon, P. M. Simmons and R. M. J. Palmer, in *Advances in Prostaglandin Thromboxane and Leukotrienes Research II* (Eds. B. Samuelsson, R. Paoletti and P. Ramwell), Raven Press, New York (1983).
32. I. Ahnfelt Ronne and E. Arrigoni-Martelli, *Biochem. Pharmac.* **81**, 2619 (1982).
33. J. Harvey and D. J. Osborne, *J. Pharm. Meth.* **9**, 147 (1983).
34. O. Radmark, C. Malmsten and B. Samuelsson, *FEBS Lett.* **110**, 213 (1980).
35. H. J. Showell, P. H. Naccache, R. I. Sha'afi and E. L. Becker, *Life Sci.* **27**, 421 (1980).
36. J. P. Atkinson, L. Simchowitz, J. Mehta and W. F. Stenson, *Immunopharmacol.* **4**, 1 (1982).
37. M. Hamberg and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **71**, 3400 (1974).
38. G. M. Bokoch and P. W. Reed, *J. biol. Chem.* **256**, 4156 (1981).
39. O. Radmark, C. Malmsten and B. Samuelsson, *FEBS. Lett.* **110**, 213 (1980).
40. B. J. Northover, *Br. J. Pharmac.* **59**, 253 (1977).
41. L. Kaplan, J. Weiss and P. Elsbach, *Proc. natn. Acad. Sci. U.S.A.* **75**, 2955 (1978).
42. R. J. Smith, W. Weirenga and S. S. Iden, *Inflammation* **4**, 73 (1980).
43. J. E. Smolen, H. M. Korchak and G. Weissmann, *Biochim. biophys Acta* **677**, 512 (1981).
44. J. O. Shaw, I. Brodersen and R. M. Lyons, *Agents and Actions* **12**, 328 (1982).
45. E. A. Ham, D. D. Soderman, M. E. Zanetti, H. W. Dougherty, E. McCauley and F. A. Kuehl, *Proc. natn. Acad. Sci. U.S.A.* **80**, 4349 (1983).
46. J. A. Salmon, P. M. Simmons and S. Moncada, *J. Pharm. Pharmac.* **35**, 808 (1983).
47. J. C. Fantone, W. A. Marasco, L. J. Elgas and P. A. Ward, *Am. J. Pathol.* **115**, 9 (1984).
48. Z. Duniec, J. Robak and R. Gryglewski, *Biochem. Pharmac.* **32**, 2283 (1983).