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In vivo AND *in vitro* EFFECTS OF ENDOTOXIN ON PROSTAGLANDIN RELEASE FROM RAT LUNG

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1 The release of prostaglandins (PGE_2, $PGF_{2\alpha}$, PGI_2) and thromboxane A_2 (TxA_2) from rat lung exposed to *E. coli* endotoxin, *in vivo* or *in vitro* has been studied.

2 Lung strips of endotoxin-treated rats demonstrated a preferential increase of TxA_2 and $PGF_{2\alpha}$ and a decrease in the ratio PGI_2/TxA_2.

3 These data suggest that changes in the proportions of arachidonic acid metabolites might play a role in the pulmonary pathophysiology during endotoxin shock.

4 Incubation of lung strips with endotoxin *in vitro* failed to stimulate prostaglandin release; paradoxically, it suppressed both the spontaneous and the ionophore-induced prostaglandin release.

5 These findings suggest that the increase in prostaglandin release by the lung following endotoxin administration *in vivo* is probably mediated by factor(s) generated in endotoxaemia and is not due to a direct action of endotoxin on the lung tissue.

Introduction

Pathological derangements of pulmonary functions have been described in human and animal endotoxaemia (Kuida, Hinshaw, Gilbert & Visscher, 1958; Harrison, Beller, Hinshaw, Coalson & Greenfield, 1959). Among the most striking phenomena are an increase in pulmonary vascular resistance and a decrease in lung compliance, both of which appear in an early stage of the endotoxaemia (Kuida *et al.*, 1958; Weil, Shubin & Biddle, 1964). In addition, it has been shown that these haemodynamic events are accompanied by an increase in prostaglandin release from the lung (Anderson, Tsagaris, Jubiz & Kuida, 1975). This observation led to the assumption that prostaglandins might be involved in the pathological derangements induced by endotoxin.

This hypothesis was further strengthened by the fact that lobar artery pressure is increased by infusion of the prostaglandin precursor, arachidonic acid, into the dog isolated lung lobe (Wicks, Rose, Johnson, Ramwell & Kot, 1976), and in addition, by the observation that endotoxin-induced pulmonary hypertension is blocked by the prostaglandin synthetase inhibitor, indomethacin (Parrat & Sturgess, 1974).

Since different prostaglandins exert different effects on pulmonary functions (Hyman, Spannhake & Kadowitz, 1978), it is of particular interest to explore whether the characteristic pulmonary derangements in endotoxaemia can be correlated with a preferential release of particular prostaglandins in the lung. In addition, the enhanced prostaglandin release from the lung during endotoxaemia may raise the question whether this phenomenon is a direct effect of endotoxin on lung tissue or an indirect consequence induced by factors generated during endotoxin shock. The present study was designed to elucidate the effect of endotoxin on the release of various prostaglandins from rat lung tissue, both *in vivo* and *in vitro*.

Methods

Male Wistar rats (300–350 g) were used in all the experiments.

In vivo *experiment*

Animals were anaesthetized with pentobarbitone sodium (50mg/kg, i.p.), and endotoxin (*E. coli* 011:B4, LPS-B, Difco, 7.0 mg/kg) was injected into the femoral vein. Forty-five minutes later a polyethylene tubing was inserted into the portal vein, the abdominal aorta was cut, and 1.0 ml of blood was collected in a tube and placed in ice. The lungs were perfused with 0.9% (w/v) NaCl solution (saline) for 5 min *in situ*. The perfused lungs were removed and a strip of 1–2 mm wide was cut along the edge of both lungs and then chopped into 5.0 mm long pieces. Four strips (randomly taken) were transferred into

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tubes containing 0.5 ml of Krebs-phosphate buffer (0.1 M, pH 7.4) and incubated for 30 min at 37°C. Incubation was stopped by transferring the tubes to ice and removing the strips. The incubation medium was stored at −20°C until assayed for prostaglandins.

The strips were dried in a dessicator for 24 h and then weighed (average dry weight was 1.0 ± 0.2 mg). PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TxB_2 were assayed in each tube as described later. The rate of prostaglandin release for each animal is an average of the release of four lung strips. The blood samples were centrifuged (5 min, 4°C, 3000 rev/min) and the serum was stored at −20°C until assayed for 6-keto-$PGF_{1\alpha}$.

In vitro *experiment*

Lung strips were prepared as described in the *in vivo* section. Eighteen strips were placed for 10 min in Krebs buffer at room temperature and then transferred to tubes for 30 min incubation at 37°C. Prostaglandin release from lung strips by the calcium ionophore A23187 was used as an internal probe for the viability and responsiveness of the lung tissue. The incubation protocols in the *in vitro* experiments were as follows: Experiment A: (1) control (Krebs buffer only): (2) Ca^{2+} ionophore (5 μg/ml); (3) endotoxin (50, 100, 200 or 400 μg/ml). Experiment B: (1) control; (2) Ca^{2+} ionophore (5.0 μg/ml); (3) endotoxin (100 μg/ml); and (4) Ca^{2+} ionophore (5.0 μg/ml) combined with endotoxin (100, 200 or 400 μg/ml). All the procedures following the incubation period, namely tissue drying and prostaglandin assay, were the same as described in the *in vivo* section. The rate of prostaglandin release for each condition is an average of the release of three different lung strips.

Prostaglandin assay

Prostaglandins were assayed by radioimmunoassay technique (Granstrom & Kindahl, 1976). The ^3H-prostaglandins were purchased from New England Nuclear, Mass. PGE_2 antibody was purchased from Accurate Chemical & Scientific Corporation, N.Y. The cross reactivity was 1.25% with $PGF_{2\alpha}$ and less than 1.0% with 6-keto-$PGF_{1\alpha}$. The cross reactivity of TxB_2 antibody with 6-keto-$PGF_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$ was less than 1.0%. 6-keto-$PGF_{1\alpha}$ antibody was prepared in our laboratory; its cross reactivity with PGE_2 was 10%, $PGF_{2\alpha}$ 7.6% and TxB_2 less than 1.0%. $PGF_{2\alpha}$ antibody was a gift from Dr Behrman, Yale University (Orczyk & Behrman, 1972).

Data analysis

Rate of prostaglandin release is presented as the amount of prostaglandin per mg dry weight in 30 min.

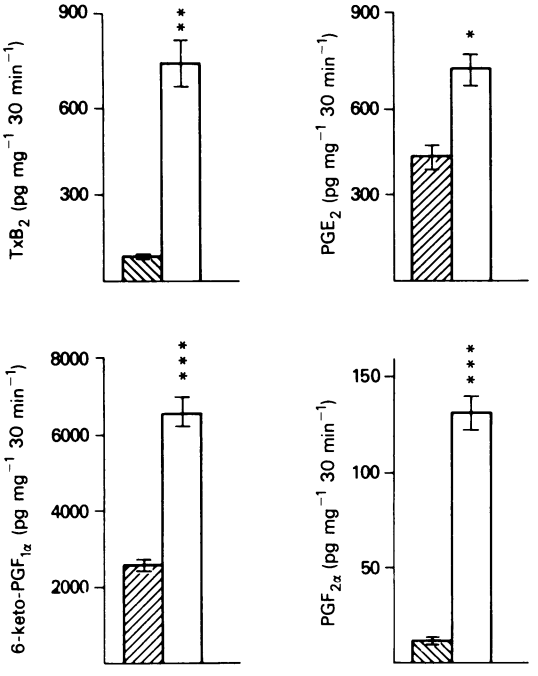

Figure 1 Effect of endotoxin *in vivo* on prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, thromboxane B_2 (TxB_2) and 6-keto-$PGF_{1\alpha}$ release from parenchymal lung strips. Hatched columns: rats injected with saline (control, $n = 12$); open columns: rats injected with endotoxin ($n = 12$). Data represent means values, vertical lines show s.e.mean. Statistical evaluation was by Student's unpaired t test: $^*P<0.05$; $^{**}P<0.005$; $^{***}P<0.001$.

Data presented in text and figures are means ± s.e.mean. Statistical evaluation was by Student's t test (paired or unpaired as denoted in the legends).

Results

In vivo *experiment*

The serum level of 6-keto-$PGF_{1\alpha}$ in the endotoxin-treated rats was 2.01 ± 0.16 ng/ml, compared with 0.82 ± 0.15 ng/ml in the control rats ($P<0.05$). This increase in serum level of 6-keto-$PGF_{1\alpha}$ following endotoxin administration confirms previous findings (Bult, Beeteus, Vercruysse & Herman, 1978).

Lung strips of control animals release prostaglandins at the following rate (pg mg^{-1} 30 min^{-1}): PGE_2 433.0 ± 78.9, TxB_2 90.0 ± 11.0, 6-keto-$PGF_{1\alpha}$ 2630 ± 316 and $PGF_{2\alpha}$ 12.0 ± 5.2 (Figure 1). Lung strips of endotoxin-treated rats released significantly more prostaglandins (Figure 1). The increase in prostaglandin output following endotoxin treatment varied considerably among the different prostaglandins, as shown in Figure 2. The release of $PGF_{2\alpha}$ increased

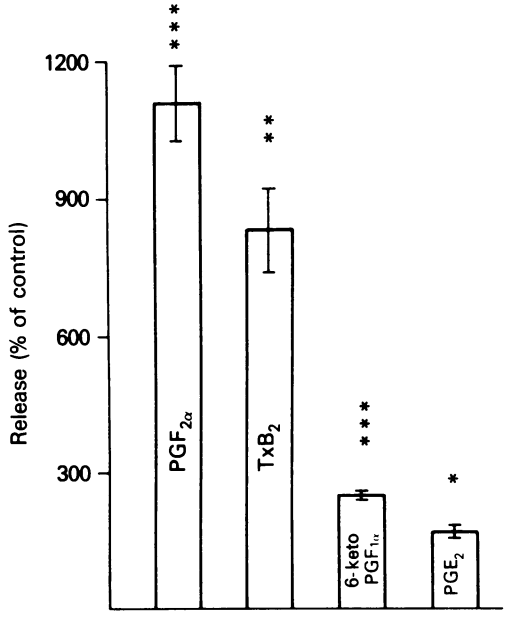

Figure 2 Percentage increase of prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, thromboxane B_2 (TxB_2) and 6-keto-$PGF_{1\alpha}$ induced by endotoxin *in vivo*. Data represent mean of % increase ($n=12$); vertical lines show s.e.mean. Statistical evaluation was by Student's *t* test: $*P<0.02$; $**P<0.005$; $***P<0.001$.

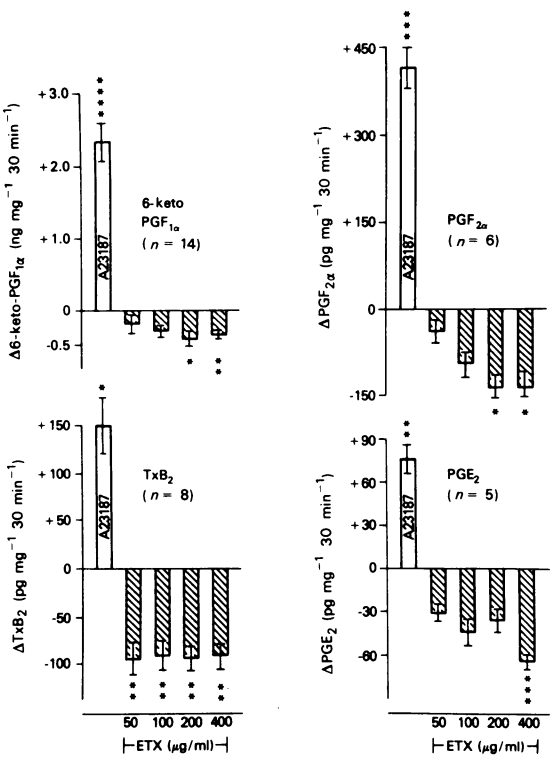

Figure 3 Effect of endotoxin or A23187 (Ca^{2+} ionophore) on prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, thromboxane B_2 (TxB_2) and 6-keto-$PGF_{1\alpha}$ release from lung strips *in vitro*. Data represent means of the change in prostaglandin release induced by incubation of lung strips with A23187 (5 μg/ml) or with endotoxin (ETX) at the following concentrations: 50, 100, 200 or 400 μg/ml; vertical lines show s.e.mean. Statistical evaluation was by Student's paired *t* test: $*P<0.05$; $**P<0.02$; $***P<0.005$; $****P<0.001$.

by 1111 ± 162%, TxB_2 837 ± 179%, 6-keto-$PGF_{1\alpha}$ 245 ± 19% and PGE_2 170 ± 25%. The increase in the release of TxB_2 was higher than that of 6-keto-$PGF_{1\alpha}$ ($P<0.005$) and PGE_2 ($P<0.005$). The increase in the release of $PGF_{2\alpha}$ was also higher than that of 6-keto-$PGF_{1\alpha}$ ($P<0.001$) and PGE_2 ($P<0.001$). However, there was no significant difference between the increase in $PGF_{2\alpha}$ and TxB_2. The ratio of 6-keto-$PGF_{1\alpha}$/TxB_2 decreased from 29.8 ± 4.4 in the control group to 8.8 ± 2.2 in the endotoxin-treated group ($P<0.001$). This was due to the greater increase in the release of TxB_2 which is induced by endotoxin.

In vitro *experiment*

Experiment A: Figure 3 shows that A23187 enhanced the release of all the prostaglandins examined. The % increase of prostaglandin release induced by A23187 was as follows: $PGF_{2\alpha}$ 262 ± 28%, 6-keto-$PGF_{1\alpha}$ 246 ± 33%, TxB_2 200 ± 32%, PGE_2 224 ± 44%. Thus, the increase in prostaglandin release induced by A23187 did not vary among the different prostaglandins. Figure 3 also demonstrates that endotoxin *in vitro* reduced the spontaneous release of prostaglandins from lung strips. The % inhibition of prostaglandin release induced by the highest dose of endotoxin (400 μg/ml) was as follows: $PGF_{2\alpha}$ 52.4 ± 16.4%, 6-keto-$PGF_{1\alpha}$ 17.6 ± 5.6%, TxB_2 45.9 ± 9.6%, PGE_2 81.6 ± 10.5%. Furthermore, the inhibitory effect of endotoxin on the release of PGE_2 and $PGF_{2\alpha}$ was dose-dependent as shown in Figure 4.

Experiment B: In order to explore further the inhibitory effect of endotoxin on prostaglandin release, the effect of endotoxin was also examined on the release of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ induced by A23187. Figure 5 shows that endotoxin significantly suppressed the A23187-induced release of both $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$. The highest dose of endotoxin (400 μg/ml) suppressed the A23187-induced release of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ by 50.8 ± 3.9% ($P<0.001$) and 34 ± 3.7% ($P<0.001$), respectively. As in Experiment A, endotoxin alone suppressed the spontaneous release of these prostaglandins from lung strips (Figure 5).

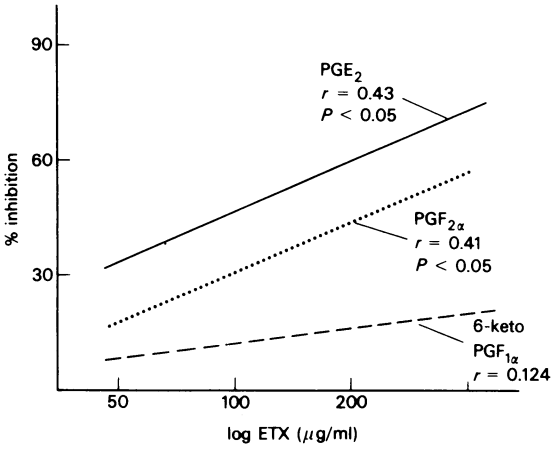

Figure 4 Dose-response curves of the inhibitory effect of endotoxin on prostaglandin release. Curves represent the regression lines of prostaglandin E_2 (PGE_2, solid line) $n = 25$; $PGF_{2\alpha}$ (dotted line) $n = 25$; 6-keto-$PGF_{1\alpha}$ (dashed line) $n = 53$.

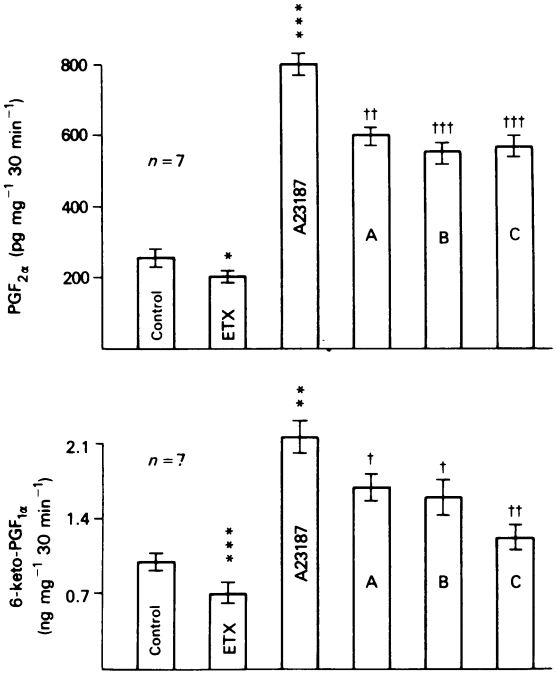

Figure 5 Effect of endotoxin on A23187 (Ca^{2+} ionophore)-induced prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and 6-keto-$PGF_{1\alpha}$ release from lung strips *in vitro*. Data represent means of prostaglandin release in the following incubation conditions: control (Krebs buffer only); ETX (endotoxin, 100 μg/ml); A23187 only; A23187 + 100 μg/ml endotoxin (column A); A23187 + 200 μg/ml endotoxin (column B); A23187 + 400 μg/ml endotoxin (column C). Data were analyzed by Student's paired *t* test as follows: Conditions A, B, and C were compared to A23187 only. Level of significance is denoted by †(dagger) as follows: $\dagger P < 0.02$; $\dagger\dagger P < 0.01$; $\dagger\dagger\dagger P < 0.001$. The effect of A23187 only and endotoxin only were compared to control. Level of significance is denoted by asterisks as follows: $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.001$.

Discussion

The present results show that administration of endotoxin *in vivo* markedly increased prostaglandin release from rat lung strips. An important finding in our work was that following endotoxin injection, the pattern of prostaglandin output by lung tissue shifts toward a more pronounced release of TxA_2 and $PGF_{2\alpha}$, compared to PGI_2 and PGE_2. In contrast, the release elicited by Ca^{2+} ionophore was of the same magnitude for all the prostaglandins measured. This suggests that a specific alteration in the pattern of prostaglandin release occurs in the lung during endotoxaemia. It is of interest that both $PGF_{2\alpha}$ and TxA_2, which exhibited the largest increase by endotoxin *in vivo*, have been previously suggested to be involved in pulmonary hypertension during endotoxaemia (Anderson *et al.*, 1975; Parrat & Sturgess, 1977; Harris, Zmudka, Maddox, Ramwell & Fletcher, 1980). An increase in plasma levels of TxB_2 during endotoxin shock was found to be temporally correlated with the increase in pulmonary artery pressure (Harris *et al.*, 1980). Anderson *et al.* (1975) observed that the injection of endotoxin into calves is followed by a concomitant increase of pulmonary artery pressure and release of $PGF_{2\alpha}$ from the lung. Furthermore, administration of the $PGF_{2\alpha}$ antagonist, polyphloretin phosphate, attenuated the endotoxin-induced pulmonary hypertension in cats (Parrat & Sturgess, 1977). In contrast to previous work, we measured the simultaneous release of PGE_2, $PGF_{2\alpha}$, TxA_2 and PGI_2. This enables us to conclude that, following endotoxin administration *in vivo*, the vasoconstrictor prostaglandins are predominantly increased. This observation provides further support for the hypothesis that vasoconstrictor prostaglandins may be involved in pulmonary hypertension in endotoxaemia.

The pulmonary changes which appear in endotoxaemia have been also ascribed to augmentation of platelet aggregation in the lung capillary bed (Bredenberg, Taylor & Webb, 1980). Platelet aggregation is facilitated by TxA_2 and inhibited by PGI_2 (Lefer, 1979). Our data show that 6-keto-$PGF_{1\alpha}$/TxB_2 ratio decreased following endotoxin administration. Thus, exposure to endotoxin induces an alteration in the balance between PGI_2 and TxA_2 which may lead to a more pronounced effect of TxA_2. This evidence implies that either an increase in PGI_2 or a decrease in TxA_2 could be beneficial in overcom-

ing the effects induced by endotoxin. Indeed, it has been recently shown that PGI_2 infusion (Fletcher & Ramwell, 1980), as well as administration of the TxA_2 inhibitor, imidazole, (Cook, Wise & Halushka, 1979) exert protective effects in endotoxin shock.

The evidence presented in this investigation might raise the hypothesis that the involvement of prostaglandins in the pathogenesis of endotoxin shock might not be the result of the increase in prostaglandins release *per se*, but might be due to an alteration in the *balance* of arachidonic acid metabolites during endotoxaemia.

In contrast to the effect of endotoxin *in vivo*, incubation of lung strips with endotoxin *in vitro* failed to stimulate prostaglandin release. In fact, endotoxin *in vitro* significantly reduced prostaglandin release.

The mechanism which underlies the inhibitory effect of endotoxin on prostaglandin release *in vitro* is obscure. However, our observation that endotoxin *in vitro* also suppresses prostaglandin release induced by calcium ionophore, indicates that endotoxin suppression of prostaglandin release may be by interference with the transmembranal flux of calcium ion. This suggestion is supported by the finding that incubation of heart homogenates with endotoxin increases Ca^{2+} efflux from the tissue homogenate to the incubation medium (Liu & Spitzer, 1977).

The physiological significance of prostaglandin inhibition by endotoxin, *in vitro*, remains to be established. However, the differential effect of endotoxin on prostaglandin release by the lungs *in vivo* versus *in vitro* suggests that the enhanced prostaglandin metabolism observed in the lung during endotoxaemia is not due to a direct effect of endotoxin on lung parenchymal tissue.

We suggest that other cell types should be examined as the possible target of endotoxin *in vivo*. Possible candidates are the immunoreactive cells, such as macrophages and lymphocytes which have been shown to respond to low does of endotoxin both *in vivo* and *in vitro* (Morrison & Ulevitch, 1978), and to produce a factor that mediates the pyrogenic effect of endotoxin (Dinarello & Wolf, 1978). Thus, it is not inconceivable that the increase in prostaglandin release induced by endotoxin *in vivo* is also mediated by a factor(s) produced by these cells. It appears that further investigations are necessary in order to elucidate the putative prostaglandin releasing factor that mediates the increase in prostaglandin release during endotoxaemia.

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