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Differential effects of prostaglandin E_2 and cyclic AMP on release of arachidonic acid metabolites from resting and lipopolysaccharide-stimulated macrophages

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1 The present study investigated the effect of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) and prostaglandin E_2 (PGE_2) on arachidonic acid metabolism in rat peritoneal macrophages.

2 Dibutyryl cyclic AMP (db-cyclic AMP) caused differential effects on the synthesis of PGE_2 and thromboxane. Although db cyclic AMP enhanced the release of PGE_2, it inhibited the release of thromboxane. This suggests that cyclic AMP may regulate cellular functions via induction of a shift in the proportion of arachidonic acid metabolites.

3 PGE_2, at low concentrations, markedly inhibited thromboxane release in nontreated macrophages, but it had virtually no effect on thromboxane release in cells treated with lipopolysaccharide (LPS). By contrast, db-cyclic AMP inhibited thromboxane release also in LPS-stimulated cells.

4 The interrelationships between PGE_2, thromboxane and cyclic AMP, and possible interference of LPS in these interactions are discussed.

Introduction

Lipopolysaccharide (LPS), the toxin of gram negative bacteria, stimulates macrophages to enhance bactericidal and tumoricidal activities, release various lysosomal enzymes and secrete a variety of mediators such as pyrogen, colony-stimulating factor and plasminogen-activating factor (review, Morrison & Ulevitch, 1978).

LPS activation of macrophages has recently been shown to involve also an increase of cellular cyclic adenosine 3′,5′-monophosphate (cyclic AMP) (McCarthy *et al.*, 1980).

Previous studies showed that increased concentrations of intracellular cyclic AMP appears generally to be associated with inhibition of specific macrophage activities such as particle uptake (Welscher & Cruchand, 1976), lysosomal enzyme release during phagocytosis (Ignarro *et al.*, 1974), induction of haeme oxygenase during erythrophagocytosis (Gemsa *et al.*, 1975) and killing of neoplastic cells (Schultz *et al.*, 1979). Based on these observations a suggestion has been made that adenylate cyclase system plays a role in regulation of macrophage activities.

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We previously reported that LPS enhances the release of the arachidonic acid metabolites: prostaglandin E_2 (PGE_2), PGI_2 and thromboxane (Tx), from peritoneal macrophages (Feuerstein *et al.*, 1981a,b). Thus, it is possible that regulation of macrophage functions by cyclic AMP also involves modulation of arachidonic acid metabolism.

This question was investigated in the present work by examining the effect of dibutyryl cyclic AMP (db cyclic AMP) on PGE_2 and Tx release in LPS-stimulated and non-stimulated macrophages.

Furthermore, we examined the effect of PGE_2, which is a known stimulator of adenylate cyclase (Gemsa *et al.*, 1975; Bonney *et al.*, 1980), on Tx synthesis in both LPS-stimulated and non-stimulated cells.

Methods

Cell culture

Fischer 344 rats (8–12 weeks) were inoculated (i.p.) with 5 ml of Freund's incomplete adjuvant. Four days later, the cells were harvested by lavage with RPMI-

1640 (50 ml), washed three times and purified on 50% Percol density gradient. Cells obtained in this manner are more than 90% macrophages, based on nonspecific esterase staining. The cells were suspended in RPMI-1640 (supplemented with penicillin, streptomycin and glutamine) to 1×10^6 cells ml^{-1}. One ml aliquots of the cell suspension were placed in tubes and incubated for 3 h in a humidified atmosphere containing 5% CO_2 in air. Thereafter, the tubes were centrifuged, the supernatant was decanted and frozen (− 20°C) until assayed for prostaglandins.

Prostaglandin assay

Prostaglandin content in the supernatant was determined by a direct radioimmunoassay (Grandstrom & Kindhal, 1976). The cross-reactivity of TxB_2 antibody with 6-keto-PGF_1, PGE_2 and $PGF_{2\alpha}$ is less than 1.0%.

Viability

Viability of the cells as tested by exclusion of trypan blue was higher than 90%.

Materials

Freund's incomplete adjuvant and *E. coli* lipopolysaccharide 055 were purchased from Difco Lab. and RPMI-1640 from Flow Lab. N^6, O^2-dibutyryl-adenosine-3′,5′-cyclic monophosphate was obtained from Calbiochem. The [^{3}H]-prostaglandins and thromboxane B_2 were obtained

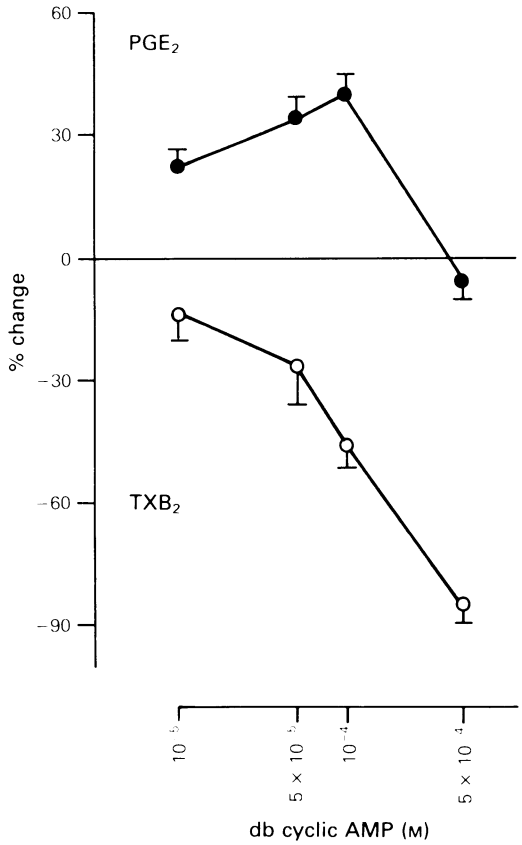

Figure 1 The effect of db cyclic AMP on release of prostaglandin E_2 (PGE_2) and thromboxane B_2 (TxB_2) from lipopolysaccharide (LPS)-stimulated macrophages. Rat peritoneal macrophages were incubated for 3 h with LPS (100 ng ml^{-1}) plus various doses of db cyclic AMP. PGE_2 (●), TxB_2 (○). Data represent means of four experiments with triplicate cultures; s.e.means shown by vertical lines.

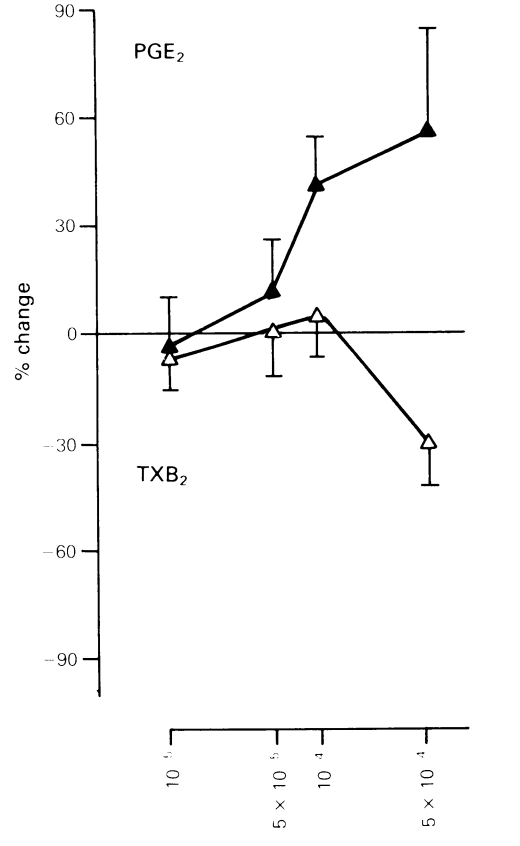

Figure 2 The effect of db cyclic AMP on release of prostaglandin E_2 (PGE_2) and thromboxane B_2 (TxB_2) from nonstimulated macrophages. Rat peritoneal macrophages were incubated for 3 h with various doses of db cyclic AMP. PGE_2 (▲); TxB_2 (△). Data represent means of four experiments with triplicate cultures; s.e.means shown by vertical lines.

from New England Nuclear and the PGE_2 antibody from Accurate Chemical and Scientific Corp. TxB_2 antibody was a gift from Dr L. Levine, Brandeis University, Boston and the 6 keto $PGF_{1\alpha}$ and $PGF_{2\alpha}$ antibodies were prepared in our own laboratory.

Results

The basal release of PGE_2 and Tx from rat peritoneal macrophage is 411 ± 59 and 939 ± 50 pg ml^{-1} 3 h^{-1}, respectively. LPS (100 ng ml^{-1}) enhanced the release of PGE_2 to $3{,}896 \pm 263$ and the release of Tx to $6{,}408 \pm 242$ pg ml^{-1} 3 h^{-1}.

Effect of db cyclic AMP on lipopolysaccharide-stimulated macrophages (Figure 1)

Incubation of LPS-stimulated macrophages with db cyclic AMP induced a differential effect on the release of PGE_2 versus Tx. Tx release was markedly inhibited, whereas PGE_2 release was mildly enhanced. The maximal differential effect of db cyclic AMP on the arachidonic acid metabolites was observed in a concentration of 10^{-4} M of db cyclic AMP. At this dose, Tx synthesis was inhibited by $46 \pm 4\%$ ($P < 0.001$), while the release of PGE_2 was increased by $40 \pm 4\%$ ($P < 0.001$).

Effect of db cyclic AMP on nonstimulated macrophages (Figure 2)

Comparison of Figures 1 and 2 shows that the mild enhancement of PGE_2 release by db cyclic AMP could be demonstrated in both LPS-stimulated and nonstimulated cells. However, the inhibition of Tx synthesis by db cyclic AMP was much less pronounced in nonstimulated cells. Addition of 10^{-4} M db cyclic AMP, which caused $46 \pm 4\%$ inhibition of Tx synthesis in LPS-stimulated cells, had no effect on Tx synthesis in nonstimulated cells. Similarly, 5×10^{-4} M db cyclic AMP suppressed Tx synthesis by $84 \pm 2\%$ and $30 \pm 10\%$ in stimulated and nonstimulated cells, respectively ($P < 0.001$).

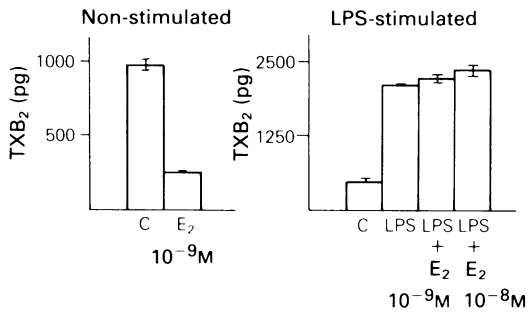

Figure 3 The effect of prostaglandin E_2 (PGE_2) on release of thromboxane B_2 (TxB_2) from non-stimulated and lipopolysaccharide (LPS)-stimulated macrophages. Rat peritoneal macrophages either nonstimulated or stimulated with LPS (100 ng ml^{-1}) were incubated with PGE_2 (E_2) for 3 h; C= control. Data represent means of four experiments with triplicate cultures; s.e.means shown by vertical lines.

Effect of prostaglandin E_2 on thromboxane synthesis (Figure 3)

Incubation of nonstimulated macrophages with PGE_2 10^{-9} M resulted in $72 \pm 5\%$ inhibition of Tx synthesis. However, we could not demonstrate any effect of PGE_2 on Tx synthesis in LPS-stimulated macrophages. The doses of PGE_2 that were tested ranged from 10^{-9} M to 10^{-6} M.

Discussion

The results of the present work demonstrate that db cyclic AMP enhances PGE_2 synthesis while it markedly suppresses Tx synthesis. This qualitative differential effect of db cyclic AMP on two different arachidonic acid metabolites indicates that the suppression of Tx synthesis by db cyclic AMP could be due to inhibition of neither the phospholipase nor the cyclo-oxygenase, but might rather be due to inhibition of Tx synthetase.

Previous studies on the effect of cyclic AMP were done on platelets. These studies are controversial, suggesting that cyclic AMP inhibits the cyclo-oxygenase (Malstein *et al.*, 1976) or the phospholipase (Lapetina *et al.*, 1977; Minkes *et al.*, 1977). Our evidence enables us to exclude the possibility that cyclic AMP affects either of these enzymes in rat macrophages.

Thus, our data indicate, for the first time, an endogenous and physiological mechanism which induces a shift in the proportion of the arachidonate metabolites. It is possible that such a selective change in the synthesis of particular metabolites, rather than inhibition of arachidonic acid release, is an endogenous mechanism for regulation of macrophage activity by cyclic AMP.

To verify the physiological relevance of the effect of db cyclic AMP, we further examined the effect of PGE_2, an agent known to increase the intracellular level of cyclic AMP, on release of Tx. This study confirmed that PGE_2 markedly inhibited Tx release in nontreated macrophages. However, we found that the effect of PGE_2 on Tx release was prominent at very low concentrations. Adolfs & Bonta (1982) showed that low concentrations of PGE_2 inhibit PGI_2-induced cyclic AMP. They further showed (Opmeer *et al.*, 1983) competition of adenylate

cyclase-coupled PGI_2 binding sites with PGE_2 in peritoneal macrophages. It is therefore possible that the effects of PGE_2 on inhibition of Tx release at low concentrations might be due to interference in the cellular effects of endogenous PGI_2. Taken collectively, this evidence indicates the possibility of interrelationships between PGE_2, PGI_2, Tx and cyclic AMP in the cellular framework of peritoneal macrophages.

An observation of major importance is that PGE_2 failed to inhibit Tx release in LPS-stimulated cells. By contrast, db cyclic AMP markedly inhibits Tx release in LPS-stimulated cells. This suggests that LPS interferes with a specific site rendering the cells unresponsive to PGE_2, but it did not interfere with the responsiveness of the cells to cyclic AMP itself. Further elucidation of the precise interactions between PGE_2, PGI_2, cyclic AMP and Tx will provide insight into the normal regulation of the release and effects of these metabolites and the mechanism by which LPS interferes in these interactions and causes stimulation of macrophages.

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