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Effect of Prostaglandin E_1 on the Pulmonary Vascular Response to Endotoxin

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13. ABSTRACT

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 PGE_1. When PGE_1 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, PGE_1 can block some of the effects of endotoxin in the circulation.

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Effect of Prostaglandin E_1 on the Pulmonary Vascular Response to Endotoxin

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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 (1,2,3,4). 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 a frequently encountered symptom 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 E_1 (PGE_1) 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 i.v. The thorax was opened through an intercostal space, the pulmonary artery and vein leading to and from the right 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.

PGE_1 (1 mg/ml) was dissolved in 95% ethanol. The compound was infused in saline in a concentration of 1 ug/ml at a rate of 0.95 ml per min in the pulmonary artery 10 min before endotoxin was administered. In control studies saline with the same amount of alcohol was given. Six mg 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 PGE_1 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 % increase in pulmonary perfusion pressure (PPP).

Blood samples were taken at 0 time and 5-10 minutes 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 ug phenolphthalein released/hr per mg of serum protein. Acidic catheptic activity was determined with hemoglobin substrate (17), expressed as ug tyrosine released/hr per mg, and prolylcarboxypeptidase (PCP) with ^{14}C-1-dimethylamino-naphthyl-5-sulfonyl-(DNS)-Pro-Phe (18,19,20). This substrate was prepared by reacting ^{14}C-DNS.Cl

with Pro-Phe. The resulting radioactive and fluorescent substrate ^{14}C-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:NH_4 OH (50:40:1) solvent (18). The fluorescent substrate and the product of its enzymic hydrolysis (^{14}C-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 S.E; Fig. 1). Prior infusion of a total of 9.5 ug PGE_1 blocked most of the increase in pressure (14% ± 5). In 2 animals PGE_1 was infused 10 min after the administration of endotoxin (Fig. 2). During the infusion of PGE_1 the increase in PPP was greatly reduced and the perfusion pressure returned almost to the pre-endotoxin level. When the infusion of PGE_1 was stopped, the pressure started to rise again.

Fig. 1.

Fig. 2

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 ($P < 0.05$ by sequential analysis) and catheptic activity ($P < 0.001$) significantly. This increase in lysosomal enzyme activity was essentially abolished by pretreatment with PGE_1 (Fig. 3).

In the blood perfusing the lobe PCP activity did not increase after shock. Administration of PGE_1 either had no influence on the level of this enzyme (Fig. 4). This ranged from 0.09 to 0.12 ($\pm$ 0.02) pmole/hr per mg of serum protein.

DISCUSSION

These studies showed that the 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 PGE_1. PGE_1 also lowered the elevated PPP when it was given after endotoxin administration. It is unlikely that the beneficial action of PGE_1 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 PGE_1 acted as a vasodilator and as a lysosomal membrane stabilizer. Recently, Weissman *et al* (11) indicated that PGE_1 can prevent the release of lysosomal enzymes from leucocytes *in vitro* by increasing the concentration of intracellular cyclic AMP. A recently published abstract attributed the *in vivo* beneficial effects of PGE_1 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 either in the perfusate collected from the lung of shocked dogs or in the dogs which received PGE_1 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 Pro^7-Phe^8 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 leucocytes 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 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 leucocytes.

Because of the clinical importance of septic shock, many drugs have been tested in laboratory animals to combat this condition. For example, non-steroidal anti-inflammatory agents blocked some of the effects of the injection of endotoxin (2,3,4) or live E. coli micro-organisms (6) in dogs. Anti-inflammatory drugs may also antagonize some prostaglandins by blocking their release, biosynthesis and action on some smooth muscles (25). PGE_1 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 PGE_1 and anti-inflammatory drugs was here provided/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 PGE_1. When PGE_1 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, PGE_1 can block some of the effects of endotoxin in the circulation.

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LEGENDS TO THE FIGURES

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 PGE_1. (B.) Vertical bars: $\pm$ 1 S.E. (n=6).

Fig. 2. Infusion of PGE_1 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. PGE_1 blocked the rise in the lysosomal enzyme level (n=5 cathepsin; n=6 β-glucuronidase).

Fig. 4. Endotoxin does not increase the prolylcarboxypeptidase activity in the blood perfusing the lobe. PGE_1 had no effect on this enzymic activity (n=6).

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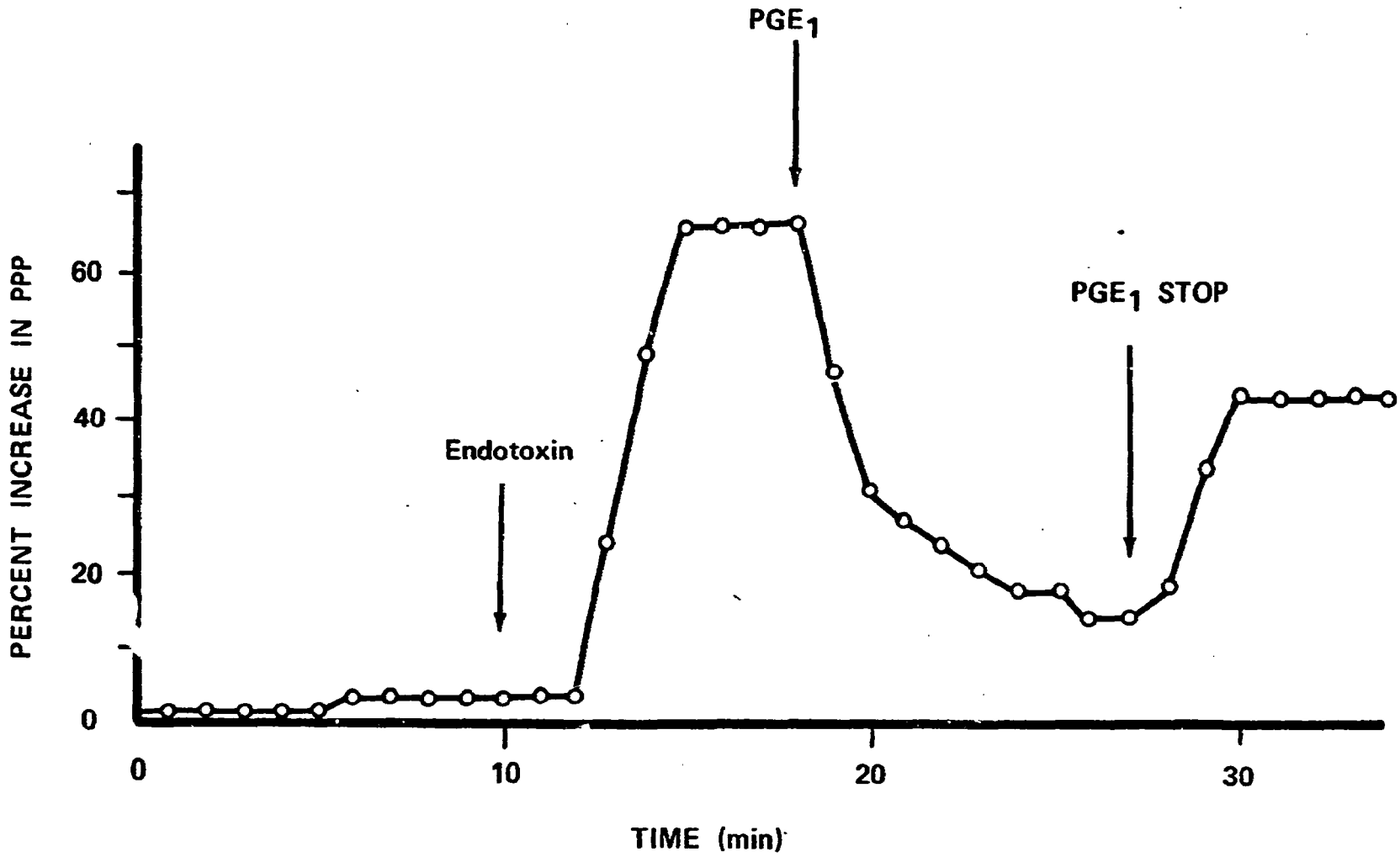
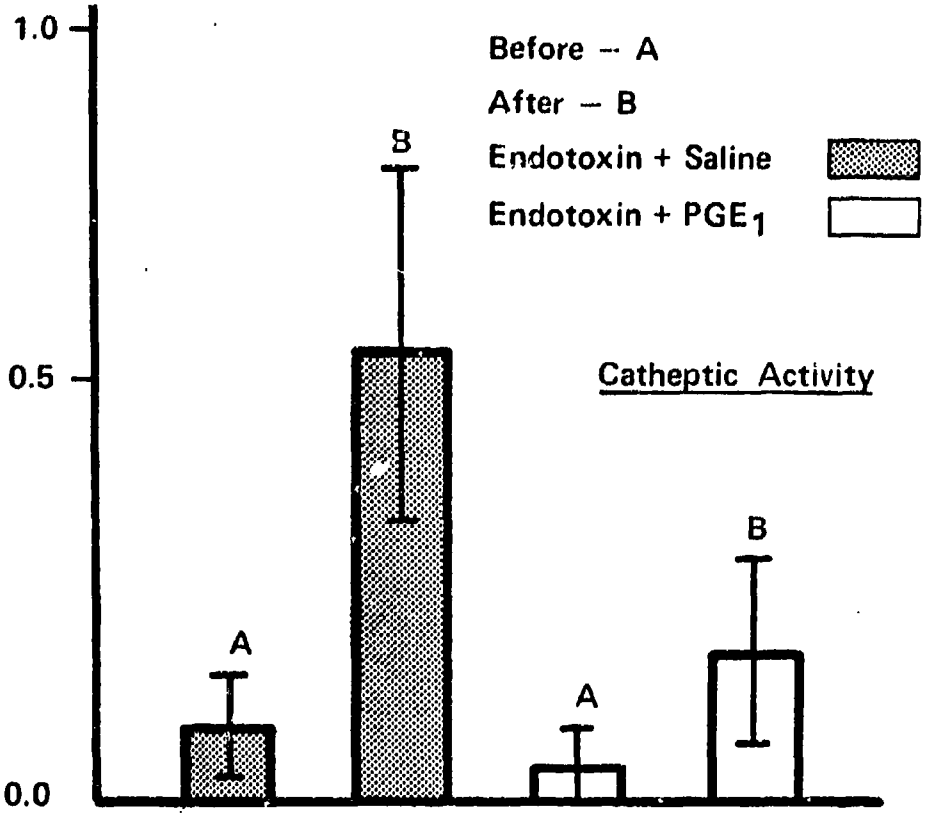
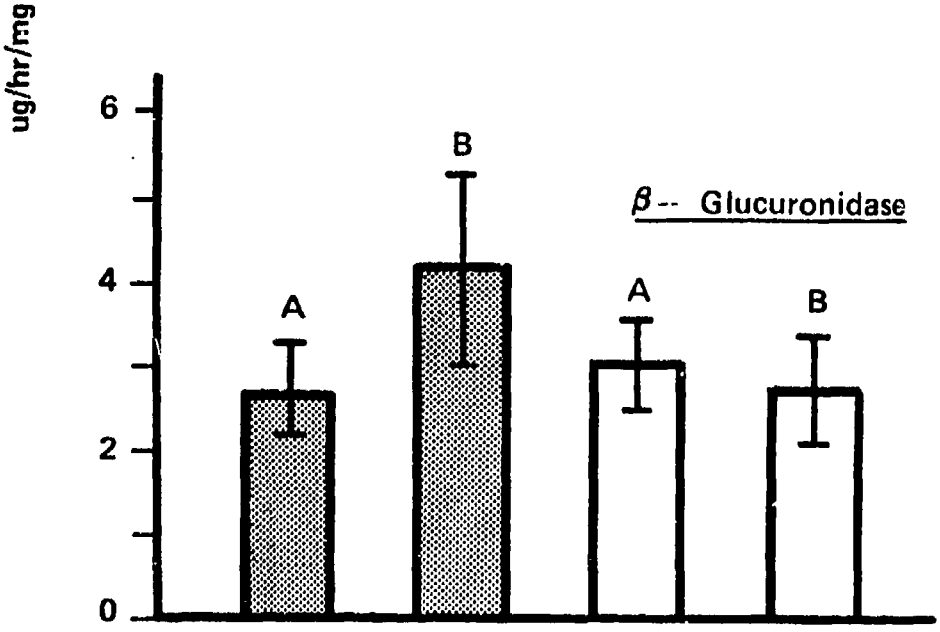
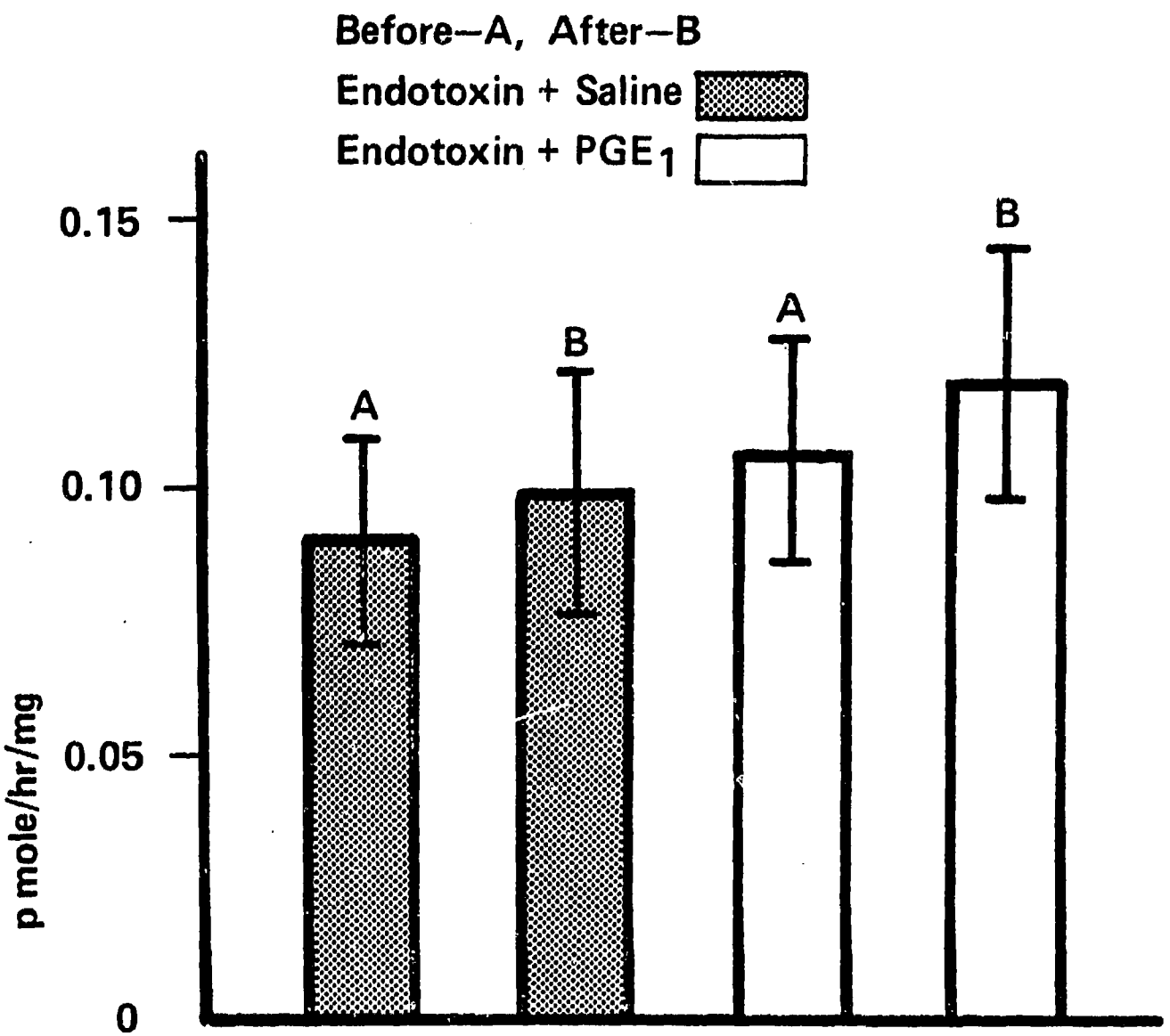
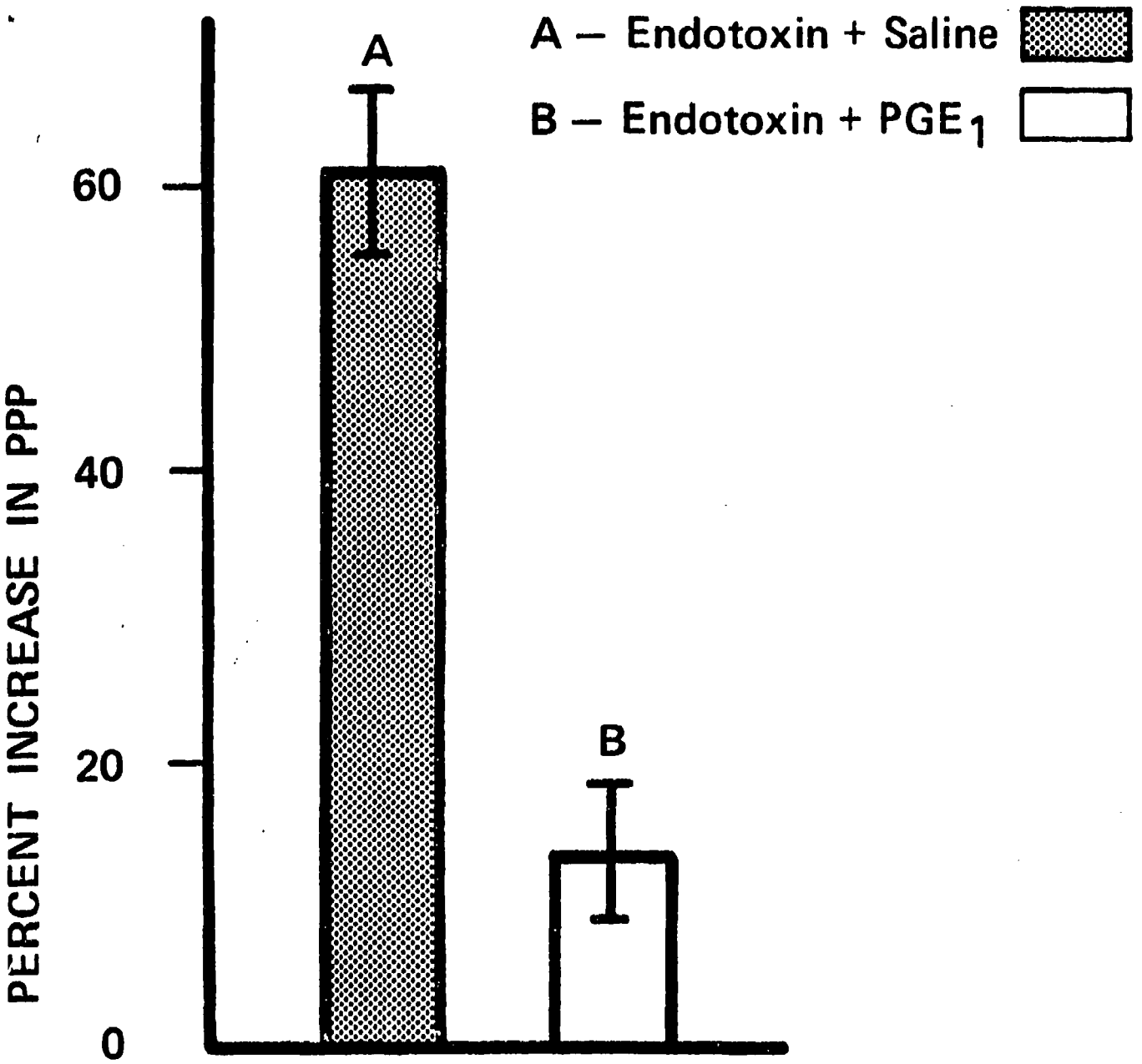

PGE1
PGE1 STOP
Endotoxin
PERCENT INCREASE IN PPP
60
40
20
0
0
10
20
30
TIME (min)


1.0
0.5
0.0
Before -- A
After -- B
Endotoxin + Saline
Endotoxin + PGE1
Catheptic Activity
A
B
A
B


ug/hr/mg
6
4
2
0
β-- Glucuronidase
A
B
A
B


Before—A, After—B
Endotoxin + Saline
Endotoxin + PGE1
0.15
0.10
0.05
0
p mole/hr/mg
A
B
A
B


A – Endotoxin + Saline
B – Endotoxin + PGE1
PERCENT INCREASE IN PPP
60
40
20
0
A
B