

Effect of Prostaglandin E₁ on the Pulmonary Vascular Response to Endotoxin¹ (36447)

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Injection of endotoxin to dogs is usually followed by a precipitous drop in systemic arterial blood pressure, rise in portal venous pressure and by increase in resistance in some vascular beds, for example in the lung (1-4). It has been also indicated, that endotoxin may cause damage to tissues and the release of subcellular constituents such as lysosomal enzymes (5, 6). The aggregation of platelets is also frequently encountered in septic and other forms of shock (7, 8, 9). Among the organs that are involved in shock, the lung has been frequently mentioned (10).

Because some prostaglandins can stabilize some cell membranes (11) through the accumulation of cyclic AMP (3',5'-adenosine monophosphate), inhibit platelet clumping (12) and are potent vasodilators (13), we tested the effects of prostaglandin E₁ (PGE₁) on the isolated lobe of the shocked lung perfused *in situ*.

Materials and Methods. Six mongrel dogs weighing 15-30 kg were anesthetized with sodium pentobarbital, 30 mg/kg iv. The thorax was opened through an intercostal space, the pulmonary artery and vein leading to and from the left lower and subsequently from the upper lobe were ligated and the circulation of the lobe was completely isolated from the rest of the lung, as previously described (14). Blood was collected in a venous reservoir and was pumped into an elevated arterial reservoir by a Sigma motor pump. From here the blood flowed by gravity into the isolated lung lobe. The height of the blood

column in the venous reservoir, the pressure in the pulmonary artery and the systemic arterial blood pressure were recorded by Statham transducers connected to a Grass polygraph. In this perfusion system the flow through the lung was identical to the flow delivered from the Sigma motor pump as long as the volume of the arterial reservoir did not change. Thus changes in pulmonary arterial pressure reflected proportional changes in the pulmonary vascular resistance.

PGE₁ (1 mg/ml) was dissolved in 95% ethanol. The compound was infused in saline in a concentration of 1 µg/ml at a rate of 0.95 ml/min in the pulmonary artery 10 min before endotoxin was administered. In control studies saline with the same amount of alcohol was given. Six milligrams of endotoxin (*Escherichia coli*, Difco) was dissolved in 2 ml of saline and added to the venous reservoir of the blood.

In each animal the lower and upper lobes of the left lung were isolated and perfused sequentially. One lobe received endotoxin and saline and was used as control, the other one was perfused with PGE₁, and endotoxin. Thus each animal served as its own control, since the sequence of the experimental and control studies was reversed repeatedly. The effect of endotoxin was expressed as percentage increase in pulmonary perfusion pressure (PPP).

Blood samples were taken at 0 time and 5-10 min after the administration of endotoxin. Proteins in plasma were measured by the biuret method (15).

The activity of β-glucuronidase was assayed with phenolphthalein glucuronate substrate (16), and expressed as micrograms of phenolphthalein released per hour per milli-

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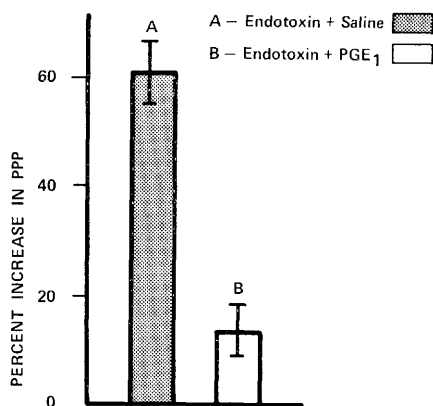


FIG. 1. Administration of endotoxin in the isolated lobe of the dog lung increases perfusion pressure (PPP) (A). This was greatly reduced by simultaneous administration of PGE₁ (B). Vertical bars: ± 1 SE ($n = 6$).

gram of plasma protein. Acidic catheptic activity was determined with hemoglobin substrate (17), expressed as micrograms of tyrosine released per hour per milligram, and prolylcarboxypeptidase (PCP) with ¹⁴C-1-dimethylamino-naphthyl-5-sulfonyl (DNS)-Pro-Phe (18–20). This substrate was prepared by reacting ¹⁴C-DNS-Cl with Pro-Phe. The resulting radioactive and fluorescent substrate ¹⁴C-DNS-Pro-Phe (3.9 nmole) was incubated with plasma (0.1 ml) at 37° in 0.1 ml 0.1 M acetate buffer of pH 4.5. Samples were taken after 5 hr of incubation and applied to silica-coated glass microfiber sheets. Thin-layer chromatography was done in *n*-butanol:*n*-amyl alcohol:NH₄OH (50:40:1) solvent (19). The fluorescent sub-

strate and the product of its enzymic hydrolysis (¹⁴C-DNS-Pro) were separated by thin-layer chromatography and localized under UV light. The spots were cut out from the sheets, placed in a vial containing a liquid scintillator and radioactivity was measured in a scintillation counter.

Results. Infusion of endotoxin in the perfused lung lobe led to vasoconstriction as shown by the increase in the volume of blood in the arterial reservoir. Endotoxin increased the PPP by 62% (± 6 SE; Fig. 1). Prior infusion of a total of 9.5 μ g PGE₁ blocked most of the increase in pressure (14% ± 5). In 2 animals PGE₁ was infused 10 min after the administration of endotoxin (Fig. 2). During the infusion of PGE₁ the increase in PPP was greatly reduced and the perfusion pressure returned almost to the preendotoxin level. When the infusion of PGE₁ was stopped, the pressure started to rise again.

The release of lysosomal enzymes in shock was also measured in the plasma collected from the perfused lobe. Administration of endotoxin raised the level of β -glucuronidase ($p < .05$ by sequential analysis) and catheptic activity ($p < .001$) significantly. This increase in lysosomal enzyme activity was essentially abolished by pretreatment with PGE₁ (Fig. 3). In the blood perfusing the lobe PCP activity did not increase after shock. Administration of PGE₁ had no influence on the level of this enzyme (Fig. 4). This ranged from 9 to 12 (± 2) pmole/hr/mg of serum protein.

Discussion. These studies showed that the

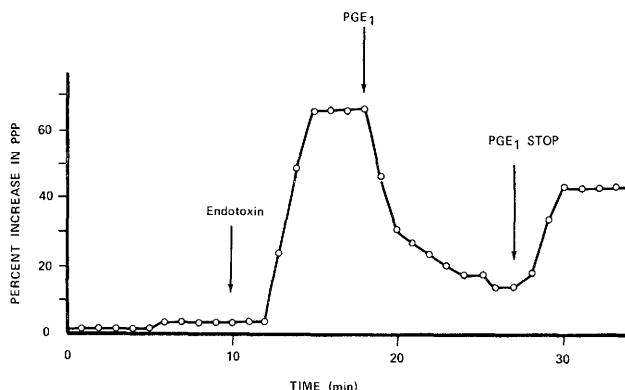


FIG. 2. Infusion of PGE₁ after endotoxin administration abolishes the effect of endotoxin.

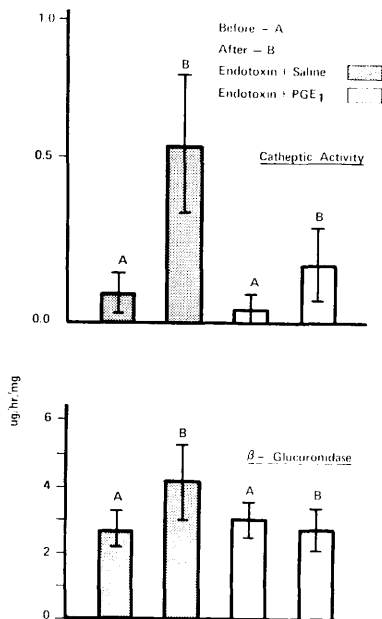


FIG. 3. Catheptic and β -glucuronidase activities increased in the blood perfusing the isolated lobe after endotoxin administration. PGE₁ blocked the rise in the lysosomal enzyme level ($n = 5$, cathepsin; $n = 6$, β -glucuronidase).

administration of endotoxin increased the perfusion pressure of the lung lobe perfused *in situ*. There was a simultaneous increase in the activity of two lysosomal enzymes in the blood plasma of the isolated lobe. Both of these effects were abolished or greatly decreased by pretreatment with PGE₁. PGE₁ also lowered the elevated PPP when it was given after endotoxin administration. It is unlikely that the beneficial action of PGE₁ was due to the abolishment of platelet aggregation (12, 21) known to be induced by endotoxin (8). Recent studies indicated that endotoxin increased pulmonary vascular resistance in the isolated lobe of the lung, even when it was perfused with blood depleted of platelets. The effect of endotoxin on the pulmonary vascular resistance does not depend on the presence of platelets in the perfusate (22). Thus in this preparation PGE₁ acted as a vasodilator and as a lysosomal membrane stabilizer. Recently, Weissman, Dukor, and Zurier (11) indicated that PGE₁ can prevent the release of lysosomal enzymes from leukocytes *in vitro* by

increasing the concentration of intracellular cyclic AMP. A recently published abstract attributed the *in vivo* beneficial effects of PGE₁ in endotoxin shock to suppression of the release of lysosomal enzymes in the dog (23).

In contrast to the two other lysosomal enzymes we studied, there was no increase in PCP activity in the perfusate either collected from the lung of shocked dogs or from dogs which received PGE₁ in addition to endotoxin. PCP is present in the lysosomal fraction of the homogenized kidney (18) and granulocytes (19). It is an enzyme that cleaves peptide bonds of penultimate proline with C-terminal amino acids (18). One naturally occurring substrate of this enzyme is angiotensin II, which is rapidly inactivated by PCP by the hydrolysis of the terminal Pro⁷-Phe⁸ bond. In the present experiments the circulation of the isolated lobe was completely separated from the rest of the circulation. Earlier studies showed that during perfusion the number of leukocytes and platelets decreased greatly in the blood perfusing the lobe (22), possibly because cells migrate from the blood into the pulmonary tissue (24). If the source of PCP had been depleted, an increase in the enzyme level after endotoxin administration could not be expected. Normally, PCP activity (18) is very low

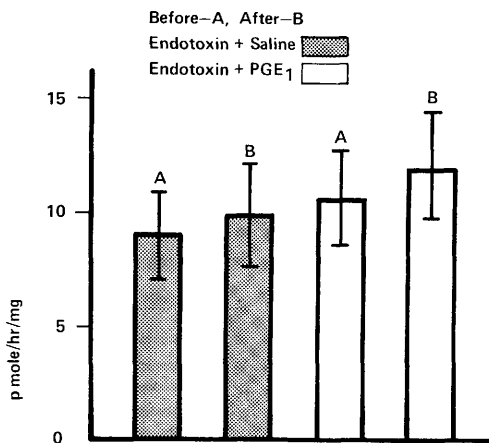


FIG. 4. Endotoxin does not increase the prolylcarboxypeptidase activity in the blood perfusing the lobe. PGE₁ had no effect on this enzymic activity ($n = 6$).

in plasma, but it increases in the systemic circulation during septic shock (20).

The two other lysosomal enzymes assayed, β -glucuronidase and the cathepsin may come from tissues other than leukocytes.

Because of the clinical importance of septic shock, many drugs have been tested in laboratory animals to combat this condition. For example, nonsteroidal anti-inflammatory agents blocked some of the effects of the injection of endotoxin (2-4) or live *E. coli* microorganisms (6) in dogs. Anti-inflammatory drugs may also antagonize some prostaglandins by blocking their release, biosynthesis and action on some smooth muscles (25). PGE₁ and anti-inflammatory drugs, however, can have a similar mode of action because both can prevent the aggregation of platelets (21, 26). Additional similarity between the effects of PGE₁ and anti-inflammatory drugs was provided here by showing that both types of compounds could block some of the effects of endotoxin on the circulation.

Summary. Endotoxin causes an increase in vascular resistance and the release of some lysosomal enzymes in the circulation of the isolated lobe of the dog lung perfused *in situ*. These effects of endotoxin were prevented by the administration of PGE₁. When PGE₁ was infused after endotoxin was given, the increase in the pulmonary perfusion pressure was greatly reduced. Thus similar to the previously tested anti-inflammatory drugs, PGE₁ can block some of the effects of endotoxin in the circulation.

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