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Possible Contribution of Prostaglandin E_2 to the Antiproliferative Effect of Phosphodiesterase 4 Inhibitors in Human Mononuclear Cells

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ABSTRACT. Phosphodiesterase (PDE) 4, mixed PDE3/4, and non-selective PDE inhibitors have been shown to inhibit the proliferation of human peripheral blood mononuclear cells (HPBM). The aim of the present study was to examine whether endogenous prostaglandins, in particular prostaglandin E_2 (PGE_2), are involved in mediating the antiproliferative actions of PDE inhibitors, by comparing their effects with drugs which elevate or mimic adenosine 3′,5′-cyclic monophosphate (cAMP) through mechanisms other than PDE inhibition. Indomethacin significantly reduced the antiproliferative effects of the PDE4 inhibitors rolipram and CDP840 and the mixed PDE3/4 inhibitor zardaverine, increasing the IC_{50} values from 2.51 μM to >10 μM, 0.81 μM to 2.82 μM, and 1.58 μM to 4.82 μM, respectively ($P < 0.05$), but did not alter the effects of theophylline. Forskolin, PGE_2, and dibutyryl cAMP also inhibited HPBM proliferation, and in the presence of indomethacin the effects of forskolin and dibutyryl cAMP were reduced (although this was not significant), whereas PGE_2 was not affected. Rolipram, CDP840, zardaverine, and dibutyryl cAMP all produced a concentration-related increase in PGE_2 production ($P < 0.05$, ANOVA), but theophylline significantly increased PGE_2 production only at the highest concentration examined, 1000 μM. The ability of indomethacin to reduce the antiproliferative effects of rolipram, CDP840, and zardaverine, together with the fact that these drugs can stimulate PGE_2 production, suggests that their antiproliferative actions may be mediated in part by stimulation of endogenous PGE_2 production. In contrast, it appears that endogenous PGE_2 is not critical for the antiproliferative actions of theophylline, forskolin, and dibutyryl cAMP in HPBM. These results establish the importance of co-ordinated regulation of the cAMP phosphodiesterase and cyclooxygenase-PGE_2 systems for the regulation of lymphocyte function in man, and have clinical implications for therapeutic approaches to diseases associated with lymphocyte dysregulation. BIOCHEM PHARMACOL **58**;9:1487–1495, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. isoenzyme selective PDE inhibitors; peripheral blood mononuclear cells; prostaglandin E_2

Lymphocyte proliferation plays a key role in the inflammatory response in bronchial asthma and other allergic diseases [1]. Intracellular cAMP‡ is an important regulator of lymphocyte activity [2], and agents which are able to elevate cAMP levels in lymphocytes have been demonstrated to possess immunosuppressive properties. Levels of cAMP in lymphocytes may be increased either by direct activation of adenylate cyclase by forskolin or by indirect activation of adenylate cyclase by PGE_2. Both PGE_2 and forskolin have been shown to inhibit the proliferation of HPBM [3, 4]. Moreover, levels of cAMP can also be elevated by inhibition of PDE enzymes. The PDE family includes at least seven different isoenzyme forms [5, 6]. The predominant PDE isoenzyme in most inflammatory cells is PDE4, but PDE3 and, more recently, PDE7 isoenzymes have also been identified in T lymphocytes and monocytes and may control cAMP breakdown in these cells [7–15]. The non-selective PDE inhibitor, theophylline, selective PDE4 inhibitors, and mixed type 3/4 PDE inhibitors inhibit human lymphocyte and mononuclear cell proliferation [16, 17]. PDE4 inhibitors have been shown to synergise with PGE_2 to inhibit the proliferation of mouse spleen cells [18], and elevation of the cellular cAMP content has been suggested to underlie this effect. In addition, we have previously observed that PDE4 inhibitors are more effective at inhibiting the proliferation of HPBM from patients with atopic dermatitis than the proliferation of HPBM from normals [17]. Spontaneous PGE_2 production is higher from monocytes obtained from atopic dermatitis patients than monocytes obtained from normal subjects [19], and it is

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‡ *Abbreviations:* ANCOVA, analysis of covariance; cAMP, adenosine 3′,5′-cyclic monophosphate; HPBM, human peripheral blood mononuclear cells; PDE, phosphodiesterase; PGE_2, prostaglandin E_2; PHA, phytohaemagglutinin; and SMEM, Spinner modified minimal essential medium.

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thus possible that the increased levels of endogenous PGE_2 produced from these cells synergise with PDE4 inhibitors to induce a leftward shift of the concentration–response curve in atopic HPBM. In the present study, we have sought to examine the possible contribution of endogenous PGE_2 to the antiproliferative effects of selective PDE4 inhibitors, mixed PDE3/4 inhibitors, and the non-selective PDE inhibitor theophylline, as well as agents which elevate cAMP through other mechanisms, by using the cyclooxygenase inhibitor indomethacin and by measuring PGE_2 production.

MATERIALS AND METHODS

Subjects

Normal healthy subjects who had no history of allergic disease and were not taking any medication were selected for this study, which was approved by the Ethics Committee of King's College Hospital.

Preparation of Human Peripheral Blood Mononuclear Cells

Peripheral venous blood (25 mL) was drawn from each volunteer and collected in tubes coated with the anticoagulant EDTA. HPBM were then isolated by Ficoll-Paque gradient centrifugation as described previously [17] prior to being suspended in SMEM containing L-glutamine (2 mM), non-essential amino acids (1% v/v), sodium pyruvate (1 mM), penicillin (100 U/mL), streptomycin (100 μg/mL), and HEPES (20 mM). Total cell counts were performed using an improved Neubauer haemocytometer and cell viability was assessed by trypan blue exclusion. The volume of cells was then adjusted to 10^6 cells/mL in culture medium containing 10% Ultroser (serum substitute). Approximately 1–1.5 $\times$ 10^6 cells were routinely obtained per millilitre of blood and cell viability was always >95%. Platelet contamination was less than one platelet per nucleated cell.

Preparation of Plates and Test Drugs

Rolipram, CDP840, zardaverine, and forskolin were all prepared as stock solutions in DMSO. Theophylline was prepared as a stock solution in saline, PGE_2 was prepared in ethanol, and dibutyryl cAMP was prepared in distilled water. All stock solutions were prepared at a concentration of 10^{-2} M. Subsequent dilutions were made in SMEM culture medium. HPBM (10^5 in 100 μL per well) were seeded in 96-well plates in the absence or presence of indomethacin (3 μM), added from a stock dissolved in 0.5% Na_2CO_3. Ten minutes later, PHA (0–20 μg/mL) in a volume of 50 μL was added to each well. The cells were then incubated at 37° in a 95% air, 5% CO_2 atmosphere. For the experiments with drugs, HPBM (10^5 in 100 μL per well) were seeded in 96-well plates in the absence or presence of indomethacin (3 μM). Ten minutes later, PHA (50 μL) was added to each well at a final concentration of 2 μg/mL simultaneously with different concentrations of drug (50 μL) or vehicle (DMSO, saline, ethanol, or distilled water, 50 μL). Control wells received culture medium (50 μL) and vehicle, DMSO, saline, ethanol, or distilled water (50 μL), which was at a final concentration in each well of no greater than 0.1%. These were the final concentrations for drug vehicle in cells that were treated with the highest concentration of drug. These concentrations of drug vehicle were found to have no significant effect on HPBM proliferation. The cells were then incubated at 37° in a 95% air, 5% CO_2 atmosphere for 24 hr.

Measurement of HPBM Proliferation and Release of PGE_2

To measure proliferation, the cells were pulsed with [^{3}H]-thymidine (0.1 μCi per well) and incubated for a further 24-hr period. They were then harvested onto glass fibre filters using a cell harvester (ICN Flow) and the incorporated radioactivity counted in a beta scintillation counter. For mediator generation experiments, the cells were incubated without thymidine for 24 hr. PGE_2 radioimmunoassay was done by transferring 100-μL aliquots of the cell-free supernatants to 3 mL polypropylene tubes and adding 200 μL polyclonal rabbit anti-PGE_2 serum diluted 1:1000 and 100 μL [^{3}H]-PGE_2 containing 10 nCi (17 pg) (Amersham, specific activity, 181 Ci/mmol) for 18 hr at 4°. Bound label was separated from free by precipitation using 200 μL dextran-coated charcoal, followed by 10-min centrifugation at 1800 *g*, 4°. Amounts of PGE_2 in the samples were obtained by interpolation using standard curves prepared with 3–5000 pg unlabelled PGE_2. Assay sensitivity was ~5 pg.

Statistical Tests

Each drug was examined on 4–9 blood samples (each from a separate individual) and each concentration of drug was examined in triplicate. Cell proliferation and PGE_2 data are shown graphically as means ± SEM as percent of control. In each case, control values represent HPBM stimulated with PHA in the absence or presence of indomethacin (3 μM) and are taken as 100%. IC_{50} values are expressed as geometric means (with 95% confidence limits) where appropriate. Differences between concentration curves obtained in the presence or absence of indomethacin were analysed using ANCOVA [20]. Differences between IC_{50} values were analysed by unpaired *t*-test. PGE_2 measurements were analysed by repeated measures of ANOVA followed by Tukey's *t*-test.

Materials

These were obtained from the following suppliers: SMEM, L-glutamine, non-essential amino acids (10×), sodium pyruvate, penicillin–streptomycin (GIBCO), HEPES, DMSO, Histopaque, trypan blue, chromotrope 2R, Erh-

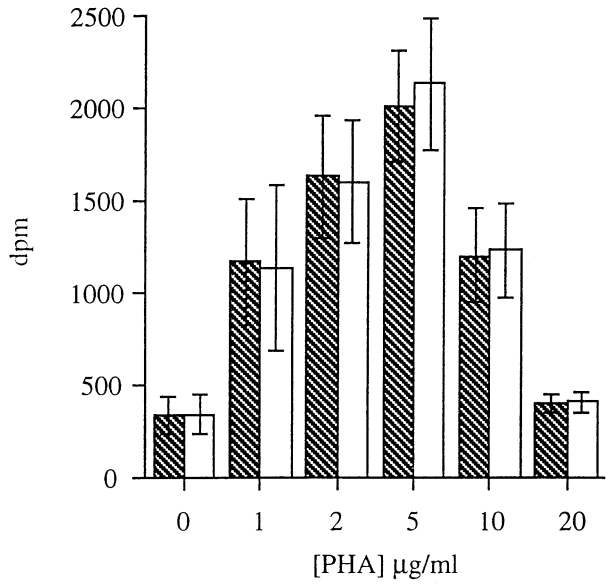

FIG. 1. Effect of PHA (0–20 μg/mL) in the presence (open bars) or absence (hatched bars) of indomethacin (3 μM) on the proliferation of HPBM. Values are means ± SEM (N = 8 per group).

lich's haematoxylin, theophylline, indomethacin, forskolin, PGE_2, dibutyryl cAMP (Sigma), Ultroser (Jones Chromatography), PHA (Biochrom), [6-^{3}H]-thymidine, [$^3H_7$]-PGE_2 (Amersham International), dextran (dextran T70, Pharmacia), charcoal (Norit GSX, BDH). We acknowledge the following kind gifts: rolipram from Schering AG, zardaverine from Byk Gulden GmbH, and CDP840 from Celltech Ltd.

RESULTS

PHA (0–20 μg/mL) produced a concentration-related stimulation of HPBM proliferation which was bell-shaped (Fig. 1). Indomethacin (3 μM) had no significant effect on the responsiveness of HPBM to PHA at any of the concentrations of PHA tested (Fig. 1). A concentration of 2 μg/mL PHA produced a submaximal (approximately EC_{90}) stimulation of proliferation in vehicle-treated (1627 ± 329 dpm) and indomethacin-treated (1599 ± 339 dpm) HPBM, compared with basal levels of 333 ± 103 dpm and 337 ± 108 dpm, respectively. This concentration of PHA (2 μg/mL) was selected for subsequent experiments with PDE inhibitors. Measurement of the PGE_2 released during 48-hr culture of the HPBM showed considerable variation of yield between subjects. For example, the amounts released by unstimulated cells obtained from 4 subjects were 5.81 ± 0.75 ng/mL (N = 8 wells tested), 4.61 ± 0.25 ng/mL (N = 12), 1.68 ± 0.13 ng/mL (N = 12), and 6.70 ± 0.39 ng/mL (N = 12). However, treatment with PHA at 2 μg/mL consistently reduced the amount of PGE_2, causing a 31% reduction of prostaglandin generation ($P < 0.002$, Table 1). Co-treatment of the cells with PHA and various doses of indomethacin produced the expected dose-dependent inhibition, with an approximate IC_{50} of 0.003 μM (Table 2). In all further experiments with indomethacin a dose of 3 μM was used, producing >98% inhibition (Tables 1 and 2).

Rolipram, CDP840, zardaverine, and theophylline all produced concentration-related inhibition of proliferation of HPBM ($P < 0.05$, ANOVA). Indomethacin reduced the antiproliferative effect of rolipram (Fig. 2A), CDP840 (Fig. 3A), and zardaverine (Fig. 4A), significantly increas-

TABLE 2. Effect of indomethacin on prostaglandin E_2 generation by human peripheral blood mononuclear cells stimulated by PHA

Treatment	PGE_2 (ng/mL)
Cells alone	5.00 ± 0.64
Cells + PHA + DMSO	3.22 ± 0.60*
Cells + PHA + indomethacin (0.003 μM)	1.55 ± 0.20†
Cells + PHA + indomethacin (0.03 μM)	0.36 ± 0.04†
Cells + PHA + indomethacin (0.3 μM)	0.12 ± 0.03†
Cells + PHA + indomethacin (3.0 μM)	0.10 ± 0.02†
Cells + PHA + indomethacin (30 μM)	0.19 ± 0.04†

Results show means ± SEM for 12 tests (except PHA/DMSO, N = 24), using cells prepared from 4 volunteers.

*Significantly reduced compared to cells not treated with PHA, $P < 0.01$.

†Significantly reduced compared to cells not treated with indomethacin, $P < 0.001$.

TABLE 1. Generation of prostaglandin E_2 by human peripheral blood mononuclear cells stimulated by phytohaemagglutinin in the presence and absence of PDE inhibitors and indomethacin

	PGE_2 (ng/mL)			
Treatment	Control		Indomethacin (3 μm)	
Cells alone	4.60 ± 0.35	(44)	ND	
Cells + PHA + vehicle	3.19 ± 0.22*	(88)	0.06 ± 0.01‡	(32)
Cells + PHA + rolipram (10 μM)	5.93 ± 0.92†	(12)	0.09 ± 0.03‡	(12)
Cells + PHA + CDP 840 (10 μM)	8.74 ± 1.28†	(8)	0.11 ± 0.04‡	(8)
Cells + PHA + zardaverine (10 μM)	8.32 ± 1.50†	(6)	0.14 ± 0.03‡	(6)
Cells + PHA + theophylline (1 mM)	10.07 ± 1.66†	(6)	0.05 ± 0.02‡	(6)

Results show means ± SEM for (N) tests in separate wells using cells prepared from 4 volunteers. ND, not determined.

*Significantly reduced compared to cells not treated with PHA, $P < 0.002$.

†Significantly increased compared to cells treated with PHA alone, $P < 0.001$.

‡Significantly reduced compared to cells not treated with indomethacin, $P < 0.001$.

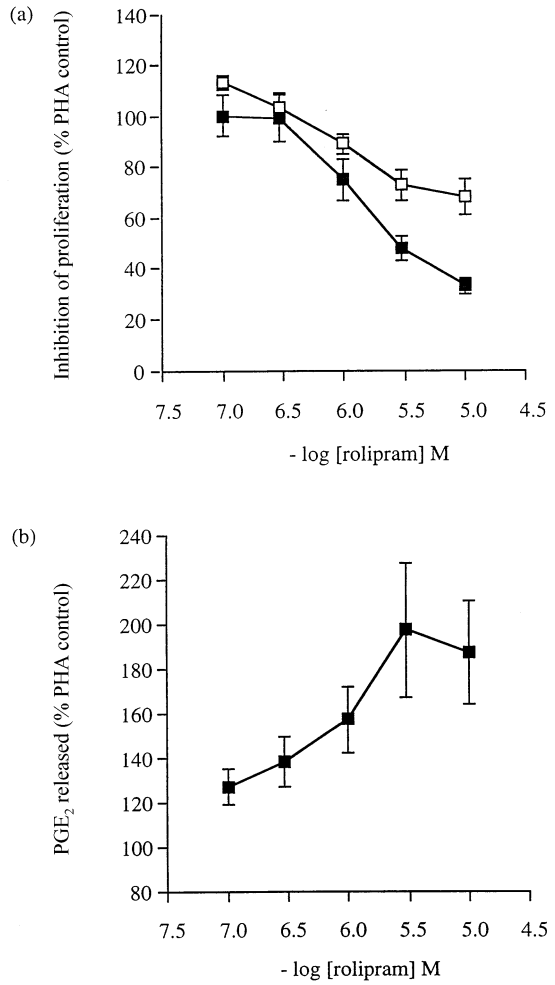

FIG. 2. Effect of rolipram (0–10 μM) on the PHA (2 μg/mL)-stimulated (a) proliferation of HPBM in the presence (open symbols) and absence of (closed symbols) indomethacin (3 μM) (N = 8) and (b) production of PGE_2 from HPBM (N = 9). Values are means ± SEM.

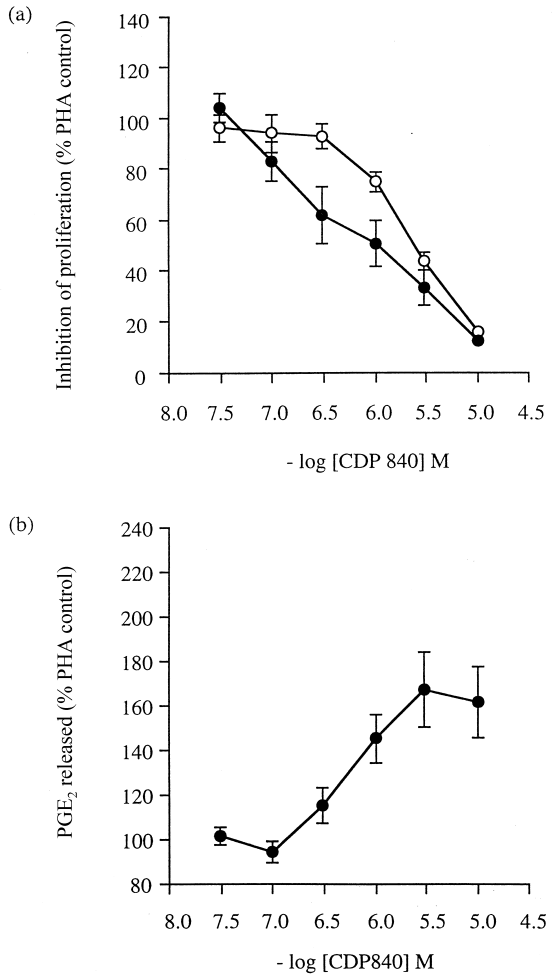

FIG. 3. Effect of CDP840 (0–10 μM) on the PHA (2 μg/mL)-stimulated (a) proliferation of HPBM in the presence (open symbols) and absence of (closed symbols) of indomethacin (3 μM) (N = 6) and (b) production of PGE_2 from HPBM (N = 8). Values are means ± SEM.

ing the IC_{50} value for rolipram from 2.51 μM (1.29–4.89) to >10 μM ($P < 0.05$), for CDP840 from 0.81 μM (95% confidence limits 0.23 and 3.16) to 2.82 μM (0.19–4.27) ($P < 0.05$), and for zardaverine from 1.58 μM (0.87–2.88) to 4.82 μM (3.06–7.59) ($P < 0.05$). In contrast, indomethacin had no effect on the IC_{50} value for theophylline, which was 288 μM (202–411) in the presence and 282 μM (186–427) in the absence of indomethacin (Fig. 5A). Similar experiments were performed to evaluate the contribution of endogenous prostaglandin generation to the antiproliferative effects of forskolin, PGE_2, and dibutyryl cAMP by carrying out proliferation assays in the presence and absence of indomethacin. All three of these agents inhibited thymidine incorporation in a concentration-related manner ($P < 0.05$, ANOVA, Figs. 6–8). Co-treatment with indomethacin did not affect the response to PGE_2 (Fig. 7). The IC_{50} values were 2.05 μM (0.36–11.78) in the absence and 1.36 μM (0.06–32.28) in the presence of indomethacin ($P > 0.05$). However, in the cases of forskolin and dibutyryl cAMP, the co-treatment with indomethacin produced a small rightward shift of the proliferation concentration–response curve, but these effects were not statistically significant. The IC_{50} values for forskolin were 1.82 μM (0.42–7.82) in the absence and 4.15 μM (0.94–18.37) in the presence of indomethacin (Fig. 6A) ($P > 0.05$, ANCOVA) and these values for dibutyryl cAMP were 28.18 μM (9.12–87.10) in the absence and 58.75 μM (28.45–121.34) in the presence of indomethacin (Fig. 8A) ($P > 0.05$, ANCOVA).

As well as testing the PDE inhibitors on HPBM proliferation, we compared their effects on PGE_2 generation during 48-hr culture, as shown in Figs. 2 to 5 (lower panels). Rolipram (Fig. 2B), CDP840 (Fig. 3B), and zardaverine (Fig. 4B) all produced concentration-dependent stimulation of endogenous PGE_2 production ($P < 0.05$, ANOVA). The greatest increases in PGE_2 production produced by rolipram, CDP840, and zardaverine were 97 ± 30%, 67 ± 17%, and 80 ± 35%, respectively. In contrast, even at the highest concentration examined (1000 μM), theophylline only increased PGE_2 production in this set of

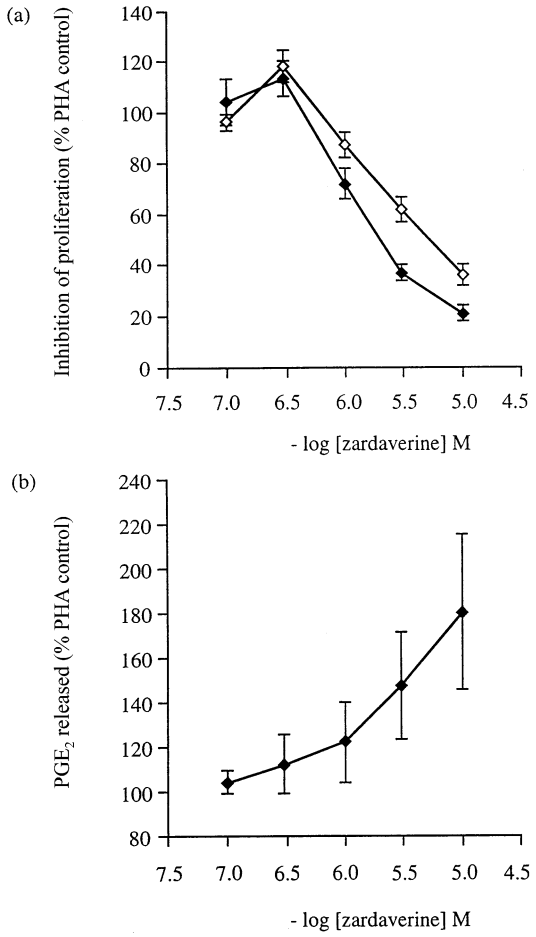

FIG. 4. Effect of zardaverine (0–10 μM) on the PHA (2 μg/mL)-stimulated (a) proliferation of HPBM in the presence (open symbols) and absence (closed symbols) of indomethacin (3 μM) (N = 7) and (b) production of PGE_2 from HPBM (N = 7). Values are means ± SEM.

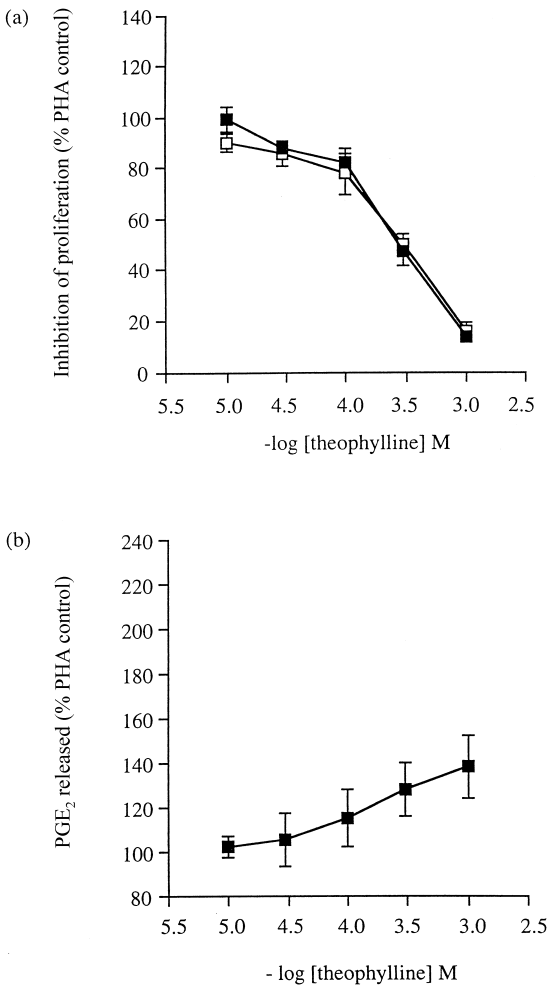

FIG. 5. Effect of theophylline (0–1000 μM) on the PHA (2 μg/mL)-stimulated (a) proliferation of HPBM in the presence (open symbols) and absence (closed symbols) of indomethacin (3 μM) (N = 6) and (b) production of PGE_2 from HPBM (N = 8). Values are means ± SEM.

experiments by 38 ± 14%, which was not significant. Forskolin had no consistent effect on PGE_2 production ($P > 0.05$, ANOVA, Fig. 6B), whereas dibutyryl cAMP produced a significant concentration-related stimulation of PGE_2 production over the concentration range 10^{-7} M to 10^{-4} M ($P < 0.05$, ANOVA, Fig. 8B).

In a separate series of tests, it was verified that 3 μM indomethacin inhibited the augmented PGE_2 generation associated with the PDE treatment (Table 1); it is notable that in this series of experiments, all four PDE inhibitors (including theophylline) produced substantial and highly significant increases in PGE_2 release, although only a single concentration of each was tested.

DISCUSSION

An increase in cAMP levels via the inhibition of PDE has been shown to inhibit the activation of a variety of cells which are thought to be involved in the inflammatory response, including macrophages, eosinophils, and T lymphocytes [21]. The type 4 PDE isoenzyme appears to be the main form of the enzyme present in inflammatory cells and has been characterised in T lymphocytes, eosinophils, neutrophils, monocytes, basophils, mast cells, and endothelial cells [reviewed in Refs. 13, 21, and 22], although as mentioned above, the type 3 and 7 PDE isoenzymes have also been identified in human peripheral blood lymphocytes [see Introduction for references]. We and others have demonstrated that type 4, mixed type 3/4, as well as non-selective PDE inhibitors can inhibit the mitogen-stimulated proliferation of HPBM [17, 23]. The present study was designed to determine, by using the cyclooxygenase inhibitor indomethacin, whether endogenous prostaglandins contribute to the antiproliferative actions of PDE inhibitors. We found that indomethacin significantly reduced the ability of the selective type 4 PDE inhibitors rolipram and CDP840, as well as the mixed type 3/4 PDE inhibitor zardaverine, to inhibit proliferation, suggesting that endogenous prostaglandins do indeed contribute to the antiproliferative effects of these agents. In contrast, the

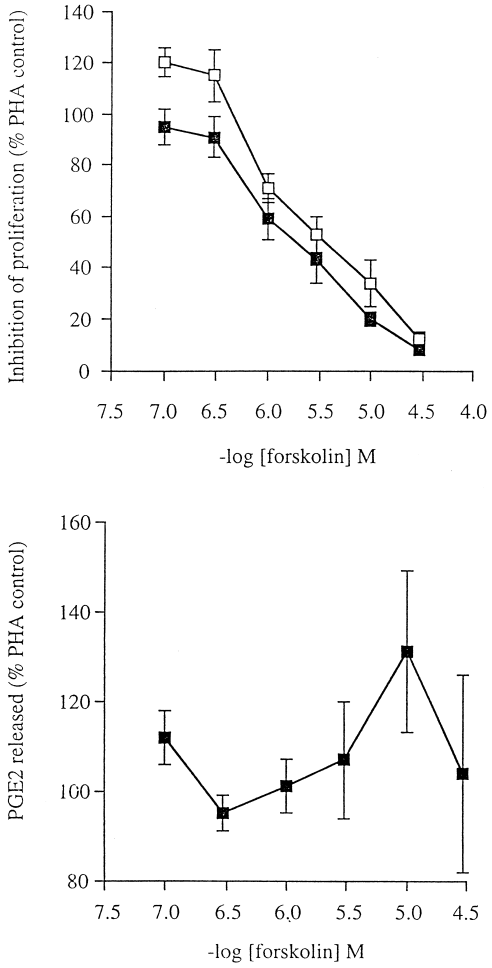

FIG. 6. Effect of forskolin (0–30 μM) on the PHA (2 μg/mL)-stimulated (a) proliferation of HPBM in the presence (open symbols) and absence (closed symbols) of indomethacin (3 μM) (N = 4) and (b) production of PGE_2 from HPBM (N = 4). Values are means ± SEM.

effects of theophylline, forskolin, and dibutyryl cAMP were not significantly affected by indomethacin, although in the cases of forskolin and dibutyryl cAMP small rightward shifts of the proliferation concentration–response curves were apparent, and the calculated IC_{50} values were more than doubled. Thus, taken overall, our results are consistent with the hypothesis that prostaglandin generation makes a significant contribution to the functional consequences of some but not all of the cAMP-raising treatments utilised in these experiments. However, it should be borne in mind that whilst nonsteroidal anti-inflammatory drugs such as indomethacin are known to inhibit the synthesis and release of prostaglandins [24], many of them also have additional effects. For example, one study demonstrated that treatment of rats with indomethacin prevented the sepsis-related depression in mitogen-induced T-lymphocyte

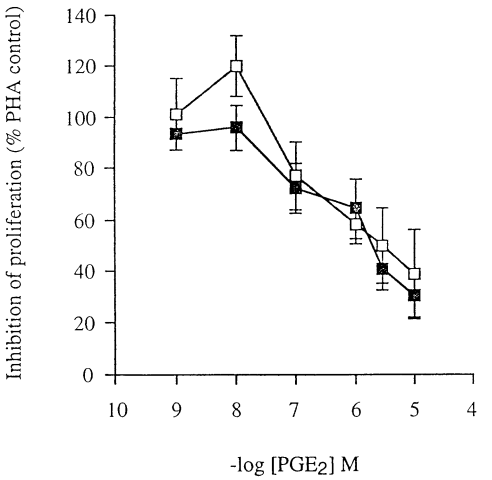

FIG. 7. Effect of PGE_2 (0–10 μM) on the PHA (2 μg/mL)-stimulated proliferation of HPBM in the presence (open symbols) and absence (closed symbols) of indomethacin (3 μM) (N = 4). Values are means ± SEM.

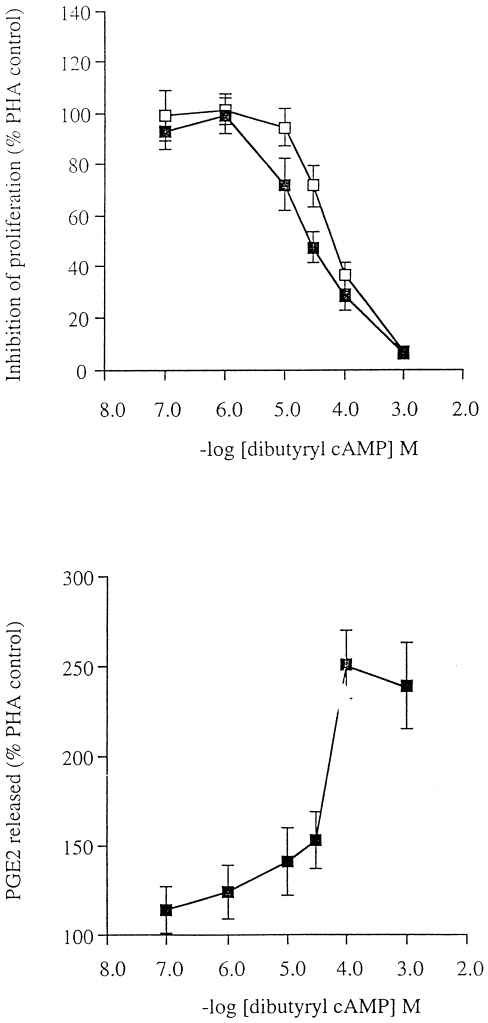

FIG. 8. Effect of dibutyryl cAMP (0–1000 μM) on the PHA (2 μg/mL)-stimulated proliferation of HPBM in the presence (open symbols) and absence (closed symbols) of indomethacin (3 μM) (N = 4) and (b) production of PGE_2 from HPBM (N = 4). Values are means ± SEM.

intracellular calcium mobilisation as well as proliferation [25]. This additional effect of indomethacin may be important, because human lymphocyte proliferation can be reduced by treatments that diminish intracellular calcium levels or antagonise the calcium/calmodulin cascade within cells [26, 27]. Indomethacin has also been demonstrated to block cAMP-dependent protein kinase at 10^{-8} M [28], to inhibit PDE at 10^{-6} M [29] and the group II secretory phospholipase A_2 at 10^{-6} M [30–32], and to interfere with the induction of inducible nitric oxide synthase [33]. With these possible difficulties in mind, we therefore sought to verify that the effects of indomethacin on HPBM proliferation are attributable to prostaglandin inhibition by measuring PGE_2 levels from PHA-stimulated cells in the presence and absence of PDE inhibitors or the other cAMP-elevating drugs.

The selective PDE4 inhibitors rolipram and CDP840, as well as the mixed type 3/4 inhibitor zardaverine, all produced a significant concentration-dependent stimulation of PGE_2 production, suggesting strongly that PGE_2 does indeed contribute to the antiproliferative actions of these PDE inhibitors. This concept—that PDE4 inhibition and associated PGE_2 generation are related and can synergise in down-regulating leukocyte function—receives strong support from the results of Brideau *et al.* [34], which appeared while this paper was under revision. Using a novel human whole blood assay, they showed that PDE inhibitors, especially those which are PDE4-selective, suppress the generation of tumour necrosis factor-α by mononuclear cells and leukotriene B_4 by neutrophils in a fashion which is blunted by indomethacin and augmented by addition of nanomolar concentrations of PGE_2. Taken together with earlier results showing that the inhibitory effect of PDE inhibitors on tumour necrosis factor-α generation by HPBM is increased by addition of exogenous prostaglandins or other activators of adenylate cyclase [35; see Table 3 therein for additional literature], it is clear that PDE exerts a powerful modulatory role within leukocytes such that the effects of cAMP stimulators are usually held in check so that their effects are limited in time. Only after PDE inhibition (an unphysiological state) can a prolonged and substantial cAMP-mediated functional effect be elicited. Even then, it may not be possible to correlate functional effects with elevation of cAMP [e.g. Ref. 35], probably because the PDE isoenzymes are compartmentalised within cells [6, 15, 22, 35, 36], such that a functionally significant

inhibition of an isoenzyme in a small compartment may not be reflected by detectable increases of cAMP concentration within the whole cell. The converse situation (decreased cAMP, increased functional effect) may also occur in mononuclear cells: T-cell proliferation following co-stimulation of CD3 and CD28 receptors is associated with the induction of PDE7, which leads to decreased cAMP and interleukin-2 gene expression [15], and in our experiments increased HPBM proliferation was associated with reduced PGE_2 generation (Tables 1 and 2).

The mechanism whereby PDE inhibition leads to increased prostaglandin generation in HPBM has not been established, but it is known that the cyclooxygenase-2 (COX-2) gene in human U937 monocytic cells contains a cyclic AMP response element [37], and that in rat mesangial cells [38] prostaglandin E_2 can itself induce COX-2 expression (a "feed-forward" amplification effect) as does dibutyryl cAMP. However, we do not know whether COX-2 induction underlies the augmented PGE_2 generation seen in the present experiments. The idea that PDE inhibition in cultured cells can enhance eicosanoid generation is also supported by the finding that Ro-20-1724, a PDE4-selective inhibitor, caused increased PGE_2 release after 24 hr in bovine airway smooth muscle cells [39]. However, satisfactory interpretation of the relationships between the cAMP phosphodiesterase and cyclooxygenase-PGE_2 systems is further complicated by the fact that acute (<1 hr) treatment of eicosanoid-generating inflammatory cells with PDE inhibitors or cAMP stimulants leads to reduced eicosanoid generation, as in human eosinophils [40], human neutrophils [41], and rat neutrophils.*

One result that is not in line with the present hypothesis is that indomethacin failed to reduce the antiproliferative effects of the non-selective PDE inhibitor, theophylline. However, this drug only markedly stimulated PGE_2 production at the highest concentration examined, 1000 μM (Fig. 5, Table 1). The reason why theophylline did not follow the general pattern is not known, although it may be relevant that in a previous study using seven PDE inhibitors, it was found that theophylline was the only substance whose capacity to affect HPBM function did not correlate with its ability to elevate cAMP levels [35]. One possibility is that although theophylline has weak PDE4 inhibitory activity, it may also act as a prostaglandin antagonist [42], a property which would preclude any contribution of prostaglandins to its antiproliferative actions in our experiments.

In conclusion, these results suggest that endogenous prostaglandin generation contributes to the antiproliferative effects of the PDE4 inhibitors CDP840 and rolipram and of the mixed type 3/4 PDE inhibitor, zardaverine, and possibly to those of forskolin and cyclic AMP itself. In contrast, prostaglandin generation does not appear to be of equivalent importance for the actions of theophylline. The overall mechanism of PDE antiproliferative activity can therefore be rationalised by supposing that the downstream effectors of these drugs respond both to "directly formed" cAMP (resulting from PDE inhibition) and to cAMP generated as a secondary consequence of receptor-mediated activity of prostaglandins (such as PGE_2) coupled to adenylate cyclase. However, additional regulation might also be exerted by desensitisation, as it is recognised that prolonged exposure to prostaglandins leads to loss of their responses in mononuclear cells [43, 44].

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