Biochemical Pharmacology, Vol. 35, No. 11, pp. 1903–1908, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

IONOPHORE-DEPENDENT GENERATION OF EICOSANOIDS IN HUMAN DISPERSED LUNG CELLS

MODULATION BY 6,9-DEEPOXY-6,9-(PHENYLIMINO)-$\Delta^{6,8}$-PROSTAGLANDIN I_1 (U-60,257)

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(*Received* 7 *October* 1985; *accepted* 17 *December* 1985)

Abstract—6,9-Deepoxy-6-9-(phenylimino)-$\Delta^{6,8}$-prostaglandin I_1, a prostacyclin analogue reported to inhibit sulphidopeptide leukotriene formation in animals, was evaluated for its pharmacological activity against eicosanoid and histamine release from human dispersed lung cells (HDLC). In the absence of drug, challenge of HDLC with A23187 (2.5 μM) increased immunoreactive eicosanoid generation by factors of 7.6 for prostaglandin (PG) D_2, 9.1 for TXB_2, 3.2 for $PGF_{2\alpha}$, 2.0 for 5-HETE, 6.3 for LTC_4, in association with a twofold increase in histamine release. When exogenous [^{14}C]-arachidonic acid was added to HDLC simultaneously with A23187 challenge, radiolabelled eicosanoids were recovered in the supernatant, but on separating the products by radio-thin layer chromatography the proportions of individual eicosanoids were not significantly different from unchallenged cells. With endogenous arachidonate, U-60,257 was a potent inhibitor of i-LTC_4 generation at 1 μM, but between 3 and 300 μM there was a concentration-related reversal of this inhibition. The effects of U-60,257 on the metabolism of exogenous [^{14}C]-arachidonic acid were also studied. Under these circumstances the drug was a potent inhibitor of both 5-HETE and 5,12-diHETE formation, without significantly affecting the formation of other mono-HETES. In agreement with previous endogenous substrate experiments there was a concentration-dependent inhibition of TxB_2 formation from exogenous arachidonic acid. These findings highlight the complex pharmacological actions of U-60,257 which appear dependent on the source of arachidonic acid substrate.

Slow reacting substance of anaphylaxis (SRS-A) has long been considered an important putative mediator of acute allergic reactions [1–9] and of particular relevance to the pathogenesis of asthma [9–13]. The structural elucidation of SRS-A as a mixture of C-6 substituted sulphidopeptide leukotriene products of the 5-lipoxygenase pathway of arachidonic acid metabolism [14–19] and the immunological generation of these substances by lung tissue [3–6, 8, 16, 17, 20] has led to the search for compounds which either inhibit the formation of, or block the pro-inflammatory actions of, these substances. Recently, 6,9 - deepoxy - 6,9 - (phenylimino) - $\Delta^{6,8}$ - prostaglandin I_1 (U-60,257, piriprost) has been described as a novel inhibitor at low micromolar concentrations of leukotriene (LT) C_4 and D_4 formation in rat peritoneal mononuclear cells and human neutrophil leukocytes [21–23]. U-60,257 also inhibits IgE-dependent bronchoconstriction induced by *Ascaris suum* in sensitized monkeys [24] and at higher concentrations it is a LT antagonist in guinea-pig ileum [21]. In a limited study on lung fragments from two subjects with birch pollen-sensitive asthma Dahlén and co-workers showed that a single high concentration of U-60,257 (100 μM) inhibited allergen-induced LTD_4 generation [25]. We have investigated the effects of U-60,257 on ionophore A23187 induced eicosanoid generation in human dispersed lung cells (HDLC) as our previous studies have shown that this drug had a surprising potentiating effect on prostaglandin (PG) D_2 release from human lung mast cells while inhibiting thromboxane (TX) B_2 release [26]. In the present series of experiments the effects of U-60,257 were evaluated with respect to the utilization of two sources of arachidonic acid (i) that derived from endogenous lipid sources and (ii) exogenous [1-^{14}C]-arachidonic acid added simultaneously with cell challenge. Radioimmunoassay (RIA) was used to quantify utilization from endogenous lipid-derived substrate, while radio-thin layer chromatography (r-TLC) was used to measure metabolism of exogenous arachidonic acid to eicosanoids.

MATERIALS AND METHODS

Materials. Papaya latex chymopapain, pronase type XIV, deoxyribonuclease (DNase) fraction V from bovine pancreas, dextran, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES), ionophore A23187, dimethylsulphoxide and gelatin type I from swine skin were purchased from Sigma Chemicals (Poole, Dorset, U.K.): human serum albumin was obtained from the Blood Products Laboratory (Elstree, Hertfordshire, U.K.). His-

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tamine acid phosphate, activated charcoal and Merck Silica gel 60 TLC plates (20 × 20 cm, layer thickness 0.025 cm) were purchased from BDH Chemicals (Poole, Dorset, U.K.). 6,9-deepoxy-6,9-(phenylimino)-$\Delta^{6,8}$-prostaglandin I_1 (U-60,257, piriprost), PGs D_2, E_2, $F_{2\alpha}$ and TxB_2 were generous gifts of the Upjohn Company (Kalamazoo, MI). Prostaglandins and U-60,257 were stored as ethanol solutions at −20°. In addition, U-60,257 was protected from light. Unlabelled LTs were generous gifts of Merck-Frosst Laboratories, Pointe-Claire, Canada. [1-^{14}C]-Arachidonic acid (57–60 mCi/mmol), [5,6,8,9,11,12,14,15 (*n*)-^{3}H]-$PGF_{2\alpha}$ (180 Ci/mmol), PGE_2 (160 Ci/mmol) and *S*-adenosyl-L-[methyl-^{3}H] methionine (60–85 Ci/mmol) were purchased from Amersham International PLC (Amersham, Buckinghamshire, U.K.). [5,6,8,9,12,14,15-(*n*)-^{3}H]-PGD_2 (100 Ci/mmol), [5,6,8,9,11,12,14,15-(*n*)-^{3}H]TxB_2 (155 Ci/mmol), 5-D-[5,6,8,9,11,12,14,15-(*n*)-^{3}H]-hydroxy-6,8,11,14-eicosatetraenoic acid (80 Ci/mmol), 15-L-[5,6,8,9,11,12,14,15-(*n*)-^{3}H]-hydroxy 5,8,11,13-eicosatetraenoic acid (15-HETE) (62 Ci/mmol), and [14,15-(*n*)-^{3}H]-LTC_4 and LTD_4 (40 Ci/mmol) and radioimmunoassay kits for TxB_2 and LTC_4 were purchased from New England Nuclear (Southampton, Hampshire, U.K.). Rabbit anti-PGD_2 serum was a generous gift from Dr L. Levine, Brandeis University, MA, U.S.A. Antiserum to $PGF_{2\alpha}$ was purchased from Miles-Yeda (Rehovot, Israel).

Measurement of 5-HETE was performed by RIA (Metachem Diagnostics, Piddington, Northants, U.K.). The cross-reactivities of these antisera to heterologous ligands have been described elsewhere [27, 28]. Sep Paks (C_{18}) were purchased from Waters Associates (Northwich, Cheshire, U.K.). All solvents were of analytical grade or equivalent.

Dispersion and separation of human lung cells. Fresh surgical specimens of lung were obtained from patients (mean age 65 ± 2.6 years) undergoing resection of bronchial carcinoma. Dispersed lung cells (HDLC) were prepared from grossly normal tissue samples using the method described previously [28]. Cells from three digestions with pronase and chymopapain (yield 1.6×10^7 nucleated cells/g wet weight lung) were combined and washed twice in Tyrode's solution buffered with 10 mM HEPES and containing 0.03% human albumin. The pooled cells were then gently resuspended in Tyrode's solution containing 0.1% gelatin and 0.2 mg/ml DNase and incubated to disperse cell aggregates at room temperature for 60 min on a mechanical roller. After this time the cells were resuspended in an appropriate volume of albumin-free Tyrode's (TGD) solution and aliquotted into Eppendorf tubes to give a final concentration of 10^7 nucleated cells per millilitre. Albumin was omitted at this stage to prevent the albumin catalyzed dehydration and isomerization of PGD_2.

Cell activation. Cells, prewarmed at 37° for 5 min, were incubated for 15 min with various concentrations of U-60,257 (1–300 μM) dissolved in 10 μl ethanol vehicle, before being challenged with 20 μl ionophore A23187 (final concentration 2.5 μM) in 12% dimethylsulphoxide vehicle. Control tubes contained TGD with 10 μl ethanol and were challenged with A23187 or aqueous dimethylsulphoxide. In all experiments the final reaction volume was 1 ml. Experiments showed that neither ethanol nor dimethylsulphoxide affected cell viability, mediator release or immunoassay of eicosanoids at the concentrations employed. In experiments to investigate the fate of exogenous arachidonic acid HDLC were simultaneously challenged with 1 μCi of [1-^{14}C]-arachidonic acid dissolved in TGD and reactions terminated after 20 min by centrifugation at 10,000 *g* for 20 sec (Beckman microfuge). Supernatant fractions for RIA were immediately frozen to −20° until assayed, while those for r-TLC were processed as described below.

Extraction and r-TLC. Supernatant fractions for r-TLC were decanted into ice-cold polycarbonate tubes and extracted at pH 8.0 on C_{18} Sep Pak cartridges as described in full elsewhere [28]. The extracts were then reduced under nitrogen to a volume of 50 μl, applied to separate tracks of a TLC plate and developed to 15 cm in toluene:1,4-dioxan:acetic acid (65:34:1.5 v:v:v) at 4°. Non-destructive quantitative analysis of the radioactive products was performed automatically using a Berthold LB284 linear analyzer interfaced to an Apple IIe microcomputer for data acquisition and processing. Radioactive peaks were identified by reference to authentic standards which were applied to separate tracks of each TLC plate.

High performance liquid chromatography (HPLC). HPLC of selected lung cell incubation extracts was performed using a Spectra-Physics SP8700 ternary pumping system and variable wavelength ultra-violet (u.v.) detector. Lipoxygenase products were analysed as described [28] using a 12.5 × 0.46 cm i.d. Techsphere 5 C_{18} column with a mobile phase comprising methanol:water:acetic acid 65:35:0.06 (v:v:v) adjusted to pH 5.3 with ammonia solution (specific gravity 0.88 g/ml). Flow rate was 1 ml/min with a column head pressure of 1200 psi. The column was calibrated using authentic standards of lipoxygenase products and additional confirmation of product identity obtained by scanning in the stopped-flow mode. Sulphidopeptide leukotrienes were quantified by guinea-pig ileum bioassay of appropriate fractions of HPLC effluent [29]. The purity of stock solutions of U-60,257 in methanol was checked using the mobile phase described above with a 12.5 × 0.46 cm Nucleosil 5 C_{18} column and u.v. detection at 235 nm. There was no appreciable decomposition of U-60,257 over the period of these experiments.

Mediator assays. RIA of eicosanoids was performed as described previously [27] using dextran coated charcoal to separate bound from free ligand. Our previous experiments had shown that at high concentrations (30–300 μM) U-60,257 interfered with the RIA of TxB_2 [26]. In the present experiments there was no interference in the RIAs, except for 5-HETE which showed non-specific cross-reaction with U-60,257. This effect was small (<0.31% at 50% maximum binding) but consistent and results for 5-HETE were therefore corrected for this effect. There was no interference with the LTC_4 immunoassay from U-60,257 at any of the concentrations used. Histamine was assayed using a microradio-

enzymatic technique employing semi-purified histamine methyl-transferase from rat kidney [30]. Lactate dehydrogenase activity was measured using the technique described by Amador *et al.* [31].

Statistical analysis. In order to allow direct comparison with our previous studies [26, 27] and to normalize data for variation in differential cell counts, results are presented as release in ng/10^6 mast cells. This normalization does not affect the conclusions regarding the pharmacological effects of U-60,257. Unless stated otherwise, all results have been corrected for spontaneous release. Values show mean ± S.E.M. Significance of differences was evaluated using Student's *t*-test (two-tailed) for paired samples, two way analysis of variance and Duncan's multiple range test.

RESULTS

Proteolytic treatment of human lung fragments dispersed a population of cells containing 5.1 ± 0.9% (mean ± S.E.M., range 2.8–8.8%, N = 6) mast cells as assessed by metachromasia of wet preparations using Kimura stain. Microscopic examination of cytocentrifuge preparations showed that the rest of the nucleated cells comprised eosonophils 0.1–1.3%, neutrophils 0.3–13%, macrophages 7–32% and lymphocytes 2–5%, with the remainder of the cells being type I and type II pneumocytes and bronchial epithelial cells. Cell viability was 91–97% measured by Trypan blue exclusion. In the absence of ionophore HDLC generated small amounts of eicosanoids from endogenous substrate as shown in Table 1. The cells also spontaneously released histamine (334 ± 61 ng/10^6 mast cells or 8.2% total histamine). Challenge of the cells with A23187 resulted in 2–9-fold increases in the generation of eicosanoids (Table 1) and increase in histamine release to 642 ± 132 ng/10^6 mast cells, 15.7% total histamine (N = 5, $P < 0.05$). The two major cyclooxygenase products generated were PGD_2 and TxB_2 with *net* generation of 16.57 ± 4.84 and 17.34 ± 5.80 ng/10^6 mast cells. These results are in accordance with our previous findings in enzymatically dispersed HDLC, although the comparatively low histamine release may be accounted for by the wide variation in "releasability" of histamine from pulmonary mast cells [27].

Table 1. The gross release of eicosanoids derived from endogenous arachidonate in dispersed human lung cells (N = 6 lungs)

Eicosanoid	Release (ng/10^6 mast cells) Unchallenged	+A23187 (2.5 μM)
PGD_2	2.51 ± 0.19	19.08 ± 4.87*
TXB_2	2.14 ± 0.38	19.48 ± 6.11*
$PGF_{2\alpha}$	1.29 ± 0.19	4.14 ± 1.07*
5-HETE	7.74 ± 1.57	15.80 ± 3.41
i-LTC_4	0.41 ± 0.08	2.57 ± 0.33†

* $P < 0.05$.

† $P < 0.001$ with respect to unchallenged (two-tailed paired *t*-test).

The data in the ionophore column have not been corrected for the spontaneous release indicated.

Experiments to investigate the fate of exogenous [^{14}C]-arachidonic acid revealed that in the presence of A23187 approximately 30% of the radioactivity recovered from the incubation medium consisted of cyclooxygenase and lipoxygenase metabolites. In 14 experiments this comprised $PGF_{2\alpha}$ 1.2 ± 0.2%; PGE_2 0.62 ± 0.20%; PGD_2 4.9 ± 1.0%; TxB_2 1.8 ± 0.3%; 5-HETE 4.9 ± 0.1%; HHT 2.4 ± 0.6%; 5,12-diHETES 2.5 ± 0.5%; mono-HETES 3.5 ± 0.6% and a polar peak 7.9 ± 2.1%. These values were not significantly different from the proportions of each compound present in supernatants from unchallenged cells. In a further study we used HPLC with guinea-pig ileum and guinea-pig neutrophil bioassay [29] to confirm the nature of the labelled lipoxygenase products generated when cells in 2 experiments were simultaneously challenged with 2.5 μM A23187 and [^{14}C]-arachidonic acid. Average net release of individual products (ng/10^6 mast cells), with proportions of total supernatant radioactivity shown in brackets were as follows: LTC_4 26.8 (4.6%); LTD_4 1.0 (1.3%); LTB_4 37.8 (6.0%); 5(S),12(R)-6-*trans*-diHETE 7.0 (1.0%); HHT 12.9 (3.6%); 15-HETE 5.7 (0.9%); 11-HETE 4.5 (0.7%); 12-HETE 19.2 (7.1%); 9-HETE 2.7 (0.5%) and 5-HETE 26.6 (3.8%).

Effects of U-60,257 on lipoxygenase product formation from endogenous arachidonate

The effects of U-60,257 on the formation of LTC_4 and 5-HETE from endogenous lipid esterified arachidonic acid were investigated in these experiments. As the antibody employed for the measurement of LTC_4 showed 55% and 8.6% cross-reaction with LTD_4 and LTE_4, as well as significant reaction with isomers of these heterologous ligands, levels are reported as immunoreactive LTC_4 equivalents (i-LTC_4). Unchallenged HDLC released only small quantities of i-LTC_4 (Table 1), which rose sixfold with A23187 challenge ($P < 0.001$). At a concentration of 1 μM, U-60,257 caused a 48% inhibition ($P < 0.02$) of net i-LTC_4 generation (Fig. 1), but at higher levels there was no statistically significant overall effect. However, in four out of six

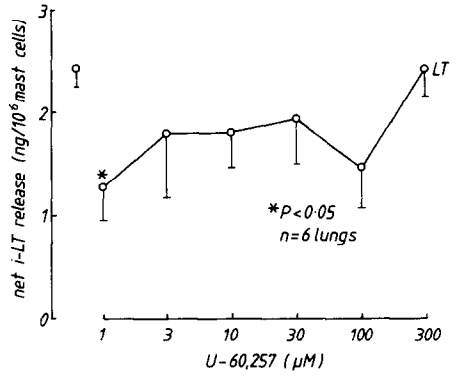

Fig. 1. The effect of U-60,257 on the net ionophore dependent release of immunoreactive LTC_4 equivalents. Control release in absence of drug is indicated on the left hand side of the figure. Data are mean ± S.E.M. in 6 lungs. *$P < 0.05$ with respect to control.

lungs the generation of i-LTC_4 in the presence of 300 μM U-60257 was 4–33% greater than in the absence of drug ($P < 0.05$). In these endogenous substrate studies there was no significant effect of the drug on 5-HETE generation. For example, in the presence of 30 μM U-60,257 the net release was 8.91 ± 5.15 ng/10^6 mast cells ($N = 6$) compared to a control value of 8.57 ± 3.81 ng.

In the absence of drug the net A23187 dependent release of histamine was 309 ± 132 ng/10^6 mast cells ($N = 5$) and this was unaffected by drug at 1 and 10 μM. However, at 100 μM the net release of histamine was potentiated slightly to 490 ± 101 ng/10^6 mast cells, 12.0% net total histamine ($P < 0.05$), but this was not correlated to the increased generation of PGD_2. Histamine release was not tested in the presence of 300 μM U-60,257. We do not know whether this increased release of histamine was due to cytotoxicity of the drug.

Effects of U-60,257 on utilization of exogenous arachidonic acid

In the second part of this study the effect of U-60,257 on the metabolism of exogenous arachidonic metabolism acid was studied by r-TLC after extraction of incubation supernatants. The proportion of free arachidonic acid in the supernatants was unaffected by low concentrations of U-60,257 (1–3 μM), but at higher concentrations the proportion of free arachidonic acid increased significantly from a control of $69.9 \pm 4.4\%$ to $83.1 \pm 3.7\%$ at 300 μM ($P < 0.05$). This was principally accounted for by reductions in the formation of 5-HETE and 5,12-diHETEs. In the 14 experiments the proportion of material with the chromatographic mobility of PGD_2 was enhanced 27–47% from control values by 1–10 μM U-60,257 (Fig. 2). In contrast, concentrations of the drug above 10 μM had statistically significant ($P < 0.05$–<0.01) inhibitory effects on TxB_2 formation (Fig. 2). The generation of both $PGF_{2\alpha}$ and 12-hydroxy-5,8,10-heptadecatrienoic acid was unaffected by the drug (data not shown).

The processing of arachidonic acid by 5-lipoxygenase was also modified by U-60,257. There was a significant inhibition of the proportion of radioactivity associated with 5-HETE and 5,12-diHETEs over the concentration range 10–300 μM (Fig. 3a). The formation of other mono-HETEs, which could not be further resolved under the TLC conditions employed, was less affected by the drug (Fig. 3b). In ionophore A23187 treated controls, $3.5 \pm 0.6\%$ of the supernatant radioactivity comprised uncharacterized mono-HETEs and this was reduced to $2.0 \pm 0.5\%$ ($P < 0.05$, $N = 14$) at 100 μM U-60,257. A feature of all chromatograms was a polar peak near the origin in r-TLC. In controls this represented $7.9 \pm 2.1\%$ of the radioactivity and was reduced to $4.0 \pm 0.7\%$ at concentrations of 30 μM U-60,257 and above ($P < 0.01$).

Effects of PGD_2 and $PGF_{2\alpha}$ on eicosanoid generation

To investigate previously reported effects of U-60,257 on TxB_2 release from HDLC [26], we examined the effects of exogenous PGD_2 and $PGF_{2\alpha}$ on the generation of TxB_2 and i-LTC_4 from endogenous substrate in HDLC. Aliquots of lung cell preparations containing $3.3 \pm 0.3\%$ ($N = 6$) mast cells and $0.3–1.0 \times 10^7$ nucleated cells/ml, were challenged with 2.5 μM A23187 with the simultaneous addition of either PGD_2, $PGF_{2\alpha}$ or ethanol vehicle

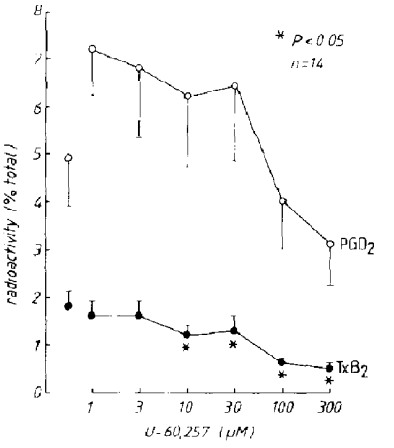

Fig. 2. The effect of U-60,257 on the formation of PGD_2 (○) and TxB_2 (●) from exogenous [^{14}C]-arachidonic acid in A23187 stimulated HDLC. Data are presented as the percentage of the total recovered supernatant radioactivity present as PGD_2 or TxB_2 and are means ± S.E.M. in 14 experiments. *$P < 0.05$ with respect to control value indicated on the left.

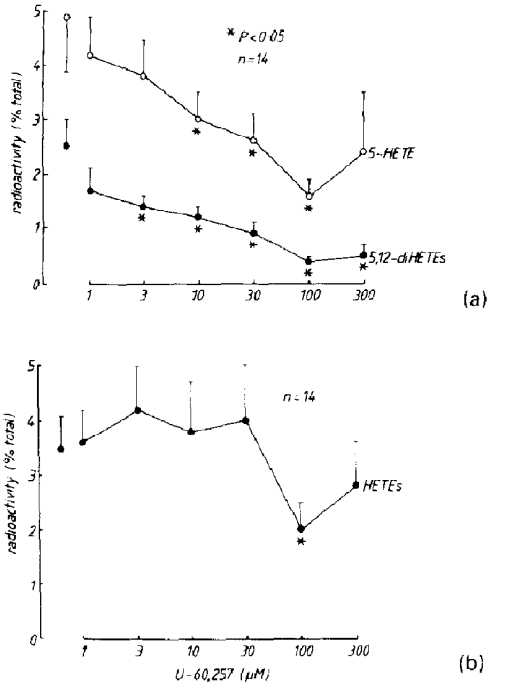

Fig. 3. (a) Inhibition by U-60,257 of the formation of 5-HETE (○) and 5,12-diHETEs (●) from exogenous [^{14}C]-arachidonic acid in the presence of A23187. Data are means ± S.E.M. in 14 experiments. *$P < 0.05$ with respect to control. (b) Effect of U-60,257 on the formation of mono-HETEs from [^{14}C]-arachidonic acid in the presence of A23187. Results are means ± S.E.M. and show the proportion of the recovered supernatant radioactivity accounted for by mono-HETEs. *$P < 0.05$ with respect to control.

(10 μl). In the absence of exogenous prostanoid the ionophore released a net (ng/10^6 mast cells) 7.53 ± 3.3 ng TxB_2, 2.67 ± 0.52 ng i-LTC_4 equivalents and 4.32 ± 2.94 ng PGD_2. Neither PGD_2 nor $PGF_{2\alpha}$, when added at concentrations of 10–300 ng/10^6 mast cells, produced any significant effects on TxB_2 or i-LTC_4 release in six lung preparations. Addition of PGD_2 or $PGF_{2\alpha}$ at the concentrations employed was not cytotoxic as indicated by measurements of lactate dehydrogenase activity.

DISCUSSION

Human dispersed lung cells have a high capacity to synthesize and release eicosanoids on calcium dependent cellular activation [8, 20, 27, 32]. Preincubation of HDLC with 1 μM U-60,257 resulted in almost 50% inhibition of ionophore-dependent i-LTC_4 generation, but at higher concentrations there was no inhibitory effect of the drug. These results contrast with the work of Dahlén and colleagues who demonstrated inhibition of sulphidopeptide LT formation with a 100 μM concentration of U-60,257 in human lung fragments [25]. Similarly, Bach and co-workers [21] have reported dose dependent inhibition of LT formation in human lung fragments over the range 2.3–230 μM. At present we do not understand why isolated cells yield different results from lung fragments, but one explanation is that higher drug concentrations are needed to produce inhibition in fragments due to the low diffusional access of the drug. However, for some compounds this is not a problem and concentration–inhibition curves are very similar in isolated cells and lung fragments [33]. Alternatively, the procedure of cell dispersion may have removed either some unidentified cell(s) or factor(s) which may be a component of the inhibitory action of U-60,257 in lung fragments.

Although U-60,257 failed to inhibit 5-HETE release from endogenous substrate separate studies demonstrated that the drug was clearly an inhibitor of 5-HETE and 5,12-diHETE formation at concentrations of 3–10 μM when exogenous substrate was used.

Our data do not identify the step(s) where U-60,257 exerts its complex actions in HDLC. In purified human neutrophils U-60,257 acts as a potent inhibitor of 5-lipoxygenase [34], but our data suggest that in the case of endogenous substrate this mechanism of action is unlikely to apply in HDLC. Our only evidence for the drug having 5-lipoxygenase inhibitory activity derives from experiments employing exogenous substrate. Interestingly, the ability of different concentrations of exogenous substrate to alter the apparent pharmacological effects of U-60,257 has been noted elsewhere [34] and may be due to complex competition between arachidonic acid, U-60,257 and 5-lipoxygenase. Bach and colleagues have suggested that U-60,257 might inhibit the incorporation of glutathione into LTA_4 [22] as the compound inhibits glutathione-*S*-transferase activity in rat basophil leukaemia (RBL-1) cells. More recent work with this cell line suggests that although U-60,257 is an inhibitor of cytoplasmic glutathione transferases, these isoenzymes are unrelated to the microsomal enzyme responsible for leukotriene formation [35]. Whether this also pertains in human lung is not known.

We have described elsewhere [26] the U-60,257 dependent inhibition of TxB_2 generation and stimulation of PGD_2 release from HDLC activated by A23187. When we examined the effects of U-60,257 on the formation of PGD_2 and TxB_2 from [^{14}C]-labelled exogenous substrate we found an inhibition of TxB_2 formation at concentrations comparable to those needed for the inhibition of TxB_2 synthesis from endogenous substrate. However, the results with PGD_2 were more complex, there being no significant potentiation at drug concentrations comparable to the previous report [26]. The present experiments also demonstrate that the inhibition of TxB_2 generation was not due to down regulation by PGD_2, and suggest that the previously reported correlation between the changes in PGD_2 and TxB_2 may be fortuitous [26]. Another explanation for the previously reported effects of U-60,257 on the formation of these cyclooxygenase products is that the drug might inhibit TX synthase whilst leaving endoperoxide formation intact. The endoperoxides could then be utilized for PGD_2 biosynthesis by mast cells [27]. A precedent exists for such transcellular metabolism of PG endoperoxides in platelets where released PGG_2 and PGH_2 may be metabolized to PGI_2 by vascular endothelial cells [36]. This explanation would be satisfactory were it not for the fact that in rat mononuclear cells U-60,257 and its methyl ester are reported not to inhibit TxB_2 [21]. Moreover, in our present experiments with exogenous substrate we have shown dissociation between changes in PGD_2 and TxB_2 formation which further argues against a direct relationship between these events [26].

In conclusion, these studies have shown that U-60,257 has complex pharmacological actions of unknown mechanism in ionophore activated HDLC. Under certain conditions with exogenous substrate the drug possessed activity consistent with an inhibitor of enzymes of the 5-lipoxygenase pathway. However, experiments investigating the utilization of endogenous substrate failed to produce convincing evidence in support of this mode of action, despite there being some inhibition of i-LTC_4 formation. Similar studies are in progress to investigate whether these effects are also observed in immunologically activated HDLC.

Acknowledgements—We thank the Medical Research Council for financial support, Dr L. S. Clegg for performing the histamine assay, Dr R. Agius for the differential cell counts and Mrs Margaret Dowling for typing the manuscript.

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