Biochemical Pharmacology, Vol. 44, No. 5, pp. 955–963, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00
Pergamon Press Ltd

DIFFERENTIAL TURNOVER OF ENZYMES INVOLVED IN HUMAN MONOCYTE EICOSANOID METABOLISM

SELECTIVE INHIBITION OF CYCLOOXYGENASE PRODUCT FORMATION BY CYCLOHEXIMIDE IN THE ABSENCE OF EFFECTS ON 5-LIPOXYGENASE OR PHOSPHOLIPASE A_2

THOMAS HOFFMAN,* YOUNG LIM LEE, ELAINE F. LIZZIO, ANIL K. TRIPATHI, EZIO BONVINI and JOSEPH PURI

Laboratory of Cell Biology, Division of Hematology, Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Bethesda, MD 20892, U.S.A.

(*Received* 22 *November* 1991; *accepted* 21 *April* 1992)

Abstract—Human monocytes treated with cycloheximide (CHX) demonstrated a concentration- and time-dependent inhibition of prostaglandin E_2 (PGE_2) synthesis and release in response to stimulation with phorbol myristate acetate, ionomycin, serum-treated zymosan, or concanavalin A. The effect of CHX required preincubation and was largely reversible within 2 hr. Thromboxane A_2 release was affected similarly but no comparable effects were observed on labeled arachidonic acid release or leukotriene B_4 generation. The PGE_2 response was also inhibited by CHX when monocytes were given exogenous arachidonic acid with or without stimulation. CHX pretreatment also comparably decreased the amount of immunoreactive cyclooxygenase in resting and stimulated monocytes. These data indicate that monocyte cyclooxygenase, in contrast to phospholipase A_2 or 5-lipoxygenase and their regulatory proteins, turns over rapidly and may be a target for up- or down-regulation by pharmacologic or (potentially) physiologic agents which affect protein synthesis or degradation.

Monocytes and macrophages are rich sources of eicosanoids. Depending on their mode of stimulation, they produce and release products of the cyclooxygenase and lipoxygenase pathways [1]. The regulation of eicosanoid formation in monocytes and macrophages, as in a variety of other tissues, seems to be exerted mainly at the level of phospholipase A_2. Agents that activate protein kinase C or increase intracellular calcium promote arachidonic acid (AA†) release [2]. In addition, two regulatory proteins, phospholipase A_2 activating protein [3] and lipocortin [4] (annexin I, formerly "lipomodulin"), are thought to control phospholipase A_2 activity in a positive or negative fashion, respectively.

A variety of evidence indicates that eicosanoid formation may also be regulated of points in arachidonic acid metabolism distal to phospholipase A_2. Recently a protein has been isolated and its gene cloned which activates 5-lipoxygenase and may regulate its translocation to the membrane [5]. This protein, also found in human monocytes, has been named 5-lipoxygenase activating protein. Levels of individual enzymes of the prostaglandin (PG) biosynthetic pathway, including cyclooxygenase (PGH synthase, EC 1.4.99.1), may be determined by a balance between their synthesis and catabolism (turn-over) [6]. Cyclooxygenase activity may also be up-regulated at the level of mRNA transcription in transformed cell lines [7].

In consideration of the fact that multiple stimuli initiate eicosanoid formation in human monocytes, it was of interest to determine whether they exert their action at the same or different points in AA metabolism. To determine whether monocytes may regulate eicosanoid production by alterations in the levels of specific enzymes involved in eicosanoid biosynthesis, we exposed monocytes to cycloheximide (CHX) prior to challenge with agents known to promote prostaglandin E_2 (PGE_2) or leukotriene B_4 (LTB_4) formation. Our findings indicated that resting monocytes had rapidly turning over cyclooxygenase compared to a more stable lipoxygenase pathway, suggesting that inhibition of cyclooxygenase synthesis could be a target for regulating the amount of PG production.

MATERIALS AND METHODS

Cells. Peripheral blood monocytes were prepared at the NIH Clinical Center by countercurrent centrifugal elutriation using techniques designed to yield large numbers of purified monocytes suitable for clinical use [8]. Briefly, peripheral blood mononuclear cells from leukapheresis were isolated by using automated Ficoll–Hypaque density gradient centrifugation. Elutriation was performed in a model J-6M centrifuge (Beckman Instruments, Palo Alto, CA) equipped with a JE-5.0 rotor operating at 1440 *g*. Monocytes were collected in Hanks' Balanced

* Corresponding author. Tel. (301) 496-4538; FAX (301) 402-2780.

† Abbreviations: AA, arachidonic; CHX, cycloheximide; Con A, concanavalin A; EBSS, Earle's Balanced Salt Solution; HBSS, Hanks' Balanced Salt Solution; IoM, ionomycin; Isc, Iscove's medium; LT, leukotriene; PG, prostaglandin; PMA, phorbol myristate acetate; and STZ, serum-treated zymosan.

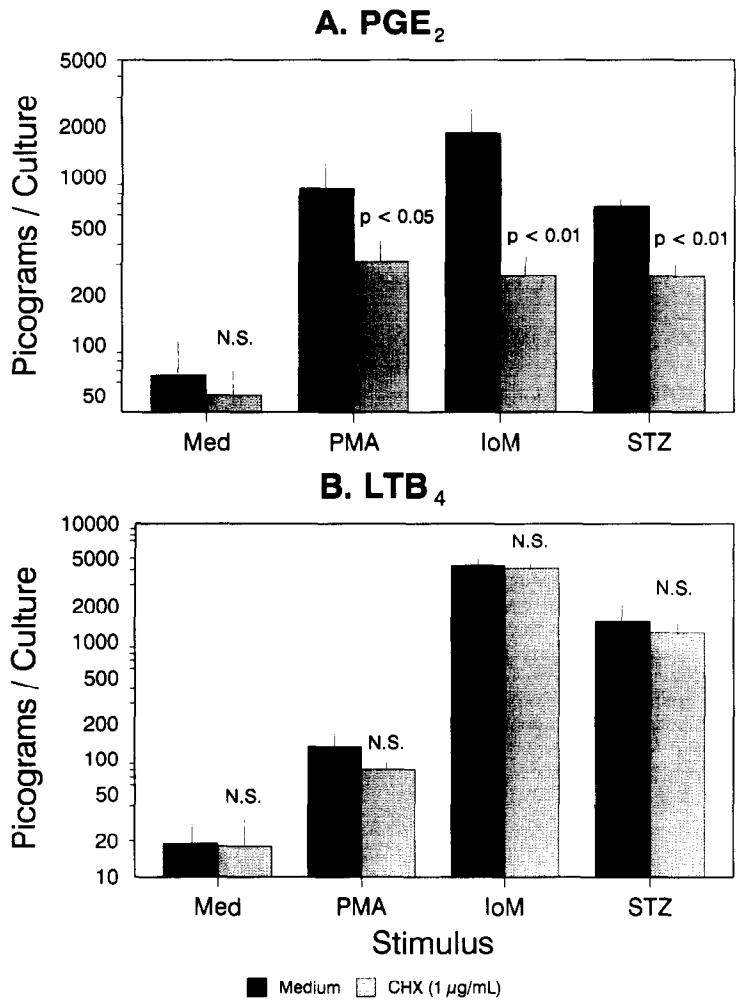

Fig. 1. Effect of cycloheximide on PGE_2 or LTB_4 release by human monocytes. Monocytes (4×10^6) were incubated at 37° in 500 μL Iscove's medium containing 1 mg/mL BSA (Isc/BSA) with or without cyclohexmide (1 μg/mL) for 30 min. An additional 500 μL of medium containing twice-concentrated stimulated agent (final concentration: 20 nM PMA, 1 μM ionomycin, or 300 μg/mL serum-treated zymosan) was added and the incubation was continued for 2 hr. Supernatants were harvested and stored at −20° prior to assay by RIA. Data are the means and SEM of data from seven consecutive donors.

Salt Solution (HBSS) without Ca^{2+} and Mg^{2+} at a flow rate of 160 mL/min. Monocytes obtained by this method were greater than 90% pure as assessed by histochemical staining. Contaminating cells consisted mainly of lymphocytes and some (<2%) granulocytes; platelets were not a source of contamination.

Reagents. Arachidonic acid, concanavalin A (Con A, Type IV), zymosan, cycloheximide, bovine [serum] albumin (BSA), and phorbol myristate acetate (PMA) were purchased from the Sigma Chemical Co. (St. Louis, MO). Ionomycin (IoM) was purchased from Calbiochem (San Diego, CA). Iscove's medium (Isc) was from Whittaker Bioproducts (Walkersville, MD). [5, 6, 8, 9, 11, 12, 14, 15-^{3}H (N)]Arachidonic acid (60–100 Ci/mmol) was bought from NEN, Inc. (Boston, MA).

Arachidonate release. Peripheral blood monocytes (4×10^6) were incubated for 4 hr at 37° in 24-well tissue culture plates (Costar, Cambridge, MA) containing 2 mL of Iscove's medium with BSA (1 mg/mL) and 2 μCi [^{3}H]AA. At the end of the incubation, cells were washed three times with Earle's Balanced Salt Solution (EBSS, Gibco, Grand Island, NY) and the medium was replaced with 500 μL of fresh medium containing stimuli at the concentrations indicated. After an additional 2 hr at 37°, the medium was removed and its radioactivity was determined by liquid scintillation counting in an LKB-Wallac 1218 Rackbeta counter calibrated for dpm measurement by external standard ratio.

Radioimmunoassay (RIA). Monocyte supernatants were prepared in 24-well plates as described above except for omission of labeled arachidonate and incubation in 1.0 mL medium. Samples were stored at −20° prior to analysis. PGE_2, thromboxane B_2, or LTB_4 assays were carried out by using RIA kits obtained from NEN, Inc. These kits cross-react

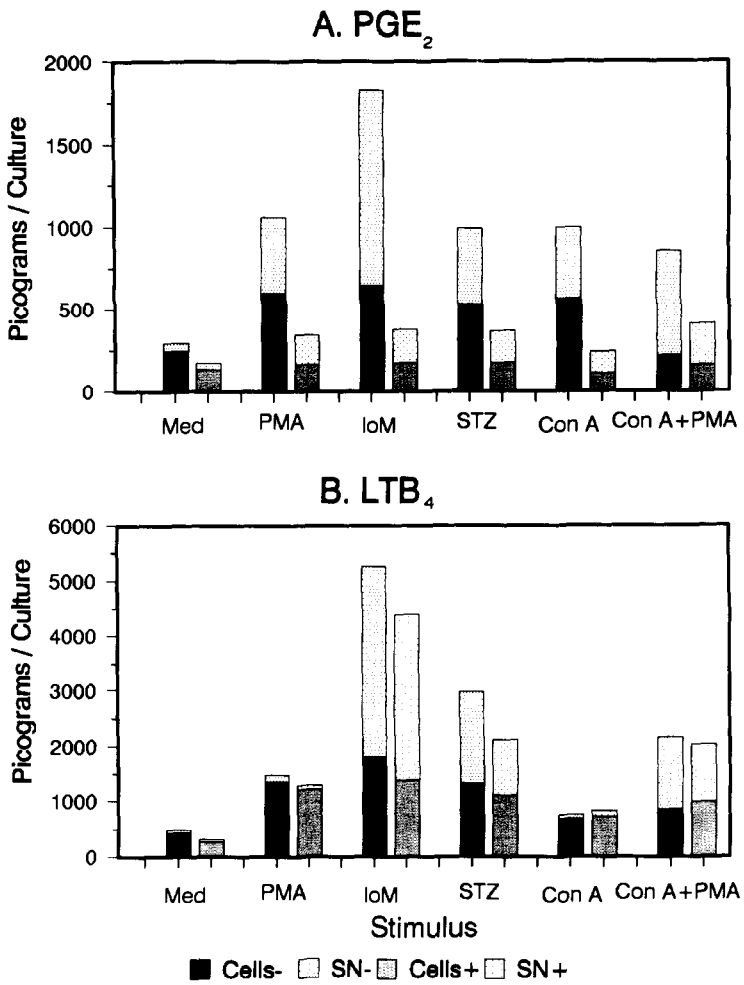

Fig. 2. Cycloheximide effect on supernatant and cell-associated PGE_2 or LTB_4 synthesis by human monocytes. Monocytes were cultured as described in Materials and Methods and the legend to Fig. 1 with (+) or without (−) cycloheximide (1 μg/mL). Con A stimulation was achieved at 30 μg/mL. At the end of the incubation, supernatants (SN) were harvested as described and the cells were lysed in distilled water and exposed to three cycles of freeze–thawing in liquid nitrogen. The data shown are the sum of supernatant and cell-associated PGE_2 (A) and LTB_4 (B) and are the geometric means of data representing monocytes from three donors. In no instance was the inhibition of PGE_2 less than 50% or that of LTB_4 greater than 15%.

non-specifically with other eicosanoids or fatty acids <1%, with the following exceptions: PGE_2 3.8% with PGE_1; LTB_4 1.3% with 20-OH-LTB_4 and 3.6% with 5,12-dihydroxyeicosatetraenoic acid. Assays were performed according to the manufacturer's instructions using standard curves constructed in Isc/BSA.

SDS–PAGE and immunoblot analysis of cyclooxygenase. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) of protein from the cell lysates was performed by adding one-half volume of 2× electrophoresis sample buffer (Novex, Encinitas, CA, Catalog No. LC-2676) and then boiling the samples for 3 min before subjecting them to electrophoresis on 8 or 14% acrylamide gels (1.5 mm). Immunoblot analysis was performed after transferring the proteins from the gels onto Immobilon-P membranes (Millipore, Bedford, MA). Blocking of the blotted membrane was accomplished during overnight incubation at 4° in 1% BSA/0.1% Tween-20. Detection was carried out by using an Elisamate kit (KPL, Inc., Gaithersburg, MD) after incubation with a 1:100 dilution of polyclonal anti-PGH synthase antibody (Oxford Biomedical Research, Oxford, MI). PGH synthesis (2 μL, Oxford) was run in parallel as a reference standard.

Statistical analysis. Analysis of variance (ANOVA) was performed by using Duncan's multiple range test component of the "means" utility of Bright Stat Pak (Bright Software, New Brunswick, NJ). Alternatively, Student's *t*-test was employed. Statistical comparisons were made on data expressed as logarithms since this technique minimizes the

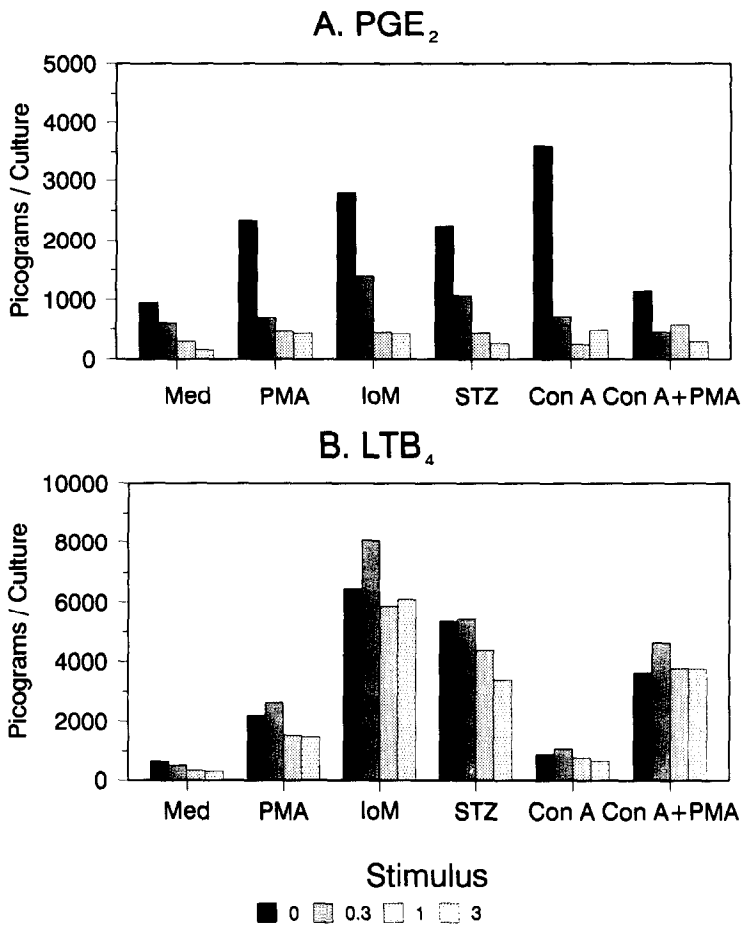

Fig. 3. Concentration-dependent response for cycloheximide effect on total PGE_2 or LTB_4 synthesis by human monocytes. Monocytes were cultured as described in Materials and Methods and the legend to Fig. 1. Con A stimulation was achieved at 30 μg/mL. At the end of the incubation supernatants were harvested as described and the cells were lysed in distilled water and exposed to three cycles of freeze–thawing in liquid nitrogen. The data shown are the sum of supernatant and cell-associated PGE_2 (A) and LTB_4 (B) from a representative experiment of three which gave comparable results.

variation of baseline values seen in assays performed on monocytes of different donors.

RESULTS

Selective inhibition by cycloheximide of monocyte PGE_2 production in response to diverse stimuli. Pretreatment of human peripheral blood monocytes with cycloheximide (1 μg/mL) for 30 min prior to challenge with soluble or particulate activators resulted in inhibition of PGE_2 release, irrespective of the stimulus (Fig. 1A). Assay of LTB_4 in aliquots of identical supernatants as those assayed for PGE_2 demonstrated no change in LTB_4 release (Fig. 1B). Under these conditions, cycloheximide inhibited the rate of [^{3}H]leucine incorporation by 87% (data not shown).

Cycloheximide effects on PGE_2 metabolism were not limited to inhibition of release. Analysis of cell-associated PGE_2 demonstrated reduction equivalent to that seen in the supernatant (Fig. 2A). Levels of LTB_4 were unaffected (Fig. 2B) even in response to PMA or Con A, agents which induce LTB_4 production but, by themselves, fail to promote LTB_4 release [9].

The inhibitory effect of cycloheximide on PGE_2 was concentration-dependent (Fig. 3A) and maximal at 1–3 μg/mL. No effect on LTB_4 was observed even at higher concentrations, except for a 35% decrease when serum-treated zymosan (STZ) was the stimulus (Fig. 3B).

Time course of cycloheximide inhibition of PGE_2 synthesis and release. The greatest inhibition of PGE_2 generation was achieved when cycloheximide was added 1 hr prior to stimulation i.e. 3 hr before harvest of supernatants. No inhibition (in fact, enhancement) was observed when cycloheximide was added 30 min after stimulation i.e. 90 min prior to harvest (Fig. 4).

Although the absence of an effect on LTB_4 apparently precluded a general toxic effect of cycloheximide, it was possible that LTB_4 might be

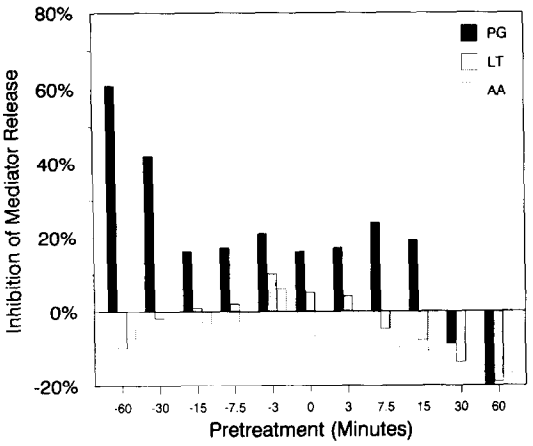

Fig. 4. Time course of initiation of cycloheximide effect on PGE_2 release. Monocytes were pretreated according to the protocol described in Fig. 1, beginning 1 hr prior to the addition of stimulus (time "0"). At the times indicated in the figure, medium, cycloheximide, or stimulus (serum-treated zymosan, 300 μg/mL) was added and the cultures were continued to the time point 2 hr after stimulation. Supernatants were harvested and assayed as described in Materials and Methods. The data shown are representative of three experiments performed with an identical protocol which gave similar results.

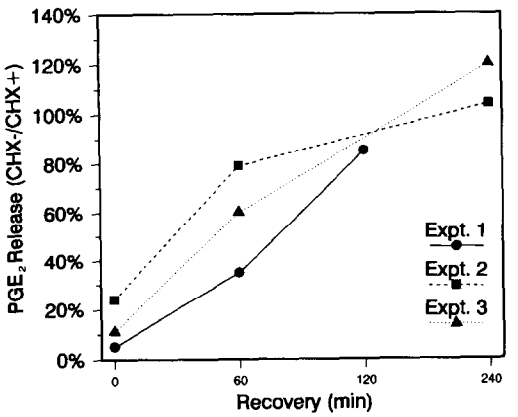

Fig. 5. Time course for monocyte recovery from the effects of cycloheximide. Monocytes were incubated as described in the legend to Fig. 1 with (+) or without (−) cycloheximide (1 μg/mL). After the indicated times, cycloheximide-containing supernatants were removed, and the monocytes were washed three times with EBSS. One milliliter of Isc/BSA with or without ionomycin (1 μM) was replaced and the cells were returned to 37°. After 2 hr, the supernatants were harvested and processed for PGE_2 analysis to determine the degree of stimulated release.

generated more rapidly than PGE_2 and therefore less likely to be impaired by the drug. To address this concern, and to obtain further data on the turnover of proteins which might be inhibited by cycloheximide, monocytes were exposed to cycloheximide (1 μg/mL) for 1 hr and washed to remove the drug. Monocytes recovered their ability to synthesize PGE_2 within 4 hr, with up to 80% recovery within 2 hr (Fig. 5).

Absence of effect of cycloheximide treatment on monocyte phospholipase A_2 activity. The inhibition of PGE_2 formation in the absence of an effect on generation of LTB_4 made it unlikely that phospholipase A_2 was affected by cycloheximide. Nevertheless, it was conceivable that 5-lipoxygenase might preferentially utilize available free arachidonate even if it were less abundant under circumstances of decreased phospholipase A_2 activity. To address these possibilities, monocytes were labeled with [^{3}H]AA and release into the supernatant was measured by liquid scintillation counting. Under identical conditions which resulted in decreased PGE_2 production and release, release of labeled AA was unimpaired (Figs. 4 and 6).

Although labeled AA release is a valid approximation of phospholipase A_2 activity, the possibility remained that compartmentalized pools of arachidonate might be preferentially labeled under the conditions employed resulting in label release even if total AA were diminished. Furthermore, other phospholipids or neutral lipids (whose turnover rates may be low and therefore would not likely be labeled) may be putative sources of free AA, including inositol phospholipids and diacylglycerols. To rule out these possibilities, eicosanoid biosynthesis was examined in the presence of exogenous (cold) arachidonate. Addition of unlabeled AA *per se* induced PGE_2 production. Exposure to cycloheximide resulted in impaired PGE_2 production even in the presence of arachidonate (Fig. 7A), although this inhibition could be overcome at the highest AA concentration. Higher concentrations of cycloheximide could be less readily overcome by AA addition (data not shown). Inhibition of PGE_2 release was observed in the presence of exogenous AA in stimulated monocytes as well (Fig. 7B). Cells treated with indomethacin, to inactivate cyclooxygenase, demonstrated only modest increases in PGE_2 release with the addition of up to 30 μM arachidonate. Under no circumstances was LTB_4 generation impaired in the presence of cycloheximide (data not shown).

Inhibition of release of additional cyclooxygenase metabolites by cycloheximide treatment. Although these data indicated that the effect of cycloheximide was exerted at the level of the cyclooxygenase, it was possible that specific inhibition in the formation of PGE_2 occurred or that a non-specific effect on the PGE_2 RIA was operative. Therefore, supernatants were assayed for another cyclooxygenase product, thromboxane A_2, which is rapidly and spontaneously converted to thromboxane B_2. The degree of inhibition of thromboxane A_2 synthesis and release was parallel to that observed for PGE_2 (Fig. 8).

Effect of cycloheximide treatment on levels of immunoreactive cyclooxygenase. The observations described above pointed strongly to a specific effect on cyclooxygenase synthesis. To rule out a specific effect on enzyme activity, cyclooxygenase levels were assayed by immunoblotting. Pretreatment with cycloheximide (3 μg/mL) for 1 hr reduced

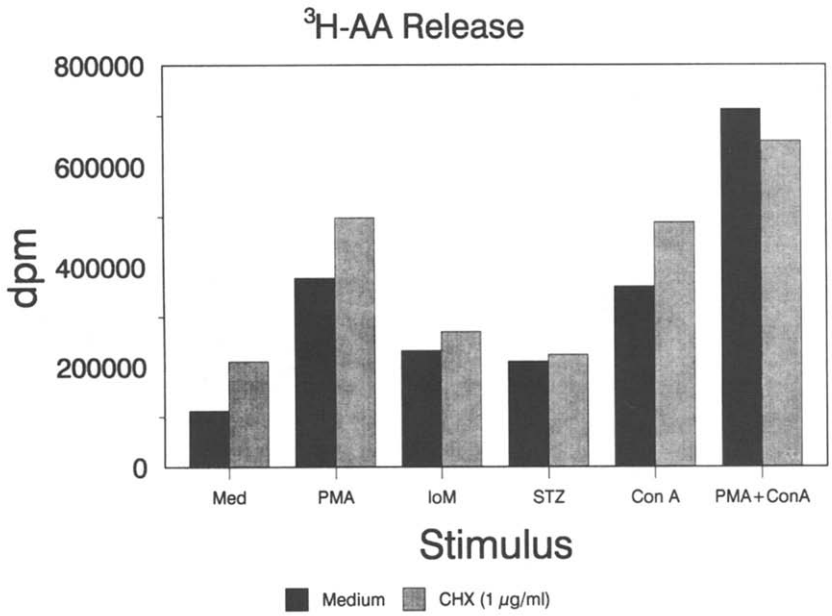

Fig. 6. Absence of cycloheximide effect on AA release from human monocytes. Monocytes (4×10^6) were incubated in Isc/BSA containing 1 μCi/mL [^{3}H]AA for 4 hr. The cells were washed three times with cold EBSS. Fresh medium (500 μL) was added which contained PMA (20 nM), ionomycin (1 μM), serum-treated zymosan (300 μg/mL), Con A (30 μg/mL), PMA + ConA, or no stimulus. After a 2-hr incubation at 37°, the medium was removed and 100 μL counted in a β-counter. Results are expressed as the mean dpm per culture from a representative experiment of three which differed only in the degree of baseline release of label. In no instance was inhibition of AA release observed.

immunoreactive cyclooxygenase by 30–50% (Table 1), consistent with the effects on stimulated or AA-induced eicosanoid production and release. Treatment with ionomycin or PMA (data not shown) resulted in no change in cyclooxygenase.

DISCUSSION

Inhibition of monocyte protein synthesis by cycloheximide selectively and reversibly impaired PGE_2 production in response to all stimuli tested. PFE_2, as well as thromboxane A_2, production was inhibited in the absence of detectable inhibition of phospholipase A_2 activity and this inhibition was evident even in the presence of exogenous arachidonate. Decreased immunoreactive cyclooxygenase in cells treated with cycloheximide correlated with inhibition of prostanoid synthesis and release.

Previous reports, including an early study in mouse macrophages [10], have described inhibition of prostaglandin biosynthesis in the presence of protein synthesis inhibitors. Other protein synthesis inhibitors, including actinomycin D which acts transcriptionally, have also been used with similar results [6]. Among the tissues used for PGH synthase inhibition studies were: dog urothelial cells [11], herpes simplex virus-transformed rat fibroblasts [12], porcine thyroid cells [13], human and bovine endothelial and rat smooth muscle cells [14, 15], murine osteoclasts [16], rat skeletal muscle, spleen, and brain [6], and human HL-60 cells [17, 18]. Commonly, phorbol ester has been used as the stimulus. In cell lines, it has been shown that cyclooxygenase regenerates after "auto-inactivation" or blockade by indomethacin [14] and that PMA may, in fact, increase cyclooxygenase activity by increasing PGH synthase synthesis [15]. A previous report indicated that cyclooxygenase activity is impaired by cycloheximide treatment of murine macrophages [10]. Our study differs in terms of being conducted in freshly isolated, non-transformed human mononuclear phagocytes, including unstimulated cells. Other similar evidence that monocyte cyclooxygenase may be regulated at the level of protein synthesis was obtained by Fu *et al.* [19], who showed that lipopolysaccharide selectively increases cyclooxygenase (but not phospholipase A_2 or 5-lipoxygenase) activity via a protein synthesis dependent mechanism which was reversed by corticosteroids.

In our monocyte system, cyclooxygenase may be a general target of protein synthesis inhibition. This conclusion is based on the following observations: (1) cycloheximide reduced PGE_2 production in response to six diverse stimuli, including ionomycin and exogenous AA, ruling out an effect on receptor turnover or proteins involved in the activation pathways, including protein kinase C; (2) 5-lipoxygenase activity and 5-lipoxygenase activating protein were both unaffected, since LTB_4 generation was constant even at high cycloheximide concentrations; (3) decreased diglyceride lipase or phospholipase C activities could not account for the

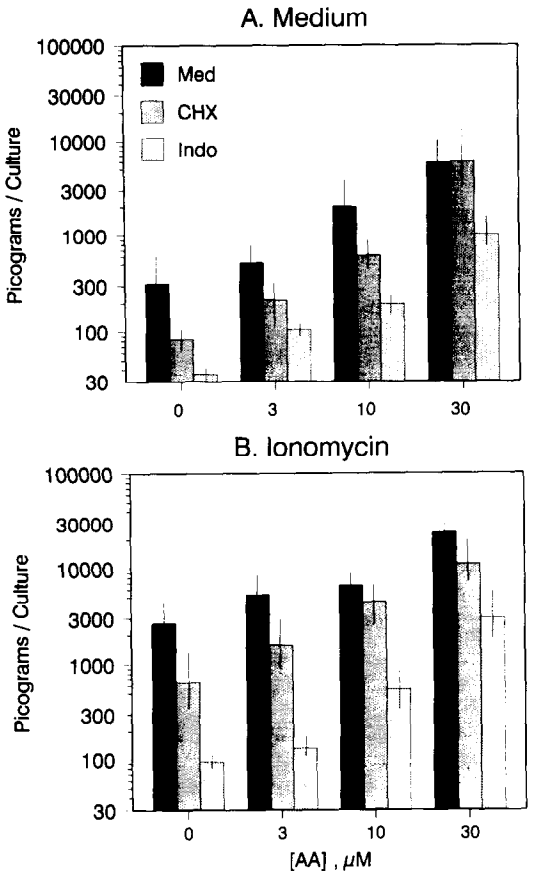

Fig. 7 Cycloheximide inhibition of PGE_2 release in the presence of exogenous AA. Monocytes were prepared as described in Materials and Methods and the legend to Fig. 1. Cycloheximide (1 μg/mL) or indomethacin (3 μM) treatment was initiated in 250 μL Isc/BSA. After 30 min at 37°, 250 μL medium containing twice-concentrated AA was added and the incubation continued for another 30 min. Ionomycin was added in an additional 500-μL aliquot to give a final stimulating concentration of 1 μM and the cells returned to the incubator for another 2 hr prior to harvest of supernatants. The data shown are the means ± SEM for PGE_2 released into the supernatant from the monocytes of four individuals studied under an identical protocol.

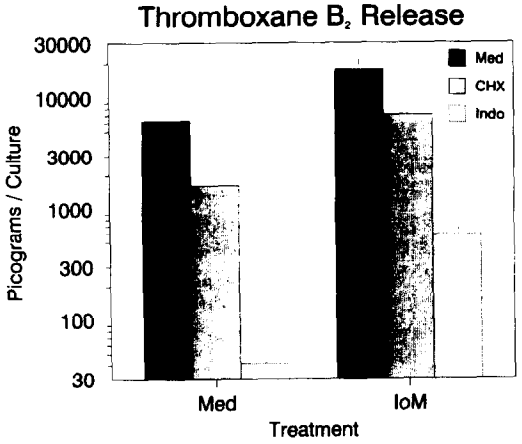

Fig. 8. Cycloheximide inhibition of thromboxane A_2 release. Monocytes were prepared as described in Materials and Methods and the legend to Fig. 1. Supernatants were harvested and assayed from thromboxane B_2, the spontaneous conversion product of thromboxane A_2. The data depict the means and SD from monocytes from four consecutive donors studied under identical conditions.

Table 1. Immunoreactive cyclooxygenase in human monocytes treated with cycloheximide

Stimulus	Immunoreactive cyclooxygenase (% of control)	
	Medium	CHX (3 μg/mL)
Medium	100 ± 5	60 ± 12
Ionomycin (1 μM)	100 ± 5	70 ± 10

Monocytes (40×10^6 cells in 5 mL Isc/HSA) were incubated for 60 min at 37° in 50-mL polypropylene tubes. Medium (5 mL) with or without cycloheximide was added and the incubation continued for an additional 2 hr. At the end of the incubation, cells were washed twice with phosphate-buffered saline, resuspended in distilled water, and submitted to two cycles of freeze–thaw in liquid nitrogen vapor. Cell lysates were stored at −20° prior to immunoblot analysis as described in Materials and Methods. The data shown are derived from the integration of the cyclooxygenase band detected by anti-PGH synthase antibody and are means ± SD from three experiments.

effects observed, since cycloheximide resulted in decreased PGE_2 even in the presence of 10 μM AA; (4) thromboxane A_2 was likewise affected; and (5) immunoreactive cyclooxygenase was reduced by cycloheximide treatment. The possibility that a direct effect of cycloheximide on PGH synthase could account for the effects observed appears remote based on evidence that cycloheximide fails to inhibit purified enzyme *in vitro* [6].

Our findings indicate that cyclooxygenase turns over relatively rapidly in resting monocytes. A 30-min pretreatment was sufficient to reduce cyclooxygenase activity, measured as PGE_2 release. Within 2 hr of cycloheximide removal, cyclooxygenase activity was restored to 80% of its original level. These observations are consistent with those made using cell lines [14], transformed cells [12], other regenerating tissues [16, 20], or macrophages [10]. 5-Lipoxygenase and 5-lipoxygenase activating protein apparently turn over more slowly since their activities, assessed in terms of LTB_4 production and release, were not affected even after 6 hr of exposure to cycloheximide (data not shown), consistent with findings in HL-60 cells where the turnover of 5-lipoxygenase activating protein is greater than 12 hr*. However, our results indicating no effect on labeled AA release and the persistence of inhibition by cycloheximide in the presence of exogenous AA

* Evans J, personal communication. Cited with permission.

stand in some contrast to those of Bomalaski *et al.* [3] and Lin *et al.* [20], respectively, in that they fail to indicate a role for phospholipase A_2 activating protein or putative protein regulators of cyclooxygenase. Possibly, the potent direct effects on phospholipase A_2 activity of the stimuli we used obfuscated the contribution of phospholipase A_2 activating protein to AA release. Alternatively, the phospholipase A_2 activity of elutriated monocytes is predominantly that of the recently described high molecular weight cytosolic phospholipase A_2 (cPLA$_2$, [21]) which may be less susceptible to the effects of phospholipase A_2 activating protein.

Together with recent observations on the synergism of protein kinase C activators and stimuli which increase intracellular calcium [2], it is possible to frame an understanding of monocyte eicosanoid synthesis and release and its potential regulation. Agents which activate phospholipase A_2 via protein kinase C or Ca^{2+}, including ligands (e.g. serum-treated zymosan) which induce receptor-mediated phosphatidylinositol bisphosphate hydrolysis [22], liberate AA which is metabolized by cyclooxygenase. Prostaglandin production is thereby regulated by the amount of enzyme available. Resulting prostaglandins are passively released into the supernatant. Leukotriene synthesis may also ensue, but is regulated at the level of synthesis and release by intracellular Ca^{2+} and 5-lipoxygenase activating protein controlling the degree of 5-lipoxygenase activation and translocation, respectively. This model may be useful in understanding the contrasting roles of lymphokines on autocoid formation. For example, interferon-γ likely acts at the level of phospholipase A_2 since it increases all arachidonic acid metabolites uniformly [23]. Evidence exists that CSF-1 may increase synthesis of cyclooxygenase in human monocytes [24], similarly to the effects of a variety of growth factors, such as epidermal growth factor [14], transforming growth factor-β [14], or platelet-derived growth factor, [20], in other cell types. We have also demonstrated both rapid and long-acting regulation of cyclooxygenase in mouse hematopoietic cells via CSF-1 receptor tyrosine kinase activity [25]. Furthermore, evidence exists that lymphokines, such as interleukin-1 [7, 26] or platelet-derived growth factor [20], may induce cyclooxygenase gene expression.

The role of monocytes as suppressor cells is thought to be mediated, at least in part, by cyclooxygenase products [27]. Recent reports have documented an increase in PG release in monocytes exposed to HIV GP 120 [28], but no data exist to rule out participation of up-regulation of cyclooxygenase in this and related processes. Ultimately, the goal of selectively reducing the effect of prostaglandins in immune suppression or inflammation may be achieved by interfering with cyclooxygenase synthesis at the transcriptional and translational levels by focused and concerted action of specific cytokines alone or in combination.

Acknowledgements—Many thanks to D. Finbloom, G. Tosato and J. Finlayson for critical review and J. Y. Chang and T. Torthy for helpful discussions. Dr. Puri was on leave from the Department of Chemical Immunology, the Weizmann Institute of Science, Rehovot, Israel. Charles S. Carter provided purified monocytes for this study.

REFERENCES

1. Goldyne ME, Burrish GF, Poubelle P and Borgeat P, Arachidonic acid metabolism among human mononuclear leukocytes: Lipoxygenase-related pathways. *J Biol Chem* **259**: 8815–8819, 1984.
2. Hoffman T, Lizzio EF, Suissa J, Rotrosen D, Sullivan JA, Mandell GL and Bonvini E, Dual stimulation of phospholipase activity in human monocytes: Role of calcium-dependent and calcium-independent pathways in arachidonic acid release and eicosanoid formation. *J Immunol* **140**: 3912–3918, 1988.
3. Bomalaski JS, Baker DG, Brophy L, Resurrection NV, Spilberg I, Muniain M and Clark MA, A phospholipase A_2-activating protein (PLAP) stimulates human neutrophil aggregation and release of lysosomal enzymes, superoxide, and eicosanoids. *J Immunol* **142**: 3957–3962, 1989.
4. Flower R, Lipocortin. *Prog Clin Biol Res* **349**: 11–25, 1990.
5. Dixon RAF, Diehl R, Opas E, Rands E, Vickers PJ, Evans JF, Gillard JW and Miller DK, Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis. *Nature* **343**: 282–284, 1990.
6. Fagan JM and Goldberg AL, Inhibitors of protein synthesis cause a rapid block in prostaglandin production at the prostaglandin synthase step. *Proc Natl Acad Sci USA* **83**: 2271–2275, 1986.
7. Maier JAM, Hla T and Maciag T, Cyclooxygenase is an immediate-early gene induced by interleukin-1 in human endothelial cells. *J Biol Chem* **265**: 10805–10808, 1990.
8. Abrahamsen TG, Carter CS, Read EJ, Rubin M, Goetzman HG, Lizzio EF, Lee YL, Hansen M and Pizzo P, Stimulatory effect of counterflow centrifugal elutriation in large scale separation of peripheral blood monocytes can be reversed by storing the cells at 37°. *J Clin Apheresis* **6**: 48–53, 1991.
9. Hoffman T, Brando C, Lizzio EF, Lee YL, Hansen M, Tripathi AK, Taplits M, Puri J, Bonvini E, Abrahamsen TG, Carter CS, Reid GK and Evans J, Calcium-dependent eicosanoid metabolism by concanavalin A-stimulated human monocytes *in vitro*: Synergism with phorbol ester indicates separate regulation of leukotriene B_4 synthesis and release. *J Immunol* **146**: 692–698, 1991.
10. Bonney RJ, Wightman PD, Dahlgren ME, Davies P, Keuhl FA and Humes JL, Effect of RNA and protein synthesis inhibitors on the release of inflammatory mediators by macrophages responding to phorbol myristate acetate. *Biochim Biophys Acta* **633**: 410–421, 1980.
11. Zenser TV, Eling Te, Duniec ZM, Wong Y-HH and Davis B, Mechanism of 12-*O*-tetradecanoylphorbol-13-acetate enhanced metabolism in dog urothelial cells. *Cancer Res* **50**: 4650–4655, 1990.
12. Heitman CRK, Waite M and Kucera LS, Studies of phospholipase and cyclooxygenase activities in herpes simplex virus type 2-transformed rat cells. *Carcinogenesis* **10**: 2059–2065, 1989.
13. Kasai K, Emoto T, Hiraiwa M, Kuroda H, Yamazaki A, Hattori Y and Shimoda S-I, Inhibition by protein kinase C inhibitor and cycloheximide of phorbol ester- and epidermal growth factor-induced arachidonic acid metabolism in cultured porcine thyroid cells. *Metabolism* **39**: 709–713, 1990.
14. Hla TT and Bailey JM, Differential recovery of prostacyclin synthesis in cultured vascular endothelial vs. smooth muscle cells after inactivation of cyclo-

oxygenase with aspirin. *Prostaglandins Essent Fatty Acids* **36**: 175–184, 1989.
15. Wu KK, Hatzakis H, Lo SS, Seong DS, Sanduja SK and Tai H-H, Stimulation of *de novo* synthesis of prostaglandin G/H synthase in human endothelial cells by phorbol ester. *J Biol Chem* **263**: 19043–19047, 1988.
16. Yamamoto S, Oshima T, Kusaka M, Yokota K, Yoshimoto T, Sumitani K, Kawata T and Kumegawa M, Molecular mechanism of cyclooxygenase regulation in cloned mouse osteoblastic cell (MC3T3-E1). In: *Advances in Prostaglandin, Thromboxane, and Leukotriene Research* (Eds. Samuelsson P, Wong PY-K and Sun FF), Vol. 19, pp. 444–449. Raven Press, New York, 1988.
17. Goerig M, Habenicht AJR, Zeh W, Salbach P, Kommerell B, Rothe DER, Nastainczyk W and Glomsett JA, Evidence for coordinate, selective, regulation of eicosanoid synthesis in platelet-derived growth factor-stimulated 3T3 fibroblasts and in HL-60 cells induced to differentiate into macrophages or neutrophils. *J Biol Chem* **263**: 19384–19391, 1988.
18. Honda A, Raz A and Needleman P, Induction of cyclooxygenase synthesis in human promyelocytic leukemia (HL-60) cells during monocytic and granulocytic differentiation. *Biochem J* **272**: 259–262, 1990.
19. Fu J-Y, Masferrer JL, Siebert K, Raz A and Needleman P, The induction and suppression of prostaglandin H_2 synthase (cyclooxygenase) in human monocytes. *J Biol Chem* **265**: 16737–16740, 1990.
20. Lin AH, Bienkowski MJ and Gorman R, Regulation of prostaglandin H synthase mRNA levels and prostaglandin biosynthesis by platelet-derived growth factor. *J Biol Chem* **264**: 17379–17383, 1989.
21. Clark JD, Milona N and Knopf JL, Purification of a 110-kilodalton cytosolic phospholipase A_2 from the human monocytic cell line U937. *Proc Natl Acad Sci USA* **87**: 7708–7712, 1990.
22. Brando C, Hoffman T and Bonvini E, HPLC separation of inositol phosphate isomers employing a reversed phase column and a micellar mobile phase. *J Chromatogr Biomed Appl* **529**: 65–70, 1990.
23. Hoffman T, Lizzio EF, Ting A, Marshall LA, Bonvini E and Jennings MK, Release of arachidonic acid metabolites by human monocytes or lymphocytes: Effect of treatment with interferon on stimulation of phorbol ester or calcium ionophore. *Clin Immunol Immunopathol* **44**: 82–92, 1987.
24. Orlandi M, Bartolini G, Minghetti L, Luchetti S, Giuliucci B, Chiricolo M and Tomasi V, Prostaglandin and thromboxane biosynthesis in isolated platelet-free human monocytes. III. The induction of cyclooxygenase by colony stimulating factor-1. *Prostaglandins Leukot Essent Fatty Acids* **36**: 101–106, 1989.
25. Puri J, Pierce JH and Hoffman T, Transduction of a signal for arachidonic acid metabolism by untriggered CSF-1 receptor induces an opposite effect to that induced by CSF-1 receptor and its ligand: Separate regulation of phospholipase A_2 and cyclooxygenase by CSF-1 receptor/CSF-1. *Prostaglandins Leukot Essent Fatty Acids* **45**: 43–48, 1992.
26. Raz A, Wyche A and Needleman P, Temporal and pharmacological division of fibroblast cyclooxygenase expression into transcriptional and translational phases. *Proc Natl Acad Sci USA* **86**: 1657–1661, 1989.
27. Bonney RJ and Davies P, Possible autoregulatory functions of the secretory products of mononuclear phagocytes. *Contemp Top Immunobiol* **13**: 199–223, 1984.
28. Wahl LM, Corcoran ML, Pyle SW, Arthur LO and Farrar WL, Human immunodeficiency virus glycoprotein (gp120) induction of monocyte arachidonic acid metabolites and interleukin 1. *Proc Natl Acad Sci USA* **86**: 621–625, 1989.