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Regulation of macrophage eicosanoid production by hydroperoxy- and hydroxy-eicosatetraenoic acids

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Resident mouse peritoneal macrophages when exposed to zymosan during the first day of cell culture synthesize and secrete large amounts of prostaglandin E_2 (PGE_2) and leukotriene C_4 (LTC_4), the respective products of cyclo-oxygenase- and 5-lipoxygenase-catalysed oxygenations of arachidonic acid. Under these conditions of cell stimulation only small amounts of hydroxyeicosatetraenoic acids (HETEs) are concomitantly produced. However, exogeneously added arachidonic acid is metabolized to large amounts of 12- and 15-HETE and only relatively small amounts of PGE_2. No LTC_4 is formed under these conditions. In contrast, resident mouse peritoneal macrophages in cell culture for 4 days synthesized less PGE_2 and LTC_4 when exposed to zymosan. However, these macrophage populations continue to synthesize 12-HETE from exogenously added arachidonic acid. Zymosan induced the secretion of a lysosomal enzyme, *N*-acetyl-β-glucosaminidase, equally in both 1- and 4-day cultures. Both 12- and 15-hydroperoxyeicosatetraenoic acids (HPETEs), the precursors of 12- and 15-HETE, were found to be irreversible inhibitors of the cyclo-oxygenase pathway and reversible inhibitors of the 5-lipoxygenase pathway in macrophages. On the other hand, 12- and 15-HETE were found to be reversible inhibitors of both pathways. Thus the oxidation of arachidonic acid to both prostaglandins and leukotrienes may be under intracellular regulation by products of 12- and 15-lipoxygenases.

INTRODUCTION

Resident mouse peritoneal macrophages synthesize and secrete products of both the cyclo-oxygenase and 5-lipoxygenase oxidative pathways (Humes *et al.*, 1977; Rouzer *et al.*, 1980). When these cells are exposed to zymosan, the principal products of these pathways are PGE_2, PGI_2 and LTC_4. In addition to these enzymic pathways, resident mouse peritoneal macrophages contain a diversity of other lipoxygenases which give rise to 5-, 8-, 9-, 11-, 12- and 15-HETEs (Rigaud *et al.*, 1979; Rabinovitch *et al.*, 1981; Scott *et al.*, 1982). In addition, these HETEs can be further oxidized to their corresponding di-HETEs (Mass *et al.*, 1982).

There are many reports showing that HPETEs and HETEs have regulatory effects on the enzymes of the arachidonic acid cascade. 12-HPETE, but not 12-HETE, inhibits platelet cyclo-oxygenase with an 50% inhibitory concentration of 3 μM (Siegel *et al.*, 1979). Lower concentrations of 12-HPETE caused an increase in 12-lipoxygenase activity, presumably by acting as the hydroperoxy activator of lipoxygenases as described by Hemler *et al.* (1978). Various other hydroperoxy compounds, including 15-hydroperoxy-PGE_1 and 15-HPETE have, in a similar manner, been shown by Egan *et al.* (1981) to inactivate the cyclo-oxygenase associated with ram vesicular gland microsomes. These workers showed that an oxygen-centred radical was released from the hydroperoxy moiety of PGG_2 during its peroxidative reduction to PGH_2. This oxidant inactivates the cyclo-oxygenase as well as other enzymes of PG synthesis. Prostacyclin synthetase is particularly sensitive to this oxidative inactivation (Ham *et al.*, 1979). In an apparently similar manner, 15-HPETE is a potent inhibitor of both the 5-lipoxygenase and 12-lipoxygenase from rabbit polymorphonuclear leukocytes and rabbit plates (Vanderhoek *et al.*, 1982).

In addition to the HPETE-mediated oxidative inactivation of cyclo-oxygenase and lipoxygenases, the corresponding HETEs are also inhibitors of these enzymes. 15-HETE has been shown to be a selective inhibitor of both the 5- and 15-lipoxygenase of rabbit polymorphonuclear leukocytes (Vanderhoek *et al.*, 1980). 15-HETE also inhibits the cyclo-oxygenase associated with ram vesicular gland microsomes (Egan *et al.*, 1981). Thus, both HPETEs and HETEs can regulate cyclo-oxygenase and lipoxygenases at the enzyme level.

These compounds have also been shown to regulate certain cellular functions. 15-HPETE has been shown to inhibit the production of 5-HETE and LTB_4 but not 11-HETE or PGE_2, by Ca^{2+} ionophore A-23187-treated human T-lymphocytes (Goetzl, 1981). 15-HETE also inhibits mitogenesis of T-lymphocytes induced by the mitogen phytohaemagglutinin or phorbol myristate acetate but had no effect on the B-lymphocyte mitogenic response to lipopolysaccharide (Bailey *et al.*, 1982). Furthermore, 15-HPETE has been shown to inhibit the generation of killer cells (Gaulde *et al.*, 1983).

These present studies demonstrate that these compounds regulate both PG and LT synthesis in mouse peritoneal macrophages. These findings suggest the possibility that the cyclo-oxygenase and 5-lipoxygenase pathways are under intracellular control by products of other lipoxygenase enzymes. Therefore, lipoxygenase

Abbreviations used: HIPS, heat inactivated porcine serum; HPETEs, hydroperoxyeicosatetraenoic acids; HETEs, hydroxyeicosatetraenoic acids; LT, leukotriene; PG, prostaglandin.

products, such as 12- and 15-HETE, must be considered as potential intracellular regulants.

MATERIALS AND METHODS

Animals

Female CFW-1 mice, 15–25 g, were purchased from Charles River Laboratories, Wilmington, MA, U.S.A. and maintained on a standard pellet diet and water *ad libitum.*

Chemicals

M-199 medium, other tissue culture reagents and porcine serum were purchased from Grand Island Biological, Grand Island, NY, U.S.A. The porcine serum was inactivated by heating at 56 °C for 30 min. Twelve-well tissue culture cluster plates (2.4 cm × 1.7 cm wells) were purchased from Linbro Division, Flow Laboratories, McLean, VA, U.S.A. Zymosan was from ICN Nutritional Biochemicals, Cleveland, OH, U.S.A. and was prepared as previously described (Bonney *et al.*, 1978). Prostaglandin E_2 was purchased from Ono Pharmaceutical Company, Osaka, Japan; Leukotrienes C_4 and D_4 were provided by J. Rokach, Merck-Frosst, Montreal, Canada. Anti-LTC_4 sera were provided by E. C. Hayes of these laboratories (Hayes *et al.*, 1983). Antisera to 12-HETE and radioimmunoassay reagents for this assay were supplied in kit form by Seragen, Boston, MA, U.S.A. [5,6,8,11,12,14,15-^{3}H(N)]PGE_2 (100–200 Ci/mmol), [14,15-^{3}H(N)]LTC_4 (20–60 Ci/mmol), [5,6,8,9,11,12,14,15-^{3}H(N)] arachidonic acid (80 Ci/mmol) and [1-^{14}C]arachidonic acid (52 mCi/mmol) were purchased from New England Nuclear, Boston, MA, U.S.A. Arachidonic acid and soybean lipoxygenase were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Triphenylphosphine was purchased from Eastman Kodak, Rochester, NY, U.S.A.

Preparation of HPETEs and HETEs

15-HPETE was prepared from arachidonic acid and soybean lipoxygenase by the method of Crawford *et al.* (1978) with modifications. 15-HPETE was reduced with triphenylphosphine to yield 15-HETE as described by Crawford *et al.* (1978). 12-HPETE and 12-HETE were prepared from a rat platelet homogenate incubated with arachidonic acid according to the procedure of Pace-Asciak *et al.* (1983). These products were purified by normal-phase h.p.l.c. (μPorasil column; Waters Associates, Milford, MA, U.S.A.) with an isocratic solvent system composed of hexane/propan-2-ol/glacial acetic acid (991:8:1, by vol.). The purity of the h.p.l.c.-purified compounds were assessed by u.v. absorbance spectrophotometry and were stored in methanolic solution at −20 °C.

Cell culture conditions

Resident peritoneal macrophages were obtained by lavage from the peritoneal cavity of mice and placed into cell culture as previously described (Bonney *et al.*, 1978).

Radioimmunoassay determinations

The macrophages were incubated overnight in 1 ml of M-199 containing 1% HIPS. After 16–20 h, the medium was removed and the adherent cells washed twice with 2 ml of M-199 devoid of serum. The cells were incubated with additions as described in 1 ml of this serum-free medium. After 2 h, the conditioned media were removed and the amounts of PGE_2, LTC_4 and 12-HETE were quantified by radioimmunoassay using the dextran-coated-charcoal binding method as previously described (Humes *et al.*, 1981). The anti-LTC_4 sera cross-reacted to an extent of approx. 50% with LTD_4 (Hayes *et al.*, 1983). However, the culture media from zymosan-stimulated resident cells contained exclusively LTC_4 (no LTD_4 was detected) as evaluated by radioimmunoassay of fractions from reverse-phase h.p.l.c. of the culture media (Bonney *et al.*, 1985).

Determination of [^{3}H]PG and [^{3}H]LT synthesis

The macrophages were incubated with 1.5 μCi of [^{3}H]arachidonic acid in 1 ml of M-199 containing 1% HIPS. After 16–20 h the medium was removed and the radiolabelled cells were washed twice with 2 ml of M-199 devoid of serum. The cells were incubated with additions as described in 1 ml of this serum-free medium. After 2 h the medium was removed, acidified to pH 4.5, extracted and chromatographed for [^{3}H]LTC_4 and [^{3}H]PGE_2 as previously described (Humes *et al.*, 1982).

[^{14}C]Arachidonic acid metabolism

Macrophages were incubated overnight in 1 ml of M-199 containing 1% HIPS. After 16–20 h, the medium was removed and the adherent cells were washed twice with 2 ml of M-199 devoid of serum. The cells were incubated with 2 μM-[^{14}C]arachidonic acid (0.2 μCi) for 2 h. The medium was removed, acidified to pH 4.5 with 0.1 M-sodium acetate buffer and extracted with two successive 3 ml portions of diethyl ether. The combined ether phases were evaporated under to dryness and the resulting residue was dissolved in acetonitrile/water (37:63). This material was chromatographed on a Waters C_{18} Bondapak h.p.l.c. column eluted with a 35–85% gradient of acetonitrile in water containing 0.058% phosphoric acid, by using the Waters gradient curve number 7 for 0–48 min and an isocratic elution for 48–60 min. The radioactive components were analysed with a Flow-ONE, Model HP, radioactivity flow detector. The identification of individual components was confirmed by comparison of their retention times with authentic standards.

Enzyme assays

Triton X-100 (1 ml of 0.1% in phosphate-buffered saline) was added to the cells, and the lysed cells were removed from the plate. Lactate dehydrogenase activity was determined in both the media and cell lysates by copper-neocuproine colorimetery coupled to the reduction of NAD (Morgenstern *et al.*, 1965). *N*-Acetyl-β-glucosaminidase activity in both the medium and cell lysates was determined by the method of Woolen *et al.* (1961) adapted to Titertek automated colorimetry. The protein content of the Triton/saline cell lysate was determined by the method of Lowry *et al.* (1951).

RESULTS

The cultures of mouse peritoneal macrophages used for these studies have been shown to be greater than 95% macrophages by both morphological and functional criteria (Bonney *et al.*, 1978). The protein content was approx. 50–100 μg of protein/culture. Cell viability in

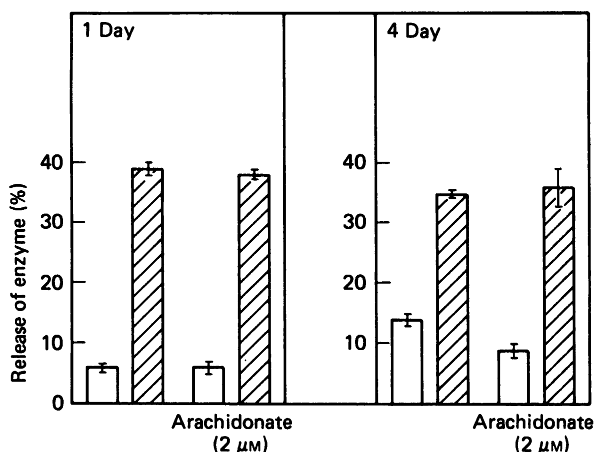

Fig. 1. Zymosan stimulates *N*-acetyl-β-glucosaminidase secretion from resident mouse peritoneal macrophages in cell culture for 1 or 4 days

Macrophages were incubated in 1 ml of M-199 containing 1% HIPS either overnight (1-day cells) or for 4 days as described in the Materials and methods section. The cells were washed with M-199 devoid of serum and then incubated in 1 ml of this medium in the presence or absence of 2 μM arachidonic acid and/or zymosan, 50 μg/ml. After 2 h the amount of *N*-acetyl-β-glucosaminidase in both the media and cell lysate was determined. The results are the average of duplicate determinations; □, no Zymosan; ▩, + Zymosan.

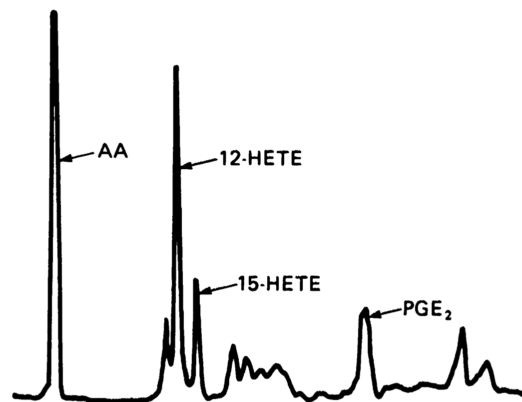

Fig. 3. Metabolism of exogenous [^{14}C]arachidonic acid (AA)

One day resident mouse peritoneal macrophages prepared as described in the legends to Figs. 1 and 2 were incubated for 2 h with 2 μM-[^{14}C]arachidonic acid. The medium was extracted and analysed for ^{14}C metabolites by h.p.l.c. as described in the Materials and methods section.

these experiments was greater than 85% as assessed by the retention of lactate dehydrogenase by the cells.

As many of the experiments on the synthesis of eicosanoids involved maintaining the cells for as long as 4 days in culture we examined the cells for their ability to release selectively the lysosomal enzyme *N*-acetyl-β-D-glucosaminidase. The amount of secretion due to zymosan-stimulation was identical on day 1 and 4 of culture and unaffected by the addition of arachidonic acid (Fig. 1). In addition the absolute amounts of enzyme activity in 1- and 4-day cultures were approximately equal (results not shown).

Production of PGE_2, LTC_4 and 12-HETE

Only low levels of PGE_2, LTC_4 and 12-HETE are synthesized in resting cultures of macrophages as measured by radioimmunoassay (Fig. 2). The addition of zymosan dramatically increased the synthesis and secretion during day 1 of culture of PGE_2(Fig. 2*a*) and LTC_4 (Fig. 2*b*) but not 12-HETE (Fig. 2*c*). However,

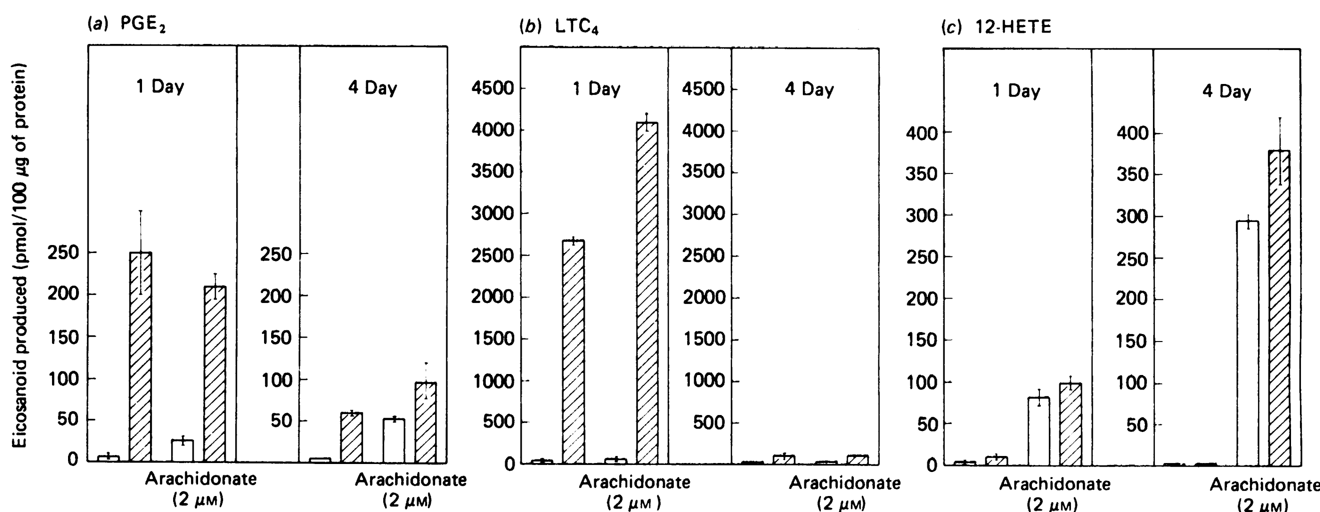

Fig. 2. PGE_2, LTC_4 and 12-HETE synthesis

Macrophages were incubated in 1 ml of M-199 containing 1% HIPS either overnight (1-day cells) or for 4 days as described in the Materials and methods section. The cells were washed with 1 ml of M-199 devoid of serum and then incubated in 1 ml of this medium in the presence or absence of 2 μM-arachidonic acid and/or zymosan, 50 mg/ml. After 2 h the medium were removed and the amounts of PGE_2, LTC_4 and 12-HETE were determined by specific radioimmunoassays. □, no zymosan; ▩, zymosan.

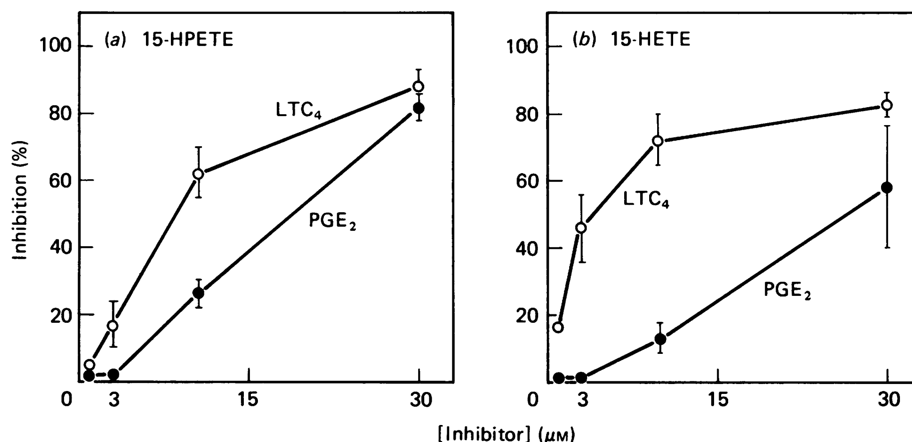

Fig. 4. 15-HPETE and 15-HETE inhibit zymosan-stimulated PGE_2 and LTC_4 synthesis

Resident mouse peritoneal macrophages were incubated overnight with 1.5 μCi of [^{3}H]arachidonic acid in 1 ml of M-199 containing 1% HIPS. The cells were washed with M-199 devoid of serum and then incubated in 1 ml of the medium containing 15-HPETE or 15-HETE. Zymosan, 50 μg/ml, was immediately added and the incubations continued for 2 h. The medium was extracted and chromatographed for [^{3}H]LTC_4 and [^{3}H]PGE_2 as described in the Materials and methods section.

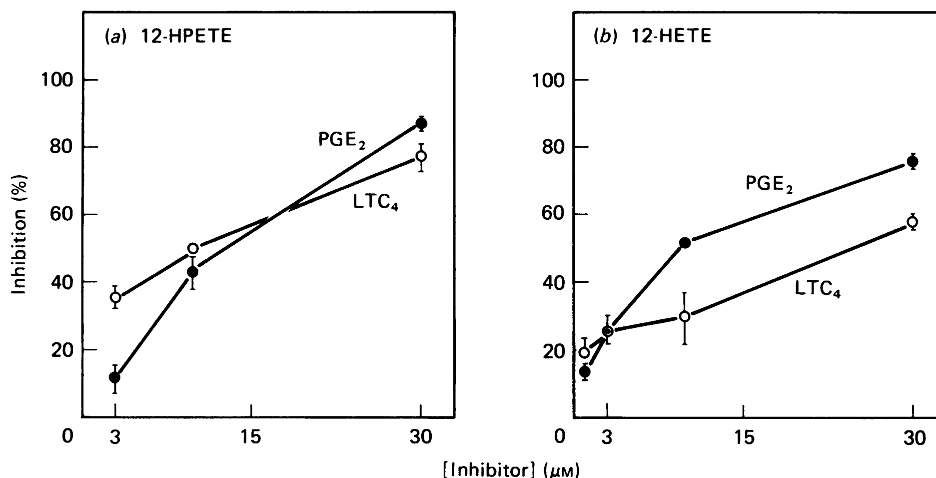

Fig. 5. 12-HPETE and 12-HETE inhibit zymosan-stimulated PGE_2 and LTC_4 synthesis

The conditions are the same as described in the legend to Fig. 4.

Table 1. LTB_4 does not inhibit PG or LT synthesis

The data represent the mean of duplicate determinations.

Compound	Concn. (μM)	Inhibition of zymosan-stimulated synthesis (%)	
		LTC_4	PGE_2
LTB_4	30	16	8
LTB_4	10	13	22
LTB_4	33	9	23
15-HETE	30	71	66
15-HPETE	30	90	86

without zymosan, the addition of 2 μM-arachidonic acid was found to stimulate greatly the synthesis of 12-HETE (Fig. 2c), slightly increase the synthesis of PGE_2 (Fig. 2a) and not to affect the synthesis of LTC_4 (Fig. 2b). After 4 days in culture, neither arachidonic acid nor zymosan were able to induce the synthesis and secretion of LTC_4 (Fig. 2c). In addition PGE_2 synthesis in response to zymosan was also diminished as compared with the synthesis in 1-day cells (Fig. 2a); however, arachidonic acid was still able to induce a small amount of PGE_2 synthesis in cells cultured for 4 days (Fig. 2a). Most importantly, arachidonic acid continued to cause a large increase in production of 12-HETE (Fig. 2c). Thus the synthesis of 12-HETE in response to exogenously added arachidonic acid in 1- or 4-day cultures is independent of synthesis of any 5-lipoxygenase products.

In order to characterize further the profile of products formed by the addition of arachidonic acid we incubated 1-day cells with 2 μM-[^{14}C]arachidonic acid for 2 h, then extracted the culture media. The h.p.l.c. analysis of the extract clearly showed that 12-HETE was the major product, although significant amounts of 15-HETE and PGE_2 were also formed (Fig. 3). A similar profile of products was found with 4-day cells.

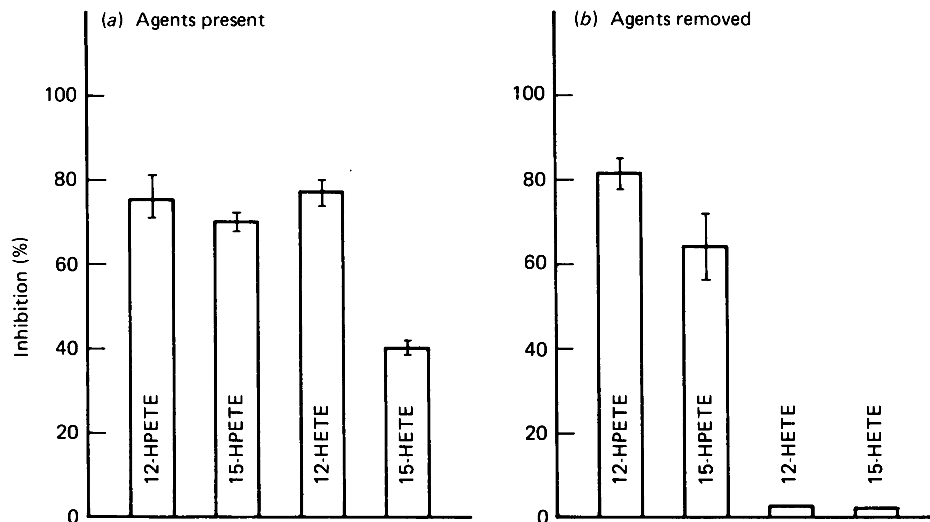

Fig. 6. HPETEs are irreversible inhibitors and HETEs are reversible inhibitors of zymosan-stimulated PGE_2 synthesis

One-day resident mouse peritoneal macrophages were prelabelled with [^{3}H]arachidonic acid as described in the Materials and methods section. After washing with serum-free M-199, the cells were exposed for 15 min to 30 μM-HPETEs or -HETEs in 1 ml of serum-free M-199. In (*a*) the cells were not washed. In (*b*) the cultures were washed five times with 2 ml of M-199 containing 1% delipidated bovine serum albumin. Zymosan (50 μg/ml) was then added. After 2 h the medium was removed and the amounts of [^{3}H]PGE_2 were determined as described in the Materials and methods section. The results are the average of duplicate determinations.

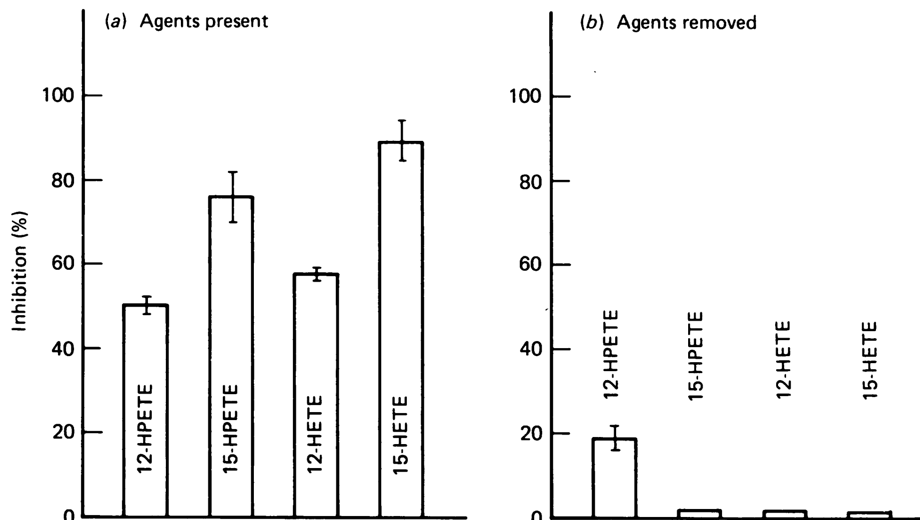

Fig. 7. Both HPETEs and HETEs are reversible inhibitors of zymosan-stimulated LTC_4 synthesis

One-day resident mouse peritoneal macrophages were prelabelled with [^{3}H]arachidonic acid. After washing with serum-free M-199 the cells were exposed for 15 min to 30 μM-HPETEs or -HETEs in 1 ml of serum-free M-199. In (*a*) the cells were not washed. In (*b*) the cultures were washed as described in Fig. 6. Zymosan (50 μg/ml) was then added. After 2 h the medium was removed and the amounts of [^{3}H]LTC_4 were determined as described in the Materials and methods section. The results are the average of duplicate determinations.

Effect of HPETEs and HETEs on zymosan-stimulated PGE_2 and LTC_4 synthesis

The exposure of [^{3}H]arachidonic acid-prelabelled cells to zymosan stimulated the synthesis of [^{3}H]PGE_2 and [^{3}H]LTC_4 and the release of these products into the culture medium. In the presence of 15-HPETE and 15-HETE, the synthesis of these products was inhibited in a concentration-dependent manner (Fig. 4). The inhibition of PGE_2 and LTC_4 synthesis by 15-HPETE was similar, approx. 5–20 μM to achieve a 50% inhibition with a maximum of 80% at 30 μM. In contrast, 15-HETE inhibited LTC_4 synthesis (50% inhibition at 3 μM) with only an approx. 50% inhibition of PGE_2 synthesis noted at 30 μM. In a similar manner 12-HPETE and 12-HETE inhibited zymosan-stimulated [^{3}H]LTC_4 and [^{3}H]PGE_2

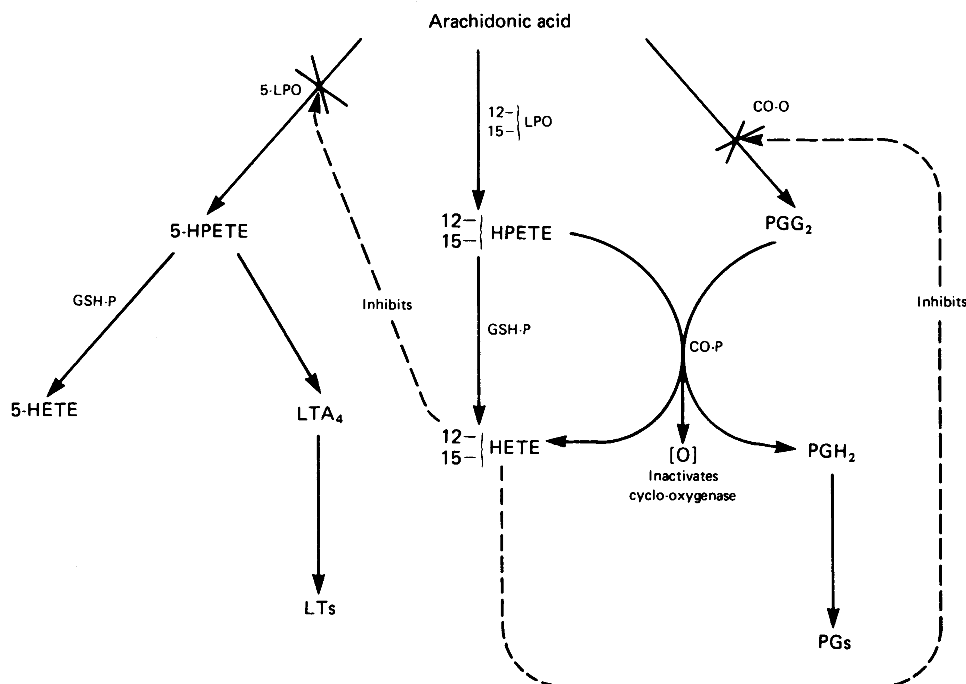

Fig. 8. Regulation of PGE$_2$ and LTC$_4$ synthesis by HPETEs and HETEs

Abbreviations used: LPO, lipoxygenase; CO-O, dioxygenase component of cyclo-oxygenase; CO-P, peroxidase component of cyclo-oxygenase; GSH-P, gluthathione peroxidase.

synthesis (Fig. 5). LTB$_4$ did not significantly inhibit the synthesis of either product at these concentrations (Table 1).

Effect of pretreatment with HPETEs and HETEs

The exposure of [^{3}H]arachidonic acid prelabelled cells to either 12- or 15-HPETE for 15 min resulted in an irreversible inhibition of zymosan-stimulated PGE$_2$ synthesis (Fig. 6). In this protocol the cells were incubated with the HPETEs (30 μM) for 15 min prior to zymosan addition. However, incubation periods as short as 5 min resulted in similar irreversible inhibition of PGE$_2$ synthesis. In contrast, the inhibition of the corresponding zymosan-stimulated LTC$_4$ synthesis was completely reversible (Fig. 7). Both 12- and 15-HETE inhibited the synthesis of LTC$_4$ and PGE$_2$ in a reversible fashion (Figs 6 and 7). The washing procedure did not appreciably change the amounts of zymosan-stimulated PGE$_2$ and LTC$_4$ synthesis (results not shown).

DISCUSSION

A proposal that one set of arachidonic acid metabolites interact and regulate the synthesis of LTs and PGs at the cellular level is speculative. However, certain biochemical facts are known which may explain these present observations describing the regulation of PG and LT synthesis in mouse peritoneal macrophages. It is clear that macrophages have the capacity to synthesize and secrete large quantities of PGs and LTs. However, the regulation of these two synthetic pathways appear to be under independent control, as certain inflammatory stimuli such as phorbol myristate acetate and lipopolysaccharide stimulate PG synthesis exclusively without correspondingly stimulating LTC$_4$ synthesis, whereas zymosan stimulates both oxidative pathways (Humes *et al.*, 1982). Both HPETEs and HETEs have been shown to modify or inhibit both cyclo-oxygenase and 5-lipoxygenase in a variety of cell and cell-free preparations (Siegel *et al.*, 1979; Vanderhoek *et al.*, 1980, 1982).

The addition of 12-HPETE or 12-HETE at the same time as zymosan resulted in a concentration-dependent inhibition of zymosan-stimulated LTC$_4$ and PGE$_2$ synthesis. A similar pattern of inhibition was observed with 15-HPETE and 15-HETE (Figs 4 and 5). However, the mechanism of the inhibition of zymosan-stimulated LTC$_4$ synthesis is clearly different from the effect on the corresponding PGE$_2$ synthesis. The addition of the HPETEs result in a reversible inhibition of LTC$_4$ synthesis but an irreversible inhibition of PGE$_2$ synthesis (Figs. 6 and 7). The corresponding HETE-mediated inhibitions of LTC$_4$ and PGE$_2$ synthesis are fully reversible. These pharmacological observations allow certain physiological predictions regarding the cellular control of LT and PG synthesis. This hypothesis is depicted in Fig. 8.

In this scheme 12- and 15-HETE are shown to be enzyme inhibitors for cyclo-oxygenase and 5-lipoxygenase enzymes, as the inhibitions mediated by these compounds are readily reversible. 15-HPETE has been shown to be a competitive inhibitor of 5-lipoxygenase in rabbit polymorphonuclear leukocytes (Vanderhoek *et al.*, 1980, 1982), from human T lymphocytes (Goetzl, 1981) and has also been shown to inhibit the microsomal cyclo-oxygenase from ram vesicular glands (Egan *et al.*, 1981).

The irreversible inhibition of PGE$_2$ synthesis by the HPETEs is in accord with studies *in vitro* on the self-inactivation of cyclo-oxygenase (Egan *et al.*, 1981).

Cyclo-oxygenase has two catalytic functions, a dioxygenase converting arachidonic acid to PGG_2 and a peroxidase component reducing PGG_2 to PGH_2 and an oxygen-centred radical. The cyclo-oxygenase–peroxidase can accept a variety of hydroperoxy substrates, including 15-HPETE (Egan *et al.*, 1981). 15-HPETE has been shown to inactivate cyclo-oxygenase, presumably as the result of being metabolized by the peroxidase component of the cyclo-oxygenase to 15-HETE and the inactivating oxidant (Egan *et al.*, 1981).

Glutathione peroxidase, a cytoplasmic enzyme, can also reduce HPETEs to HETEs but without the generation of an oxygen radical. As the 5-lipoxygenase is also apparently a soluble enzyme, one would predict that glutathione peroxidase would protect cytosol enzymes from HPETE inactivation. Therefore, the reversible inhibition of the LT pathway by HPETE may be due to the competitive inhibitory effects of the HETEs that are formed.

We have previously reported that populations of peritoneal macrophages elicited with various agents such as thioglycollate broth or *Corynebacterium parvum* have a diminished capacity to synthesize PGs when exposed to zymosan in cell culture (Humes *et al.*, 1980). Rouzer *et al.* (1980) also have shown that *Corynbacterium parvum*-elicited peritoneal macrophages produce less LTC_4 than do resident macrophages. In a similar manner to macrophages elicited *in vivo*, macrophages in cell culture for 4 days were unable to synthesize as much LTC_4 or PGE_2 when exposed to zymosan as compared with identically treated cells in culture for only 1-day. However, cultures of 4-day macrophages were able to metabolize exogenous arachidonic acid to PGE_2. Thus, the diminished response of these cells to produce PGE_2 when stimulated with zymosan is more complex than simply the inactivation of cyclo-oxygenase. Alterations of other controlling mechanism such as substrate availability from phospholipid pools and/or phospholipase activation may also be affected in 4-day cells.

The 4-day old cells, however, continued to synthesize somewhat more 12-HETE as compared with macrophages in culture for only 1 day. These observations are consistent with the notion that the continued synthesis of HPETEs and HETEs caused the diminished synthesis of PGs and LTs in cells remaining in culture for extended periods of time. The synthesis of HPETEs and HETEs in macrophage populations elicited *in vivo* that have a diminished capacity to synthesize PGs and LTs is presently under investigation.

The physiological significance of exogenous arachidonic acid as well as various HPETEs and HETEs is not known. However, in one pathological situation, namely psoriasis, largely elevated amounts of both free arachidonic acid as well as 12-HETE have been found in the involved epidermis (Hammarstrom *et al.*, 1975). In contrast with the extremely elevated levels of arachidonic acid and 12-HETE, the levels of prostaglandins in the psoriatic lesion were only modestly elevated. Of interest is the report that topically applied glucocortoids lowered the elevated amounts of arachidonic acid in the involved tissue and caused a visual improvement of the disease (Hammarstrom *et al.*, 1977).

Lipoxygenase products thus have the potential to act as intracellular regulators to modulate LT and PG synthesis as well as other cellular functions. Recently Chang *et al.* (1985) also reported effects of 5-, 5-lactone-, 12- and 15-HETEs on LT and PG synthesis in resident mouse peritoneal macrophages. In contrast to their studies, we have evaluated and contrasted both HPETEs as well as HETEs. Our studies clearly show that there are marked differences between HPETEs and HETEs in their mechanism of modification or inhibition of both LT and PG synthesis.

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