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# Stimulation of release of prostaglandin $D_2$ and thromboxane $B_2$ from perfused rat liver by extracellular adenosine

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In isolated perfused rat liver, adenosine infusion (50 $\mu$M) led to increases in glucose output and portal pressure and a net $K^+$ release of $3.7 \pm 0.21$ $\mu$mol/g, which was followed by an equivalent net $K^+$ uptake after cessation of the nucleoside infusion. These effects were accompanied by a transient stimulation of hepatic prostaglandin $D_2$ and thromboxane $B_2$ release. The $Ca^{2+}$ release observed upon adenosine infusion (50 $\mu$M) was $23.5 \pm 5.2$ nmol/g, i.e. 10–20 % of the $Ca^{2+}$ release observed with extracellular ATP (50 $\mu$M). Indomethacin (10 $\mu$M) prevented the adenosine-induced stimulation of glucose output and the increase in portal pressure by 79 and 63 % respectively, and completely abolished the stimulation of prostaglandin $D_2$ release. The thromboxane $A_2$ receptor antagonist BM 13.177 (20 $\mu$M), the phospholipase $A_2$ inhibitor 4-bromophenacyl bromide (20 $\mu$M) and the cyclo-oxygenase inhibitor ibuprofen (50 $\mu$M) also decreased the glycogenolytic and vasoconstrictive responses of the perfused rat liver upon adenosine infusion by 50–80 %. When the indomethacin inhibition of adenosine-induced prostaglandin $D_2$ release was titrated, a close correlation between prostaglandin $D_2$ release and the metabolic and vascular responses to adenosine was observed. These findings suggest an important role for eicosanoids in mediating the nucleoside responses in the perfused rat liver. Since eicosanoids are known to be formed by non-parenchymal cells in rat liver [Decker (1985) Semin. Liver Dis. 5, 175–190], the present study gives further evidence for an important role of eicosanoids as signal molecules between the different liver cell populations.

## INTRODUCTION

The effects of extracellular adenosine are mediated by binding to specific $P_1$-purinoceptors, which in turn either activate or inhibit adenylate cyclase (for reviews, see Burnstock, 1979; Williams, 1987). In perfused rat liver, adenosine as well as the purine nucleotide ATP have been found to increase glucose release and to evoke vasoconstriction (Buxton *et al.*, 1986, 1987*b*; Häussinger *et al.*, 1987*a*). Adenosine stimulates glycogenolysis in isolated hepatocytes via activation of glycogen phosphorylase (Hoffer & Lowenstein, 1986; Stanley *et al.*, 1987). Several studies showed a stimulation of cyclic AMP accumulation by adenosine in rat liver cells (Bartrons *et al.*, 1984; Buxton *et al.*, 1987*b*). $P_1$-purinoceptors of the $A_2$-subtype, which are characterized by their affinity for the adenosine analogue 5′-*N*-ethylcarboxamidoadenosine, have been identified in rat liver plasma membrane (Londos *et al.*, 1980). However, it is still controversial whether the activation of hepatic glycogen phosphorylase in isolated rat hepatocytes is exclusively due to binding of adenosine to external $A_2$-adenosine receptors linked to adenylate cyclase (Stanley *et al.*, 1987). Adenosine has also been shown to decrease gluconeogenesis from lactate in liver cell preparations (Lavoinne *et al.*, 1987) and the concentration of fructose 2,6-bisphosphate in rat hepatocytes (Bartrons *et al.*, 1984), and to inhibit the glucagon-dependent expression of phosphoenolpyruvate carboxykinase in rat liver (Christ *et al.*, 1987). The mechanisms of adenosine action in perfused rat liver have not been clearly understood yet, since cyclo-oxygenase inhibitors such as indomethacin were shown to suppress the adenosine response in perfused rat liver, whereas the adenosine-dependent hepatic cyclic AMP production was not inhibited by indomethacin (Buxton *et al.*, 1987*b*). Prostaglandins and thromboxanes are released from perfused rat liver upon stimulation with vasoactive nucleotides such as ATP, UTP and the diadenine dinucleotides $Ap_3A$ and $Ap_4A$, and were shown to mediate the nucleotide responses, at least in part (Häussinger *et al.*, 1988*b*; Tran-Thi *et al.*, 1988*b*; Busshardt *et al.*, 1989).

As eicosanoids are capable of stimulating hepatic glycogenolysis (Buxton *et al.*, 1987*a*; Häussinger *et al.*, 1987*c*, 1988*a*; Altin & Bygrave, 1988; Casteleijn *et al.*, 1988*a*) and non-parenchymal cells are known to be the major sites of hepatic eicosanoid production (Birmelin & Decker, 1984; Decker, 1985), the question arises as to how far eicosanoids released from non-parenchymal cells are involved in the actions of extracellular adenosine in perfused rat liver.

## MATERIALS AND METHODS

### Haemoglobin-free liver perfusion

Livers of male Wistar rats (120–180 g body wt.), fed on a stock diet *ad libitum* (Altromin, Lage, Germany) were perfused *in situ* in a non-recirculating system as described previously (Sies, 1978). The perfusion fluid was bicarbonate-buffered Krebs–Henseleit saline plus L-lactate (2.1 mM) and pyruvate (0.3 mM), equilibrated with $O_2/CO_2$ (19:1). The flow rate was 3.5–4.5 ml/min per g of liver and was kept constant throughout the individual perfusion experiment. The temperature was 37 °C. If not otherwise indicated, the $K^+$ and $Ca^{2+}$ concentrations in the perfusion medium were 5.9 mM and 1.25 mM respectively. When the effect of adenosine on extracellular $Ca^{2+}$ was monitored, the $Ca^{2+}$ influent concentration was lowered to 0.3 mM in order to increase the signal/noise ratio for the $Ca^{2+}$-sensitive electrode. Additions of agonists were made by micro-pumps directly before the portal vein. Agonists were dissolved either in Krebs–Henseleit perfusion medium or, for indomethacin, ibuprofen and BPB, in DMSO. In control experiments without these antagonists, DMSO was infused. In experiments with DMSO the perfusion medium contained 0.1 % BSA.

Abbreviations used: AGEPC, acetylglycerol ether phosphocholine; $Ap_3A$, diadenosine 5′,5″-$P^1$,$P^3$-triphosphate; $Ap_4A$, diadenosine 5′,5″-$P^1$,$P^4$-tetraphosphate; BM 13.177, 4-[2-(benzenesulphonamido)ethyl]phenoxyacetic acid, generic name sulotroban; BPB, 4-bromophenacyl bromide; DMSO, dimethyl sulphoxide.

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### Effluent perfusate assays

The glucose concentration in the effluent perfusate was determined as described in Bergmeyer (1983). The portal pressure was recorded continuously with a pressure transducer (Hugo Sachs Electronics, Hugstetten, Germany). The pH in the effluent perfusate was registered with a glass electrode. The $K^+$ and $Ca^{2+}$ concentrations in the effluent perfusate were monitored throughout the perfusion experiments with ion-selective electrodes (Radiometer, Munich, Germany). The electrodes were calibrated by infusion of known amounts of KCl and $CaCl_2$ respectively. Data on $K^+$ and $Ca^{2+}$ release were calculated by planimetry. If present, a baseline drift was taken into account.

Concentrations of prostaglandin $D_2$ and thromboxane $B_2$ in the effluent perfusate were determined with radioimmunoassay kits (Amersham Buchler, Braunschweig, Germany).

### Statistics

Data are given as means ± S.E.M. Statistical significance was calculated by applying the Mann–Whitney–Wilcoxon test. $P$ values $< 0.05$ were considered as statistically significant.

### Materials

Adenosine, ATP and all enzymes and coenzymes were from Boehringer (Mannheim, Germany). The radioimmunoassay kits were provided from Amersham Buchler. BM 13.177 was kindly given by Dr. K. Stegmeier of Boehringer. BPB, ibuprofen, indomethacin and prostaglandin $F_{2\alpha}$ were from Sigma (Munich, Germany). All other chemicals were from Merck (Darmstadt, Germany).

## RESULTS

### Effects of extracellular adenosine in perfused rat liver

Infusion of adenosine into perfused rat liver at 50 $\mu$M concentration resulted in a transient elevation of portal pressure from $3.9 \pm 0.3$ $cmH_2O$ to a peak value of $12.8 \pm 1.1$ $cmH_2O$ ($n = 6$) 1.5 min after adenosine infusion (Fig. 1). The hepatic glucose release increased from basal values of $0.95 \pm 0.11$ $\mu$mol/min per g to a maximum of $2.16 \pm 0.25$ $\mu$mol/min per g ($n = 6$) after 2.5 min of adenosine infusion. Within 5 min, glucose output and portal pressure returned to values only slightly above basal. Simultaneously, there was a prolonged $K^+$ release of $3.70 \pm 0.21$ $\mu$mol/g ($n = 7$), which was followed by a slow $K^+$ uptake of $3.69 \pm 0.40$ $\mu$mol/g ($n = 7$) after withdrawal of the adenosine stimulus. The observed $K^+$ release across the hepatic plasma membrane upon adenosine addition is different from the biphasic $K^+$ movements observed with ATP, UTP, prostaglandin $E_2$ and prostaglandin $F_{2\alpha}$ in the perfused rat liver (Häussinger *et al.*, 1987*a*,*c*).

The net $Ca^{2+}$ release from the perfused rat liver was calculated to be approx. $23.5 \pm 5.2$ nmol/g ($n = 4$) after 10 min infusion with adenosine (50 $\mu$M). Thus the net $Ca^{2+}$ release observed with the purine nucleoside adenosine under these conditions (extracellular $Ca^{2+}$ concn. was 0.3 mM) was much lower in comparison with the effect obtained with ATP at 20 $\mu$M concentration, which is capable of releasing $114 \pm 3$ nmol of $Ca^{2+}$/g from perfused rat liver (Häussinger *et al.*, 1987*a*). Lowering the extracellular $Ca^{2+}$ concentration to 0.3 mM (to facilitate extracellular-$Ca^{2+}$ measurements) affected neither the adenosine-induced increase in glucose output and portal pressure nor the observed $K^+$ release upon adenosine addition.

### Stimulation of prostaglandin and thromboxane release by adenosine

Addition of adenosine to perfused rat liver led to a transient increase in prostaglandin $D_2$ release into the effluent perfusate (Fig. 2*a*). The average prostaglandin $D_2$ concentration increased from basal values of $0.80 \pm 0.39$ to a maximum of $28.3 \pm 4.5$ ng/min per g ($n = 4$) within 1 min after onset of the adenosine infusion. Although adenosine infusion was continued, the prostaglandin $D_2$ release decreased rapidly to 3 ng/min per g during the late infusion interval and returned to basal levels after cessation of the adenosine infusion.

Thromboxane $B_2$ release from perfused rat liver increased from basal values of $0.1 \pm 0.02$ to a peak of $2.7 \pm 0.5$ ng/min per g ($n = 3$), as observed 1 min after onset of the adenosine infusion (Fig. 2*b*). The time course of the adenosine-induced stimulation of thromboxane $B_2$ release from perfused rat liver resembles the pattern of thromboxane release observed with extracellular ATP and the diadenine nucleotides $Ap_3A$ and $Ap_4A$, as described previously (Häussinger *et al.*, 1988*b*; Busshardt *et al.*, 1989). Thus the question arises whether the adenine-nucleotide-

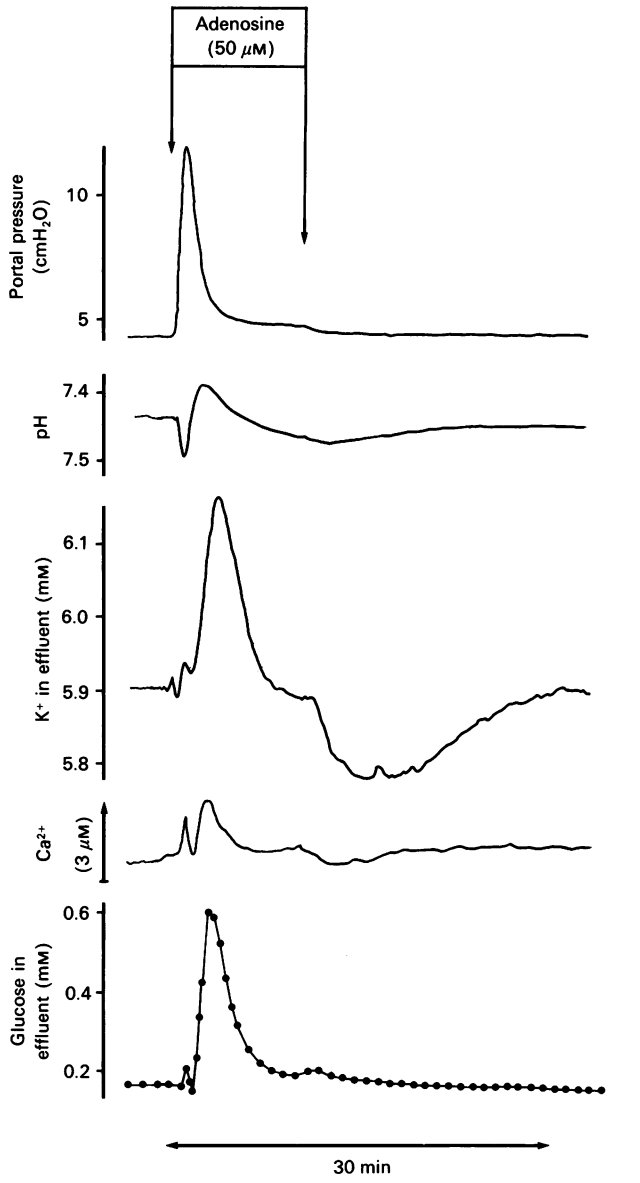


**Fig. 1. Effects of adenosine infusion on portal pressure, pH, $K^+$, $Ca^{2+}$ and glucose concentrations in the effluent perfusate in the perfused rat liver**

The $K^+$ and $Ca^{2+}$ concentrations in the perfusion medium were 5.9 mM and 0.3 mM respectively. Results are shown of a representative experiment from a series of six perfusions.

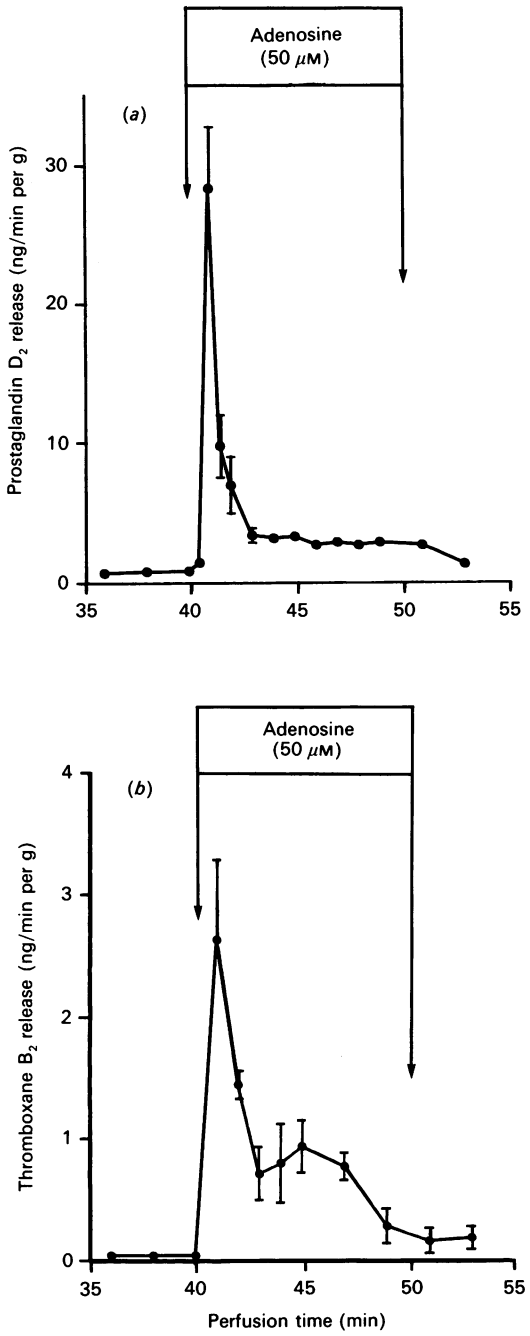


**Fig. 2. Release of prostaglandin $D_2$ (*a*) and thromboxane $B_2$ (*b*) upon stimulation of perfused rat liver with adenosine**

Data are given as means ± S.E.M. ($n = 3$–4).

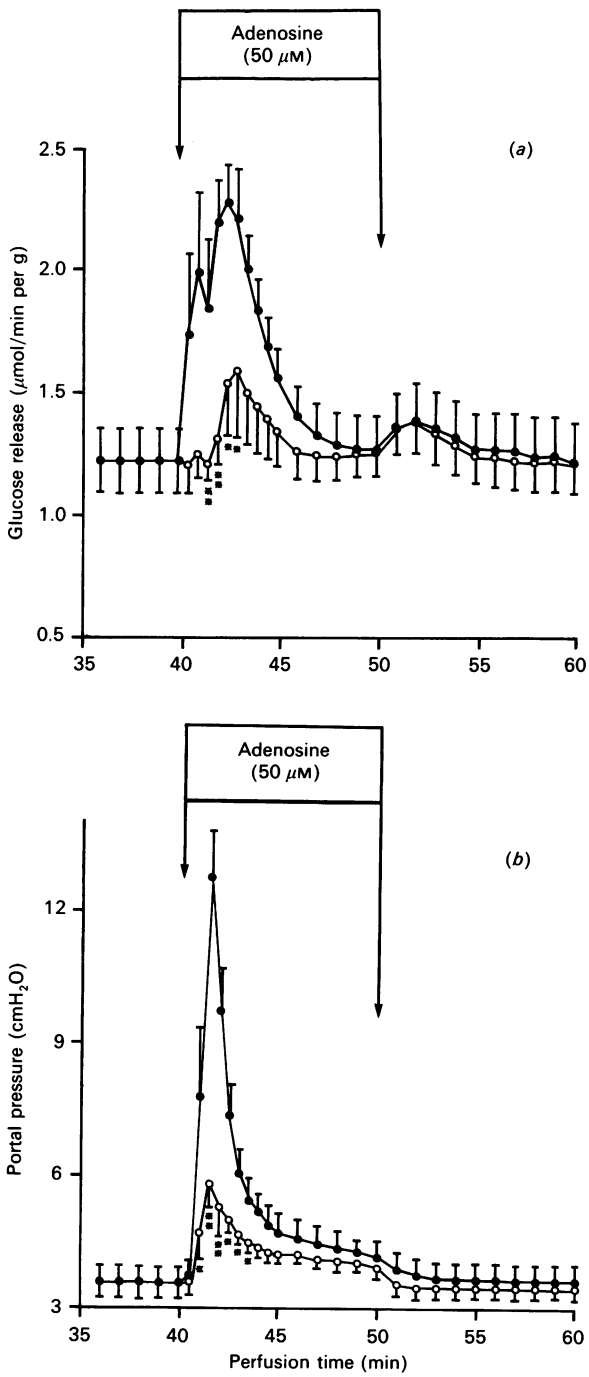


**Fig. 3. Effect of indomethacin on the stimulation of glucose release (*a*) and increase in portal pressure (*b*) in perfused rat liver upon adenosine infusion**

Perfusions were performed with (○) and without (●) indomethacin infusion (10 μM) 10 min before addition of adenosine (50 μM). In order to dissolve indomethacin, all experiments were carried out in presence of DMSO, which was infused at a rate of 100 μl/min into the influent perfusate. Neither the basal portal pressure nor the basal glucose release were affected by DMSO or indomethacin. Data represent means ± S.E.M. from 4–6 experiments: $*P < 0.05$; $**P < 0.005$, for statistically significant difference from the corresponding control.

dependent effects on hepatic eicosanoid production could be partially mediated by adenosine, since extracellular adenine nucleotides can be rapidly degraded to adenosine by ectonucleotidases (Sasaki *et al.*, 1983; for a review, see Gordon, 1986).

### Effect of inhibitors of eicosanoid synthesis and of eicosanoid-receptor antagonists on adenosine responses in perfused rat liver

Indomethacin (10 μM) inhibited the adenosine-induced stimulation of hepatic glucose output (Fig. 3*a*) and increase in portal pressure (Fig. 3*b*) by 79 and 63 % respectively. As for the control, neither DMSO nor indomethacin themselves affected the basal glucose release and the portal pressure, whereas indomethacin diminished the basal rate of hepatic prostaglandin $D_2$ release to levels only slightly above the detection limit. In control experiments with DMSO, a $K^+$ release of 2.12 ± 0.38 μmol/g ($n = 6$) upon adenosine addition was measured; the adenosine-

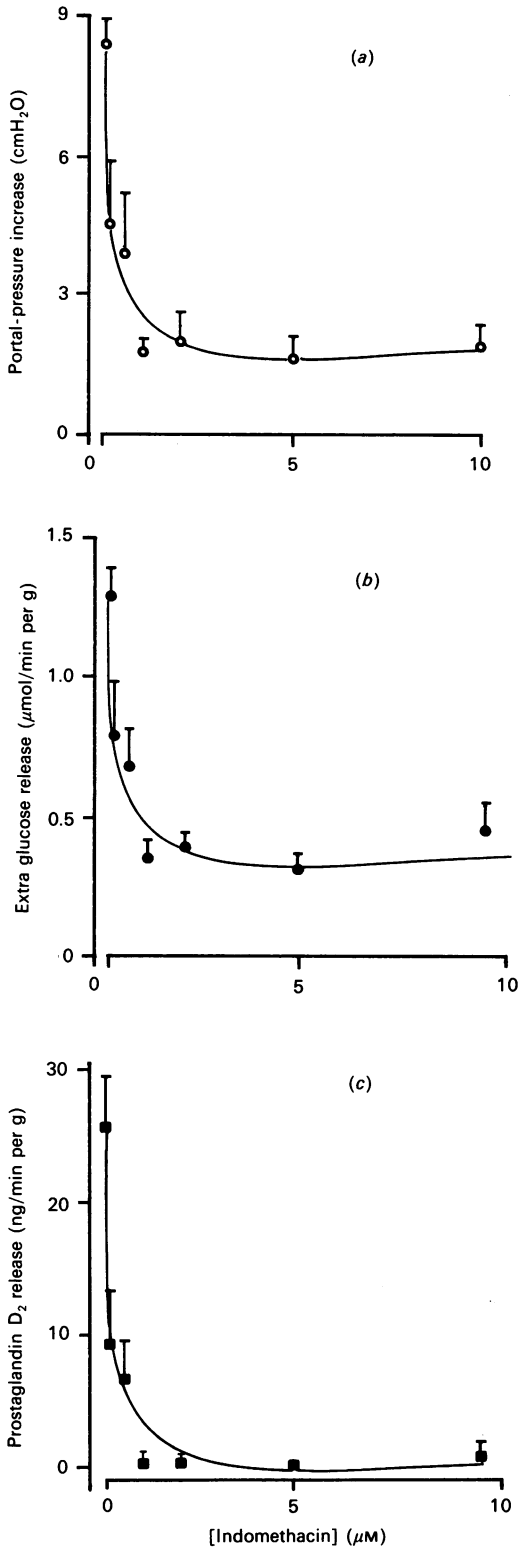


**Fig. 4. Inhibition by indomethacin of adenosine-induced portal-pressure increase (*a*), stimulation of glucose release (*b*) and prostaglandin $D_2$ efflux (*c*) from perfused rat liver**

Perfusions were performed with indomethacin infusion (0, 0.1, 0.5, 1, 2, 5 and 10 $\mu$M) 10 min before addition of adenosine (50 $\mu$M). The basal prostaglandin efflux decreased slightly in the presence of indomethacin. Maximal stimulation of glucose release and prostaglandin $D_2$ efflux from perfused rat liver was determined in effluent samples taken every 30 s after infusion of adenosine and occurred 2.5–3 min and 1–1.5 min respectively after addition of adenosine. Data represent means ± S.E.M. from 3–9 experiments.

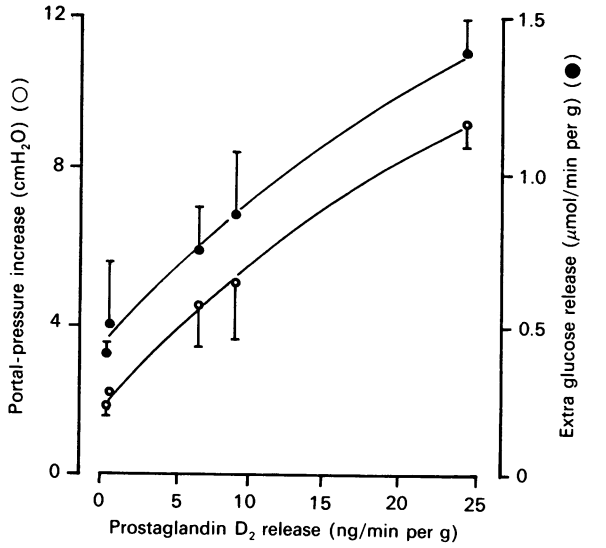


**Fig. 5. Relationship between adenosine-dependent stimulation of prostaglandin $D_2$ release and the concomitant increases in portal pressure and glucose release**

Experiments were performed with different concentrations of indomethacin (10, 1, 0.5, 0.1 and 0 $\mu$M). Conditions were as in Fig. 3. Data represent means ± S.E.M. from 4–9 different perfusion experiments.

induced $K^+$ release during indomethacin infusion (10 $\mu$M) was 2.40 ± 0.22 $\mu$mol/g ($n = 4$). This suggests that indomethacin does not inhibit the adenosine-induced $K^+$ release, whereas the glycogenolytic and haemodynamic effects of adenosine are largely inhibited. In order to exclude unspecific inhibitory effects of indomethacin, adenosine responses were studied at different indomethacin concentrations. Maximal inhibition of the haemodynamic and glycogenolytic effects of adenosine was observed at indomethacin concentrations of about 1 $\mu$M (Figs. 4*a* and 4*b*). This indomethacin concentration was sufficient to suppress completely the adenosine-induced prostaglandin $D_2$ release (Fig. 4*c*). There was no difference in the inhibitory action on adenosine responses at 1 and 10 $\mu$M indomethacin concentrations. At sub-maximal concentrations of indomethacin (< 1 $\mu$M), the effects of adenosine on portal pressure and glucose

**Table 1. Effects of various eicosanoid-synthesis inhibitors and eicosanoid-receptor antagonists on adenosine responses in perfused rat liver**

Livers were perfused for 30 min before addition of the inhibitors. Adenosine (50 $\mu$M) was infused at 40 min. The basal glucose release and the basal portal pressure before infusion of adenosine were 1.14 ± 0.03 $\mu$mol/min per g and 4.0 ± 0.1 $cmH_2O$. They were not significantly affected by infusion of the antagonists. Data are given as means ± S.E.M. and represent the peak values for the adenosine-dependent increases of portal pressure and extra glucose release as observed 1–1.5 and 2.5–3 min after addition of the agonist, respectively.

| | Portal pressure increase (cm $H_2O$) | Extra glucose release ($\mu$mol/min per g) | ($n$) |
|---|---|---|---|
| Control | 9.3 ± 0.6 | 1.29 ± 0.10 | (14) |
| Indomethacin (10 $\mu$M) | 2.1 ± 0.5 | 0.45 ± 0.19 | (4) |
| Indomethacin (1 $\mu$M) | 2.0 ± 0.3 | 0.35 ± 0.06 | (3) |
| BM 13.177 (20 $\mu$M) | 4.9 ± 1.2 | 0.52 ± 0.14 | (6) |
| BPB (20 $\mu$M) | 1.2 ± 0.3 | 0.16 ± 0.12 | (4) |
| Ibuprofen (50 $\mu$M) | 2.8 ± 0.2 | 0.33 ± 0.09 | (3) |

release rose in parallel with the stimulation of prostaglandin $D_2$ release (Fig. 5). On the other hand, indomethacin (10 $\mu$M) did not influence the haemodynamic response of perfused liver to prostaglandin $F_{2\alpha}$ at 2 $\mu$M concentration [$5.9 \pm 0.9$ $cmH_2O$ ($n = 4$) versus $6.1 \pm 1.2$ $cmH_2O$ ($n = 4$) in control experiments without indomethacin]. Ibuprofen, an inhibitor of cyclo-oxygenase, decreased the glycogenolytic and vasoconstrictive responses of the liver to adenosine by 64% and 70% respectively (Table 1). This is in contrast with recent results by Lapointe & Olson (1989), who described an almost complete inhibition of AGEPC-induced prostaglandin $D_2$ formation by ibuprofen, but not of the metabolic and vascular responses of the perfused rat liver to AGEPC.

BPB, an inhibitor of phospholipase $A_2$ (Volwerk *et al.*, 1974), also blocked adenosine actions in liver by about 80% (Table 1).

BM 13.177 (20 $\mu$M), an antagonist of the thromboxane $A_2$ receptor (Stegmeier *et al.*, 1984), inhibited the adenosine-dependent increases in glucose output and portal pressure by 60 and 48% respectively (Table 1). Thus the inhibition of vascular and metabolic responses to adenosine by BM 13.177 is much more pronounced than that obtained with ATP, UTP and $Ap_4A$ in perfused rat liver (Häussinger *et al.*, 1988*b*; Busshardt *et al.*, 1989). In the presence of BM 13.177 (20 $\mu$M), the adenosine-induced $K^+$ release decreased slightly from $3.70 \pm 0.21$ ($n = 6$) to $2.82 \pm 0.30$ $\mu$mol/min per g ($n = 5$).

## DISCUSSION

The present data suggest a role of eicosanoids in mediating the effects of extracellular adenosine in perfused rat liver. This is supported by the observations that: (i) adenosine addition leads to a 20–30-fold stimulation of prostaglandin $D_2$ and thromboxane $B_2$ release from perfused rat liver (Fig. 2); (ii) the time course of prostaglandin and thromboxane release after adenosine infusion shows a pattern similar to that observed in metabolic and vascular responses (Fig. 1); (iii) the peak in prostaglandin $D_2$ and thromboxane $B_2$ release precedes the maximum in portal pressure increase by about 0.5 min and the maximum increase in glucose output by about 1.5 min; (iv) the adenosine effects are effectively inhibited by addition of indomethacin even at sub-micromolar concentrations (Figs. 3–5), the cyclo-oxygenase inhibitor ibuprofen, the phospholipase $A_2$ inhibitor BPB and the thromboxane-$A_2$-receptor antagonist BM 13.177 (Table 1); and (v) there is a strong dependence of the haemodynamic and glycogenolytic effects of adenosine on the extent of prostaglandin formation, as shown in titration experiments with indomethacin (Figs. 4 and 5).

These findings are in line with previous reports from this laboratory, which demonstrated a stimulation of eicosanoid release from perfused rat liver by extracellular ATP, UTP and the diadenine nucleotides $Ap_3A$ and $Ap_4A$ (Häussinger *et al.*, 1988*b*; Busshardt *et al.*, 1989). Addition of prostaglandins and thromboxanes to perfused rat liver evokes marked glycogenolytic and vasoconstrictive responses (Buxton *et al.*, 1987*a*; Häussinger *et al.*, 1987*c*, 1988*a*; Altin & Bygrave, 1988; Iwai *et al.*, 1988). It has also been shown that prostaglandins and thromboxanes in liver are primarily synthesized by non-parenchymal cells, namely endothelial and Kupffer cells, which are capable of releasing these eicosanoids into the sinusoidal space (Birmelin & Decker, 1984; Decker, 1985). Subsequent studies revealed that prostaglandins released from non-parenchymal liver cells may directly stimulate glycogenolysis in parenchymal liver cells (Häussinger *et al.*, 1987*c*, 1988*b*; Casteleijn *et al.*, 1988*a*,*b*). The concentrations of prostaglandin $D_2$ in the effluent perfusate, of 1–10 nmol/l, as observed with adenosine, were about 3–4 orders of magnitude lower than those required to mimic glycogenolytic responses by prostaglandin infusion (see, e.g., Buxton *et al.*, 1987*a*; Häussinger *et al.*, 1987*c*). The discrepancy at first glance may contradict a role of eicosanoids in mediating the adenosine effects. However, eicosanoids are rapidly inactivated during a single liver passage (Häusinger & Stehle, 1988). Further, only 40% of infused prostaglandin $D_2$ can be recovered in the effluent perfusate, and a substantial amount is released into bile (Tran-Thi *et al.*, 1988*a*). Thus the intra-acinar eicosanoid concentration in the vicinity of putative prostaglandin receptors may be much higher than reflected by the effluent concentration. Although prostaglandin $D_2$ and thromboxane $B_2$ were the only eicosanoids measured in our study, it is well conceivable that other prostanoids are formed under the influence of adenosine, and are more potent effectors on hepatic metabolism. It is not clear whether the glycogenolytic response to adenosine is due to direct action of eicosanoids on parenchymal cells or is mediated by vasoconstriction. Vasoconstriction leads to hypoxia, which itself can activate hepatic glycogenolysis (Ismail & Hems, 1978; Theen *et al.*, 1982; Wölfle *et al.*, 1983).

The thromboxane $A_2$ analogues U-46619 and ONO-11113 increase portal pressure and glucose output in perfused rat liver (Fisher *et al.*, 1987; Altin & Bygrave, 1988; Häussinger *et al.*, 1988*a*; Iwai *et al.*, 1988). It is likely, in view of the effects of BM 13.177 (Table 1) and the thromboxane $B_2$ release (Fig. 2*b*), that thromboxanes are involved in mediating the vascular and metabolic responses to adenosine. It has been shown that the thromboxane $A_2$ analogue U-46619 is not capable of stimulating glycogen phosphorylase in isolated hepatocytes (Fisher *et al.*, 1987; Häussinger *et al.*, 1988*b*). Therefore it might be possible that the stimulation of glycogenolysis by these thromboxane analogues is due to vasoconstriction. This could at least in part also be true for adenosine-induced vasoconstriction.

BPB, an inhibitor of arachidonic acid formation from phospholipids, also exerted inhibitory effects with respect to adenosine action, but did not interfere with the glycogenolytic effects of ATP in perfused rat liver (Iwai & Jungermann, 1987). The cyclo-oxygenase inhibitor ibuprofen blocked adenosine responses in perfused rat liver. This is different from findings by Lapointe & Olson (1989), who showed that complete inhibition of prostaglandin formation by ibuprofen had no effect on the metabolic and vascular responses to AGEPC.

With respect to adenosine actions in isolated perfused rat liver, our data suggest a mediating role of eicosanoids. In addition, a direct effect of adenosine on liver parenchymal cells must be taken into account, because cyclo-oxygenase inhibitors only blocked about 70% of the adenosine responses.

An interesting observation was the $K^+$ release observed upon addition of adenosine to perfused rat liver, which has also been described for ATP, UTP and phenylephrine in perfused rat liver (Häussinger *et al.*, 1987*a*,*b*) and for different $Ca^{2+}$-mobilizing hormones in isolated hepatocytes (Burgess *et al.*, 1979). As the $K^+$ release upon adenosine addition remained basically unaffected by infusion of indomethacin and BM 13.177, it must be suggested that mechanisms different from hepatic eicosanoid production are responsible for the observed $K^+$ fluxes in perfused rat liver. The $Ca^{2+}$ efflux from perfused rat liver ($23.5 \pm 5.2$ nmol/g) during adenosine infusion 50 $\mu$M) was low in comparison with that observed with extracellular ATP at 50 $\mu$M concentration (200 nmol/min per g). These findings are in line with previous studies in isolated hepatocytes, which showed only small increases in intracellular $Ca^{2+}$ at high extracellular adenosine concentrations (Charest *et al.*, 1985; Sistare *et al.*, 1985; Staddon & McGivan, 1985), whereas ATP is capable of mobilizing substantial amounts of intracellular $Ca^{2+}$. It is unclear whether the small $Ca^{2+}$ release reflects prostaglandin action on

parenchymal liver cells, which has been shown to stimulate $Ca^{2+}$ efflux (Häussinger *et al.*, 1987*c*; Altin & Bygrave, 1988).

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