J. Pharm. Pharmacol. 1981, 33: 401–402
Communicated January 12, 1981

22–3573/81/060401–02 $02.50/0

3-Deazaadenosine, a transmethylase inhibitor, suppresses the effect of lipopolysaccharide on release of prostacyclin and thromboxane

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Lipopolysaccharide (LPS), the toxin of Gram-negative bacteria has been shown to activate macrophages and induce various metabolic processes of these cells, among them an increase of prostaglandin release (Morrison & Ulevitch 1978). However the mechanism of this effect is still unknown.

Recently it has been suggested by Hirata & Axelrod (1980) that phospholipid methylation is involved in transmission of biological signals when ligands bind to various cell types. They demonstrated that phospholipid methylation is involved in IgE mediated histamine release from leukaemic basophils, lectin-induced lymphocyte mitogenesis and in the coupling of β-adrenoceptor with adenylate cyclase (Hirata & Axelrod 1980). However, no study has been made on whether stimulation of macrophages by LPS involves activation of methylation processes.

In an attempt to examine this possibility we studied the effect of 3-deazaadenosine (3-DZA), a methyltransferase inhibitor (Chiang et al 1977; Crew et al 1980) on the LPS-induced prostaglandin and thromboxane release from rat peritoneal macrophages.

Method

Cell culture. Fischer 344 rats (8–12 weeks) were inoculated (i.p.) with 5 ml of Freund's incomplete adjuvant (Difco Lab). Four days later the cells were harvested by lavage with RPMI-1640 (50 ml), washed three times with RPMI and purified on 50% Percol density gradient. Cells so obtained are more than 90% macrophages, based on nonspecific esterase staining. The cells were suspended in RPMI-1640 (supplemented with penicillin and streptomycin 100 u ml^{-1}, with glutamine 2 mM) to 1×10^6 cells ml^{-1}. One ml amounts of the cell suspension were placed into tubes and incubated for 3h in a humidified atmosphere containing 5% CO_2 in air. Thereafter, the tubes were centrifuged, and the supernatant decanted and frozen (−20 °C) until assay for prostaglandins.

Prostaglandin assay. Prostaglandin content in the supernatant was determined by a direct radioimmunoassay (Granstrom & Kindhal 1976). The [^{3}H]prostaglandins were purchased from New England Nuclear, Mass. PGE_2 antibody was purchased from Accurate Chemical & Scientific Corporation, N.Y. The antibody for 6-keto-$PGF_{1\alpha}$ prepared in our laboratory has cross reactivity with PGE_2 of 10%, $PGF_{2\alpha}$ 7·6% and TxB_2 less than 1·0%. Antibody for TxB_2 was kindly provided by Dr L. Levine, Brandeis University, Boston, Mass. The cross reactivity of TxB_2 antibody with 6-keto-$PGF_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$ is less than 1·0%.

Reagent. *Escherichia coli* lipopolysaccharide 055, Difco. Indomethacin and quinacrine dihydrochloride were purchased from Sigma Chemical Co., St Louis. 3-Deazaadenosine was purchased from Southern Research Institute, Alabama.

The viability of the cells as tested by exclusion of trypan blue was higher than 90%. Data in text and Figures represent means ±s.e.m. Student's *t* test was used for statistical evaluation.

Results

The basal release of $PGF_{2\alpha}$, PGE_2, 6-keto-$PGF_{1\alpha}$ and TxB_2 from rat peritoneal macrophages is shown in Table 1. TxB_2 and 6-keto-$PGF_{1\alpha}$ were the major products of these cells. The rate of release of both TxB_2 and 6-keto-$PGF_{1\alpha}$ is significantly higher than that of PGE_2 and $PGF_{2\alpha}$ ($P < 0.001$). Incubation of the cells with LPS (0·1 μg ml^{-1}) significantly enhanced the release of all arachidonate metabolites assayed (Table 1).

The relationship between prostaglandin release and activation of phospholipase A_2 as well as the cycloxygenase was shown by Yorio & Bentley (1978). In the current model (Fig. 1a) that indomethacin potently inhibited the LPS-induced release of PGE_2, 6-keto-$PGF_{1\alpha}$, and TxB_2 dose-dependently (IC50 is 5×10^{-9} for 6-keto-$PGF_{1\alpha}$ and 10^{-8} for TxB_2 and PGE_2). Quinacrine, an inhibitor of phospholipase A_2 (Yorio & Bentley 1978) also suppressed the LPS-induced TxB_2 and 6-keto-$PGF_{1\alpha}$ release, the IC50 is 10^{-6} for both these metabolites.

Table 1. Effect of LPS on prostaglandin and TxB_2 release from rat peritoneal macrophages. Peritoneal cells (1×10^6) were incubated for 3 h in RPMI-1640 (control) or in RPMI + LPS (0·1 μg ml^{-1}). Data represent means ± s.e.m. of quadruplicate cultures of an experiment representative of three other experiments with similar results. * $P < 0.001$ (by Student-*t*-test) significance of the difference between control and LPS.

	Control (ng)	LPS (ng)
$PGF_{2\alpha}$	1·22 ± 0·05	*5·66 ± 0·27
PGE_2	2·34 ± 0·08	*7·07 ± 0·32
6-Keto-$PGF_{1\alpha}$	3·81 ± 0·19	*6·88 ± 0·43
TxB_2	4·80 ± 0·25	*9·23 ± 0·60

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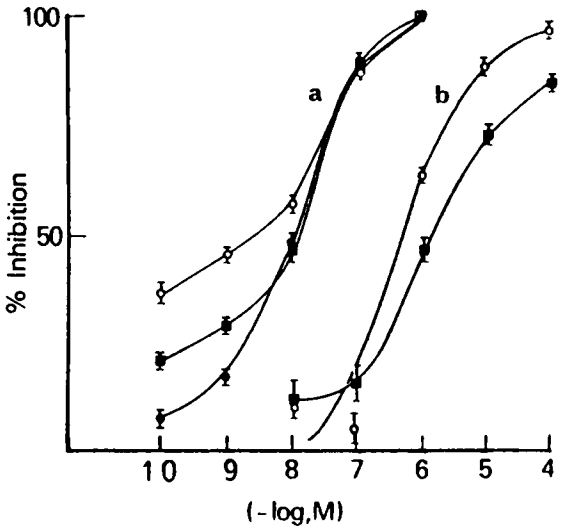

FIG. 1. Effect of (a) indomethacin (b) 3-DZA on LPS-induced release of PGE_2, 6-keto-$PGF_{1\alpha}$ and TxB_2 from rat peritoneal macrophages. 10^6 cells were incubated for 3 h with LPS (0·1 $\mu g\ ml^{-1}$) + various doses of indomethacin or 3-DZA. Data represent mean ± s.e.m. of quadruplicate cultures of one experiment representative of three other experiments with similar results.
●–● PGE_2 ○–○ 6-keto-$PGF_{1\alpha}$ ■–■ TxB_2.

The effect of 3-DZA, a transmethylase inhibitor, was examined on the release of TxB_2 and 6-keto-$PGF_{1\alpha}$ induced by LPS. Fig. 1b demonstrates that 3-DZA suppressed the effect of LPS on both TxB_2 and 6-keto-$PGF_{1\alpha}$ release dose-dependently, the IC50 being 7×10^{-7} for 6-keto-$PGF_{1\alpha}$ and $1{\cdot}3 \times 10^{-6}$ for TxB_2. These results demonstrate the potency of 3-DZA in blocking prostaglandin and TxB_2 released from macrophages.

The results are averages of quadruplicate cultures of one experiment, representative of three other experiments with similar results.

Discussion

Prostaglandins along with other factors have been previously shown to be released from macrophages upon stimulation with LPS (Morrison & Ulevitch 1978). We have now demonstrated that LPS-induced prostaglandin release can be blocked by indomethacin, a cycloxygenase inhibitor and by quinacrine, a phospholipase A_2 inhibitor. We have also shown that 3-DZA, a transmethylase inhibitor, blocks the effect of LPS on 6-keto-$PGF_{1\alpha}$ and TxB_2 release from macrophages. This finding suggests that the increased release of arachidonic acid metabolites elicited by LPS, may be mediated by transmethylation processes. The nature of these methylation processes cannot be inferred from the results and remains to be established.

Recent work on basophils indicates that there is a close association between phospholipid methylation and the release of arachidonic acid from phospholipids (Crews et al 1980). Furthermore 3-DZA has been shown to block the release of [^{14}C]arachidonic acid from previously labelled phosphatidylcholine in leukaemic basophils (Crews et al 1980). Our data are in accord with these observations and further demonstrate that the transmethylation process is a crucial mediator in the release of arachidonic acid metabolites from macrophages upon activation with LPS.

Prostaglandin release from macrophages has been shown to be correlated with increased phagocytosis and lysosomal enzyme release from these cells (Hsueh et al 1979; Scott et al 1980). Thus, the effectiveness of 3-DZA on blocking LPS-induced prostaglandin release lays the ground for studying the role of the transmethylation processes in mediation of biological functions of macrophages.

The present results also show that TxB_2 and 6-keto-$PGF_{1\alpha}$ are the major arachidonic acid metabolites produced by rat peritoneal macrophages, and that their rate of release is significantly higher than that of PGE_2 and $PGF_{2\alpha}$. This finding invites further investigation of the role of TxA_2 and PGI_2 in immune reaction and in inflammation.

In rabbit alveolar macrophages (Hsueh et al 1979) and in mice peritoneal macrophages (Bonney et al 1978), PGE_2 was reported to be the major metabolite converted from exogenous labelled arachidonic acid. In contrast to those reports, our present findings provide evidence of the *endogenous* release of four different prostaglandins assayed by a direct radioimmunoassay. We believe that this approach gives a more accurate picture on the physiological endogenous production of prostaglandins by these cells. Further characterization of the profile of arachidonic acid metabolites by this method is necessary, in order to clarify whether TxA_2 and PGI_2 are also the predominant arachidonic acid metabolites also in other immunoreactive cells.

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