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Possible involvement of prostaglandins in vasoconstriction induced by zymosan and arachidonic acid in the perfused rat liver

Peter Dieter*, Joseph. G. Altin and Fyfe L. Bygrave

Department of Biochemistry, The Faculty of Science, Australian National University, Canberra, ACT 2600, Australia

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Exposure of perfused livers to zymosan, arachidonic acid or phenylephrine but not to latex particles, stimulates hepatic constriction. The effects of arachidonic acid are rapid, reach a maximum after 2–3 min and then decline. They are blocked by the cyclooxygenase inhibitor indomethacin but not by the lipoxygenase inhibitor nordihydroguaiaretic acid. This suggests a role for prostaglandins in this action. Zymosan progressively increases hepatic pressure after a lag time of about 1 min. Perfusion of bromophenacyl bromide, indomethacin and nordihydroguaiaretic acid only partially inhibits the zymosan-induced vasoconstriction. None of these inhibitors affect the phenylephrine-induced response. Repeated infusion of arachidonic acid leads to homologous desensitization of the response whereas the response of the liver to phenylephrine is unaffected. The present data indicate that prostaglandins, produced and released within the liver, affect vasoconstriction in this organ.

Arachidonic acid; Latex particle; Prostaglandin; Vasoconstriction; (Perfused liver)

1. INTRODUCTION

It is known that blood flow through the hepatic vasculature can be regulated by constriction of the portal venules and by sinusoids [1–3]. Mechanisms, including those induced by hormones such as catecholamines, α- and β-adrenergic agonists, serotonin, dopamine, angiotensin and vasopressin are known to regulate the hepatic blood flow [4]. The role of eicosanoids, which are known to be vasoactive in other systems [5], has not been investigated in the liver to any great extent. In 1939 Von Euler [6] showed that a crude prostaglandin preparation increases portal venous pressure in anesthetized cats. A vasodilatory action of PGE_2 in the hepatic arterial vascular bed of the dog has been described [7]. Later Mendlovic and co-workers [8] presented some evidence that the action of platelet-activating factor (PAF) on hepatic blood flow may be mediated by eicosanoids. However the origin of the eicosanoids remained totally unclear.

It is well established that the liver consists of several cell types with hepatocytes, endothelial cells, Kupffer cells and fat storing cells being the most predominant. Of these, the sinusoidal cells, namely the Kupffer and endothelial cells, are known to produce eicosanoids [9–11]. By contrast, the parenchymal cells appear to be involved more in the degradation rather than in the synthesis of eicosanoids [12,13].

Here, we have examined the vascular effects of phagocytosable material like zymosan and latex particles in perfused rat liver; these are known to interact rather selectively with Kupffer cells [14].

Correspondence address: P. Dieter, Department of Biochemistry, The Faculty of Science, Australian National University, Canberra, ACT 2600, Australia

* Permanent address: Biochemisches Institut, Herrmann Herder Strasse 7, D-7800 Freiburg, FRG

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We have also examined the vascular effects of arachidonic acid which is converted to eicosanoids by both Kupffer and endothelial cells [10,11]. The results described are consistent with the notion that prostaglandins are produced and released within the liver and that they mediate the vascular effect induced by zymosan and arachidonic acid.

2. EXPERIMENTAL

2.1. *Animals and perfusion*

Wistar-strain albino rats (250–350 g body wt) having free access to food were anaesthetized with sodium pentobarbitone (50 mg/kg). Livers were perfused with Krebs-Henseleit bicarbonate medium [15] containing 1.3 mM $CaCl_2$ and equilibrated with O_2/CO_2 (19:1) in a flow-through mode as in C [16]. After a preperfusion of 15 min, stimuli were infused for the appropriate times. Any inhibitors tested were infused 15 min before the administration of the stimuli and thereafter for the duration of the experiment.

2.2. *Analytical procedures*

Perfusate oxygen concentrations were continuously monitored with a Clark-type oxygen electrode as detailed [16]. Portal vein pressure, which can be used as an index of intrahepatic pressure or vasoconstriction [17,18], was determined by measuring the changes in the level of the perfusion buffer in an open glass capillar tube (4 mm ∅) connected to the portal inflow. Basal portal perfusion pressure was 3.3 ± 0.3 mmHg. A difference of 10 mmHg was determined to correspond to a change in flow rate of about 8 ml/min at constant pressure. The uptake of zymosan and latex particles by the liver was assessed by measuring the differences in absorbances at 540 nm of the inflow and outflow media. Perfusion experiments shown are representative experiments that were selected from at least two to five experiments which gave essentially identical results.

2.3. *Chemicals and materials*

Zymosan, latex particles (1 μm ∅), arachidonic acid, phenylephrine, indomethacin, nordihydroguaiaretic acid (NDGA), bovine serum albumin (fraction V), catalase and superoxide dismutase were obtained from Sigma (St. Louis, USA). Bromophenacyl bromide (BPB) was purchased from Aldrich (Milwaukee, USA). All other chemicals were of analytical grade.

Zymosan suspensions were kept at 95°C for 30 min to destroy endogenous phospholipase A_2 activity. Bovine serum albumin was made fatty acid-free as described [19]. Arachidonic acid was diluted in perfusion buffer containing 10% bovine serum albumin and sonicated for 2–3 min. NDGA was dissolved in DMSO and infused directly. Indomethacin and BPB were dissolved in DMSO and slowly added to the perfusion buffer containing 0.1% bovine serum albumin. Perfusion with DMSO (final concentration 0.03%) or bovine serum albumin (0.1%) alone did not induce any change in hepatic pressure.

3. RESULTS

3.1. *Effect of arachidonic acid, zymosan and latex particles on hepatic portal pressure*

The change in hepatic portal pressure induced by infusion of arachidonic acid (100 μM), zymosan (150 μg/min), latex particles (150 μg/min) and phenylephrine (2 μM) into the perfused rat liver is shown in fig.1. Phenylephrine induces a rapid increase in hepatic pressure which remains elevated until the hormone is removed and then declines very rapidly to the original level. The pressure changes induced by arachidonic acid during the

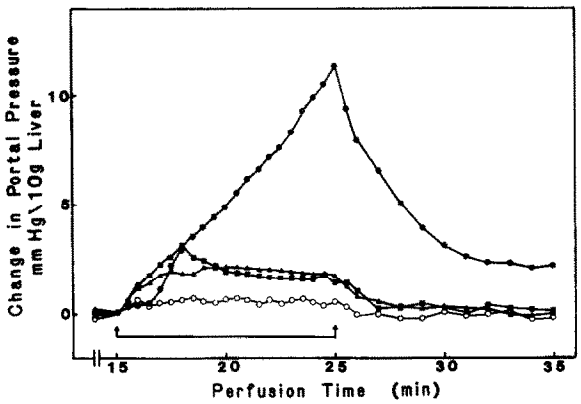

Fig.1. Effects of phenylephrine, arachidonic acid, zymosan and latex particles on hepatic portal pressure. Rat livers were perfused for 10 min (arrowed) in the presence of 2 μM phenylephrine (▲), 100 μM arachidonic acid (■), 100 μg/min zymosan (●) and 100 μg/min latex particles (○). For further details see section 2.

first 2–3 min of infusion are very similar to those induced by phenylephrine. However a notable difference is that the pressure increase induced by arachidonic acid decreases after about 3 min even though it is still being infused. The pressure then remains constant until the arachidonic acid is removed and then declines to the original level. Zymosan infusion leads to a significant increase in hepatic portal pressure after a lag period of 0.5–1 min; thereafter the hepatic pressure increases constantly up to 10 min of infusion. Removal of zymosan leads to a rapid decline in hepatic pressure, but in contrast to the removal of arachidonic acid and phenylephrine, the hepatic pressure does not return to its original level after the removal of zymosan. Although about 71 ± 12 and 84 ± 5% of zymosan and latex particles, respectively, are taken up by the liver, no significant pressure change was induced by the latter.

3.2. *Effects of various inhibitors on the arachidonic acid- and zymosan-induced change in hepatic portal pressure*

To determine whether cyclooxygenase and/or lipoxygenase products are involved in the observed pressure changes, the livers were perfused with inhibitors of these enzymes prior to the addition of the stimuli. Perfusion with the inhibitors alone did not result in any significant change in pressure. Also the overall shape of the curves as shown in fig.1 is not altered by preperfusion with the inhibitors (not shown). Fig.2a shows that the infusion of BPB, a phospholipase A_2 inhibitor [20], indomethacin, a cyclooxygenase inhibitor [21], NDGA, a lipoxygenase inhibitor [22], or the combination of these two inhibitors, does not alter the pressure changes induced by phenylephrine. The changes in hepatic pressure induced by zymosan are inhibited to 40–50% by each of these compounds (fig.2b). In contrast to its effects on zymosan, BPB does not inhibit the change in portal pressure induced by arachidonic acid (fig.2c). However after the infusion of indomethacin, or indomethacin plus NDGA, arachidonic acid is no longer able to induce any change in hepatic pressure. NDGA alone, in contrast to indomethacin alone, produces a stimulatory effect on the hepatic pressure change induced by arachidonic acid (fig.2c).

Since it is known that zymosan also induces a release of superoxide in cultured Kupffer cells [10,23] we attempted to assess whether this anion is involved in the zymosan-induced pressure changes. In these experiments, superoxide dismutase (50 U/min) and catalase (50 U/min), which together convert O_2^- into O_2 and H_2O, were infused 2 min prior to and together with zymosan. No significant change in the zymosan-induced pressure increase was observed suggesting that superoxide anions are not involved in this action of zymosan.

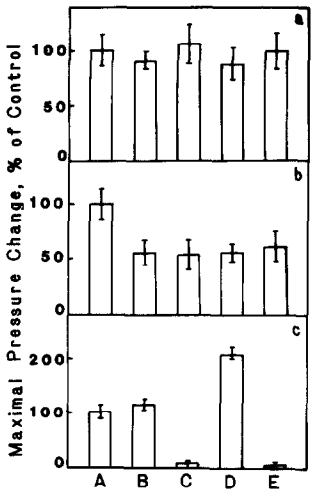

Fig.2. Effect of various inhibitors on the increase in hepatic portal pressure induced by phenylephrine (a), zymosan (b) and arachidonic acid (c). 100 μM BPB (B), 10 μM indomethacin (C), 50 μM NDGA (D) or indomethacin and NDGA together (D) were infused 15 min prior to the infusion of the stimuli and thereafter for the duration of the experiment. A represents the maximal response induced by the stimuli alone as in fig.1. 100% corresponds to a maximal portal pressure change of 2.2, 5.1 and 3.0 mmHg/10 g liver induced by phenylephrine, zymosan and arachidonic acid, respectively. Values are means ± SE for 2–4 experiments. For further details see section 2.

3.3. *Effect of repeated administrations of phenylephrine, zymosan and arachidonic acid on the hepatic portal pressure*

The results in fig.2 suggest that the mechanisms by which phenylephrine, zymosan and arachidonic acid induce hepatic vasoconstriction may be different. To assess this, repeated infusions with phenylephrine, arachidonic acid and zymosan were

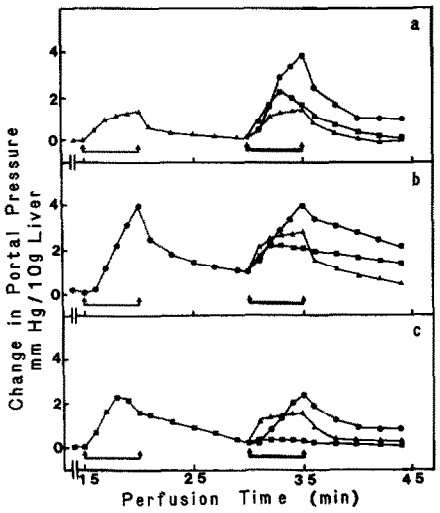

Fig.3. Effect of repeated infusions of phenylephrine, arachidonic acid and zymosan on the hepatic portal pressure. Phenylephrine, zymosan and arachidonic acid were infused initially for 5 min in a, b, c respectively (thin arrow); corresponding responses: (▲) phenylephrine, (●) zymosan, (■) arachidonic acid. At 30 min either the same agent, or one of the other two agents (as indicated), was infused for a further 5 min (thick arrow). For further details see section 2.

carried out. For these experiments one of these agents was infused for 5 min and subsequently either the same or one of the other two agents was infused for 5 min after an intervening recovery period of 10 min. Fig.3a shows that the initial infusion with phenylephrine does not alter the hepatic portal pressure increase induced by a subsequent infusion of phenylephrine, arachidonic acid or zymosan. When zymosan is infused first, the subsequent phenylephrine-induced change in portal pressure is not altered. Moreover the change in hepatic pressure induced by a subsequent infusion with zymosan or arachidonic acid, is inhibited by 25–35 and 40–60%, respectively (fig.3b). An initial infusion with arachidonic acid also does not lead to a change in the response induced by a subsequent infusion of phenylephrine (fig.3c). However almost no change in portal pressure can be measured after a subsequent infusion of arachidonic acid, whereas the response induced by a subsequent infusion of zymosan is inhibited by 40–60% (fig.3c).

4. DISCUSSION

Infusion of zymosan and arachidonic acid into rat liver produces an increase in portal vein pressure. Since it has been shown that portal vein pressure is directly related to hepatic constriction [15,16], these results suggest that zymosan and arachidonic acid induce constriction of the hepatic vasculature. Our results also suggest that the mechanism of action of these agents is most probably different from that induced by α-adrenergic agonists like phenylephrine. In contrast to the zymosan- and arachidonic acid-induced increase in portal pressure, the increase in pressure induced by phenylephrine is not affected by the inhibitors BPB, indomethacin and NDGA (fig.2). Repeated infusions of phenylephrine result in the pressure changes also being repeated, whereas those induced by subsequent infusions with zymosan and arachidonic acid show marked desensitization (fig.3).

The ability of indomethacin to block almost totally the arachidonic acid-induced response suggests a role for prostaglandins in this action. This assumption is strongly strengthened by the fact that administration of NDGA, a lipoxygenase inhibitor, led to an increase in the arachidonic acid-induced pressure change. Arachidonic acid itself seems to be vasoinactive since in the presence of indomethacin almost no pressure change could be observed during infusion with arachidonic acid alone.

In contrast to the arachidonic acid-induced increase in portal pressure, that induced by zymosan is only partially inhibited by indomethacin, but is inhibited to about the same extent by BPB and NDGA (fig.2). This suggests that only a part of the pressure change induced by zymosan is mediated by eicosanoids. The other part may be due to a direct effect of zymosan on the perfusion flow and/or to a shape change of the Kupffer cells which is known to occur after phagocytosis of zymosan [14].

These data thus suggest a role of prostaglandins in the action of zymosan and arachidonic acid. However the origin of the prostaglandins remains to be clarified. We suggest that zymosan and arachidonic acid act primarily in Kupffer cells and endothelial cells. These cells would release prostaglandins after contact with these agents which in

turn would react with the hepatic vasculature inducing the observed pressure changes. The following findings are in agreement with this hypothesis: (i) Cultured Kupffer cells and endothelial cells are able to release eicosanoids after contact with zymosan or with arachidonic acid [9–11]. (ii) Supernatant fractions of zymosan-stimulated Kupffer cells are able to contract rabbit femoral arteries [13]. (iii) In contrast to Kupffer cells and endothelial cells, hepatocytes seem to be more involved in the degradation rather than in the synthesis and release of eicosanoids [12,13]. (iv) Zymosan and latex particles ($\geqslant 0.8\ \mu M$) are selectively taken up by Kupffer cells [14]. (v) Latex particles which were vasoinactive (fig.1) also do not lead to a pronounced release of eicosanoids in cultured Kupffer cells (Dieter, P., unpublished), although they are taken up by the liver and Kupffer cells [10,14]. (vi) Prostaglandins directly infused into the liver show vasoactivity [6,7]. Further investigations are required to establish which prostaglandins mediate the vasoconstriction induced by zymosan and arachidonic acid.

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