Stimulation of uric acid release from the perfused rat liver by platelet activating factor or potassium

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The stimulation of hepatic glycogenolysis by platelet activating factor (AGEPC) or increased perfusate potassium concentration ($[K^+]_o$), but not phenylephrine, causes a transient increase in uric acid release into the effluent perfusate of perfused rat livers. Uric acid was identified in chromatograms of perfusate samples using reversed-phase h.p.l.c., which show a peak which co-elutes with authentic uric acid, and by the fact that the A_{293} of perfusate samples decreases in the presence of uricase. Uric acid release is dose-dependent with respect to both AGEPC and $[K^+]_o$, and is blocked completely by prior exposure of the perfused liver to 5 mM-allopurinol, a specific inhibitor of xanthine oxidase (XOD). Allopurinol inhibits the increase in portal vein pressure induced by AGEPC, increased $[K^+]_o$ or phenylephrine; the inhibitory effect increases with increasing concentrations of the agents. Also, allopurinol inhibits the second phase of O_2 uptake and glucose release characteristic of concentrations of AGEPC or increased $[K^+]_o$ equal to or greater than their reported half-maximal concentration for glucose release. The ratio of xanthine dehydrogenase (XDH) to XOD activity in extracts of freeze-clamped perfused livers is not affected by treatment of the livers with AGEPC or increased $[K^+]_o$. The results suggest that uric acid production may be an indicator of ischaemia within localized hepatic sinusoids, and that allopurinol partially protects the hepatocyte from the effects of AGEPC or increased $[K^+]_o$ by inhibiting XOD-dependent superoxide production. We propose that the second phase of the glycogenolytic response to these agents results from ischaemia and subsequent reperfusion. Activation of XOD *in vivo* and hence O_2 -derived free radical production may be involved in the response of the liver to vasoactive agonists under a variety of pathophysiological conditions.

INTRODUCTION

Oxygen-derived free radical production mediated by xanthine oxidase (XOD) has been implicated in low flow or ischaemia-induced injury in a variety of tissues [1–4]. Tissue damage is thought to result from free radicals generated during reoxygenation (reperfusion) of the ischaemic tissue [1–6]. Evidence indicates that XOD, which utilizes molecular oxygen as electron acceptor, is formed from xanthine dehydrogenase (XDH), which utilizes NAD^+ as electron acceptor, during oxygen-limiting conditions [1,4,7]. XDH conversion to XOD appears to be mediated by a proteolytic cleavage, which may be calcium-dependent [2,4,7]. Pronounced ischaemic injury has been correlated with net calcium influx into the intracellular compartment, increased adenine nucleotide metabolism resulting in decreased intracellular ATP and increased AMP concentrations, and increased blood levels of purine metabolites [8–13]. Pretreatment of the organism with either allopurinol, a specific inhibitor of XOD, or superoxide dismutase, a scavenger of superoxide, has been shown to alleviate tissue damage resulting from low flow ischaemia and reperfusion injury [1,3,5,8,14,15]. These observations have led to the proposal that XOD-generated free radicals are involved in the tissue injury process (e.g. see [2]).

In addition to superoxide, XOD generates uric acid

from xanthine/hypoxanthine. In rats, as in most mammals other than primates, uric acid is further oxidized to allantoin by the peroxisomal enzyme uricase [16,17]. Serum levels of uric acid are necessarily much lower in the rat than in, for example, man. Experimental or pathological conditions which lead to increased serum levels of uric acid include haemorrhagic shock, which results in low-flow-induced ischaemia [8–11]. The source(s) of the uric acid have been proposed to be mainly the pancreas and the liver, although these measurements have not been made in isolated perfused organs [9]. Since allantoin levels increase during hypoxic conditions [9], it seems unlikely that the increased uric acid levels result from inhibition of the uric acid utilizing enzyme, uricase.

Previous work has demonstrated that exposure of the perfused rat liver to platelet activating factor (AGEPC) or increased perfusate potassium concentration ($[K^+]_o$) results in transient increases in portal vein pressure, glucose release and oxygen uptake [18–23]. Neither of these agents have significant effects on adenine nucleotide levels in extracts of the perfused liver, nor a stimulatory effect on glucose release from isolated hepatocyte suspensions [18,23,24]. At greater than 2×10^{-10} M-AGEPC, or 50 mM- $[K^+]_o$, the kinetics of the glucose release and oxygen uptake responses become multiphasic, although a temporal relationship between these responses

Abbreviations used: AGEPC, platelet-activating factor (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine); XOD, xanthine oxidase; XDH, xanthine dehydrogenase; $[K^+]_o$, perfusate potassium concentration.

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cannot be distinguished clearly [18,19,22,23]. The glycogenolytic effects of these agents have been suggested to result from primary interaction at the site of the reticuloendothelial cells, leading to vasoconstriction of the portal sinusoids and concomitant local ischaemia of the hepatocyte parenchyma, and/or release of a glycogenolytic mediator of reticuloendothelial origin, although direct evidence for this later hypothesis is not available to date [18,23,25].

In this report we show that AGEPC or increased $[K^+]_o$ stimulate a transient release of uric acid from the perfused rat liver. Further, allopurinol inhibits this release in addition to inhibiting the increase in portal pressure, and the second phases of both glucose release and oxygen utilization. We propose that the first and second phases of glucose release result from two independent mechanisms, and that the second phase likely results from ischaemia-induced tissue injury mediated by oxygen-derived free radicals generated by XOD.

METHODS

Materials

The following reagents were purchased from: Sigma Chemical Co. (allopurinol, adenosine, cytosine, hypoxanthine, inosine, phenylephrine, uric acid, uricase from porcine pancreas, xanthine), Bachem Feinchemikalien, Bubendorf, Switzerland (AGEPC), and Boehringer-Mannheim (bovine serum albumin, defatted, Fraction V). Ammonium phosphate, methanol and acetonitrile were of h.p.l.c. grade, and all other materials used were of the highest purity available from commercial sources.

Methods

Livers from fed male albino rats (Sprague-Dawley; Harlan, Houston, TX, U.S.A.) (175–225 g body wt.) were perfused using a volume constant apparatus as previously described [26]. The perfusion circuit was a one-pass system, the perfusate being gassed constantly with a mixture of O_2/CO_2 (19:1) and maintained at 37 °C. The flow rate was set at the beginning of each perfusion at 240–300 ml/h per g of liver. Portal pressure (mmHg) was monitored using a Statham P231D transducer located inline immediately prior to the portal inlet cannula, and linked to a Grass 7P1A/70AC polygraph. The oxygen concentration of the effluent perfusate was monitored continuously with a Clark-type electrode inline immediately following the vena cava exit cannula. The potassium concentration in some experiments was increased by adding KCl with an equivalent decrease in the NaCl content of the perfusion medium to maintain a constant osmotic concentration. AGEPC was infused as a concentrated solution (3.5×10^{-9} – 7.0×10^{-6} M) prepared in 0.25% defatted bovine serum albumin/0.9% NaCl. Perfusate containing allopurinol, 5 mM final concentration, was prepared by dissolving the allopurinol in distilled water at 37 °C, equilibrating with O_2/CO_2 prior to adding the Krebs–Henseleit salts. Effluent perfusate samples were collected and assayed for glucose content [27] and the results are expressed as μmol of glucose released/h per g of liver. Purine nucleosides and bases were measured in 0.1 ml effluent samples using a reversed-phase h.p.l.c. system at a flow rate of 0.8 ml/min [28]. This system was modified to a constant slat/pH system (150 mM- NH_4HPO_4) for the determination of

uric acid. All h.p.l.c. measurements were made on a Varian model 5060 h.p.l.c., using a 280 nm detector and a C-18 column (25 cm $\times$ 4 mm i.d.). Uric acid also was measured in effluent perfusate samples (1.0 ml) using the decrease in A_{293} in the presence of uricase and an absorption coefficient of $12.6 \text{ mm} \cdot \text{cm}^{-1}$ [29]. XOD and XDH activities were measured in tissue extracts from livers freeze-clamped prior to or at various times after the onset of infusion of 50 mM- K^+ or 1×10^{-9} M-AGEPC. Tissue extraction and assay was performed using the method of Chambers *et al.* [1]. Some livers were extracted with 0.1 M-Tris/HCl, pH 8.1 [7] instead of 0.05 M-potassium phosphate, pH 7.8, containing 1 mM-phenylmethanesulphonylfluoride and 10 mM-dithiothreitol [1]. Each experiment was performed at least three times with nearly identical results. Such representative results, or means $\pm$ S.E.M., are presented where appropriate for maintaining clarity.

RESULTS

Preliminary experiments showed that difference absorption spectra of effluent perfusate samples collected from perfused rat livers revealed a peak at 293 nm which increased to a maximum between 3 and 5 min of exposure to either 1×10^{-9} M-AGEPC, or 50 mM- $[K^+]_o$ (results not shown). H.p.l.c. of the perfusate, using a constant salt and pH buffer system, revealed a peak at a retention time of 4.13 ± 0.08 min ($n = 10$). This peak increased transiently to a maximum in the samples collected at 4.5 min of exposure to AGEPC (Fig. 1a), and at about 2.0 min for $[K^+]_o$ (results not shown). Fig. 1(a) shows the elution profile of authentic uric acid (2×10^{-9} mol/ml), and perfusate samples taken after 4.5 min of exposure of the livers to 1×10^{-9} M-AGEPC, 5 min prior to exposure to AGEPC, or after 4.5 min in the presence of AGEPC and 9.5 min exposure to 5 mM-allopurinol. These results show that the material absorbing at 293 nm in the perfusate co-eluted with authentic uric acid and that this material was not present in samples of perfusate obtained from livers pretreated with the XOD inhibitor allopurinol. These results also indicate that the perfusate was relatively free of other purine nucleotides and their metabolites except after exposure to allopurinol. The elution profile of samples taken during exposure to allopurinol alone reached a steady state within 2 min of exposure, and was not significantly different from that shown in Fig. 1(a) (lower trace) (results not shown). Although other peaks were observed using an isocratic gradient of ammonium phosphate and methanol/acetonitrile [28], these peaks were minor compared with the uric acid peak, and none of these peaks were altered following exposure of the liver to AGEPC or increased $[K^+]_o$ (results not shown).

Uric acid has an absorption maximum in the u.v. at 293 nm [29]. Fig. 1(b) (upper trace) indicates that the addition of uricase to a cuvette containing 2.5×10^{-8} mol of uric acid/ml resulted in a decrease of the A_{293} . In the remaining traces 1 ml of perfusate from the same samples used in Fig. 1(a) was added to cuvettes containing uricase. The results show that the perfused livers released, in the absence of AGEPC, 1.2×10^{-9} mol of uric acid/ml of perfusate (third trace), and this increased by 17-fold to 2×10^{-8} mol/ml in the presence of AGEPC (second trace). Perfusate from livers pretreated with allopurinol and then AGEPC showed no change in A_{293} (fourth

Fig. 1. Determination of uric acid in perfusate by reversed-phase h.p.l.c. (a) or uricase assay (b)

Traces show authentic uric acid (upper trace), and perfusate from livers exposed to 1×10^{-9} M-AGEPC (trace 2), control livers (trace 3), and livers exposed to 5 mM-allopurinol and AGEPC (lower trace).

trace). Allopurinol eluted in a broad band at about 13.7 min (results not shown). Similar results were obtained using perfusate samples collected during exposure of the liver to increased $[K^+]_o$ (results not shown). The combined results of Fig. 1 indicate that uric acid production and release is stimulated by AGEPC or increased $[K^+]_o$ in the perfused rat liver.

Figs. 2 and 3 show that uric acid release from perfused rat livers stimulated by AGEPC is dose-dependent, and appears to be related temporally to the second phases of AGEPC-induced changes in O_2 uptake and glucose release. This is most apparent at 1×10^{-9} M-AGEPC, where, as has been reported previously [18], two distinct phases of O_2 uptake and glucose release occur (Fig. 2). The first phase of O_2 uptake is characterized by a rapid uptake of O_2 corresponding to a maximum decrease in perfusate $[O_2]$ of about $20 \mu M$. This is followed by a rapid decrease of O_2 uptake to a minimum at about 2.5 min after exposure to AGEPC, and corresponding to a maximum increase in the perfusate O_2 of about $90 \mu M$ below basal $[O_2]$. In combination with a slower recovery period of about 6.5 min, this decrease in O_2 uptake defines the second phase. As the AGEPC concentration was decreased, the second phase of O_2 uptake became less prominent, until, at 5×10^{-11} M-AGEPC, there occurred but a single initial increase in O_2 consumption (compare upper traces, Figs. 2 and 3). Conversely, the first phase became larger (magnitude and extent) with decreasing AGEPC concentration. At 1×10^{-9} M-AGEPC uric acid release paralleled the increase in O_2 uptake and glucose release in their second phases (Fig.

2). The onset of increased uric acid release occurred at about 2 min, which is about the time that the peak of the first phase of glucose release, and the half-maximal response of both the increase in portal pressure and decrease of the second phase of O_2 uptake occurred. The time taken to reach half of the maximal rate of release of uric acid (about 3.5 min) is about the same as that for the increase in rate of the second phase of glucose release. The peak of uric acid release occurs at 5 min, roughly equivalent to the time taken to reach half the maximal extent of the final return to basal O_2 uptake and the portal pressure decrease. At lower concentrations of AGEPC a clear description of the temporal relationship between uric acid release and the other responses cannot be observed, although it is apparent that uric acid release lags behind the onset of O_2 uptake, portal pressure increase and glucose release (Fig. 3).

Figs. 2 and 3 also illustrate the effects of 5 mM-allopurinol on the metabolic and haemodynamic responses of the perfused liver to AGEPC. The results show that allopurinol blocked completely the release of uric acid into the perfusate in the absence or presence of all concentrations of AGEPC tested. However, the metabolic and haemodynamic responses were inhibited only partially by allopurinol, and this effect decreased with decreasing concentrations of AGEPC. In addition, inhibition of glucose release and O_2 uptake is apparent only for the secondary phases of these responses. The second phase of glucose release was blocked completely (Figs. 2 and 3a). Similarly, the second phase of O_2 consumption was blocked completely at 2×10^{-10} M- and

Fig. 2. Effect of 1×10^{-9} M-AGEPC in the absence (—, □, △, ○) or presence (---, ■, ▲, ●) of 5 mM-allopurinol on O_2 consumption, portal pressure, urate and glucose production in the perfused rat liver

5×10^{-11} M-AGEPC. At 1×10^{-9} M-AGEPC both the magnitude and the recovery time of the second phase of O_2 uptake were reduced by nearly 50% and 75%, respectively (Fig. 2). Table 1 summarizes O_2 uptake data from a series of perfused livers exposed to 1×10^{-9} M-AGEPC in the absence ($n = 4$) or presence ($n = 4$) of 5 mM-allopurinol. The results indicate that the magnitude of the first phase was not affected significantly by allopurinol, whereas that of the second phase was reduced by approx. 50%. The combined results of Figs. 2 and 3 and Table 1 show that allopurinol blocked completely the release of uric acid and the second phases of glucose

release and O_2 consumption from the AGEPC-stimulated perfused livers.

Attempts were made to determine whether uric acid release, and the inhibition of this response by allopurinol, are phenomena specific for AGEPC. Increased $[K^+]_o$ has been shown previously to have similar metabolic and haemodynamic effects on the perfused liver and to lack effects on isolated hepatocyte suspensions, as does AGEPC [22,23]. Conversely, phenylephrine, an α -adren-ergic agonist, stimulates O_2 uptake, increases portal pressure and glucose release in the perfused liver, but is equally effective in stimulating O_2 uptake and glucose release in suspensions of isolated hepatocytes [18,24].

Fig. 4 shows that 50 mM- $[K^+]_o$ causes a 7-fold increase in uric acid release from the perfused liver within 3 min of exposure. Allopurinol blocked completely this release, along with complete inhibition of the increase in portal pressure and the second phase of O_2 uptake. The maximal increase in glucose release was inhibited by about 50% in the presence of 5 mM-allopurinol. At 15 mM- $[K^+]_o$ the haemodynamic and metabolic responses were qualitatively similar to those shown for 5×10^{-11} M-AGEPC, both in the absence or presence of 5 mM-allopurinol (results not shown).

Fig. 5 illustrates that, in contrast to AGEPC or increased $[K^+]_o$, 10 μ M-phenylephrine (a concentration which results in maximal stimulation of glucose release in the perfused rat liver) had no significant effect on uric acid release from the perfused liver. Whereas allopurinol did not affect significantly glucose release, the small decrease in O_2 uptake intermediate between the two peaks characteristic of phenylephrine-induced O_2 uptake was eliminated, and the portal pressure increase was inhibited by about 65%. Allopurinol had no effect on glucose release or O_2 uptake stimulated by 0.1 μ M-phenylephrine (results not shown). At this low concentration of phenylephrine, no increase in portal pressure was observed in the absence or presence of allopurinol. These results indicate that, although the portal pressure response to phenylephrine may be mediated by a similar mechanism as that which evokes the responses to AGEPC or increased $[K^+]_o$, the glucose release response likely occurs solely through a mechanism which is independent of the haemodynamic responses.

To determine whether AGEPC or increased $[K^+]_o$ have effects on the total amount of XDH and XOD activities, homogenates of freeze-clamped livers were assayed for their ability to generate uric acid from xanthine in the absence or presence of NAD^+ . Our results indicated that the ratio of XDH to XOD activities was not affected significantly by prior exposure of the perfused livers to AGEPC or increased $[K^+]_o$, either in the absence or presence of 5 mM-allopurinol (results not shown). Although the ratio of XDH/XOD was significantly higher when the tissue was extracted in the presence of phenylmethanesulphonyl fluoride and dithio-threitol, there was still no significant difference between livers perfused in the absence or presence of 1×10^{-9} M-AGEPC or increased $[K^+]_o$.

DISCUSSION

This report presents evidence suggesting that the glycogenolytic agents AGEPC and increased $[K^+]_o$ stimulate O_2 -derived free radical production mediated by XOD within the perfused rat liver. The results are the

Fig. 3. Effects of 2×10^{-10} M- (left panel) or 5×10^{-11} M-AGEPC (right panel) in the absence (—, □, △, ○) or presence (---, ■, ▲, ●) of 5 mM-allopurinol on O_2 consumption, portal pressure, urate and glucose production in the perfused rat liver

Table 1. Effect of 5 mM-allopurinol on the magnitudes of the first and second phases of O_2 consumption of the perfused rat liver to 1×10^{-9} M-AGEPC

	Decrease in perfusate $[O_2]$ (μM)		n
	Phase 1	Phase 2	
– Allopurinol	22.4 ± 8.0	87.0 ± 10.2	4
+ Allopurinol	23.9 ± 6.1	42.9 ± 4.6	4

first direct indication that a major portion of the metabolic effects of AGEPC or increased $[K^+]_o$ result from vasoconstriction with attendant tissue hypoxia within the liver parenchyma. These conclusions are based on the observations that: (1) AGEPC or increased $[K^+]_o$ stimulated the release of uric acid, a product of XOD reaction, from the perfused liver; (2) the metabolic and haemodynamic effects of AGEPC or increased

$[K^+]_o$ were reduced or completely blocked by the xanthine oxidase inhibitor allopurinol; (3) in contrast with AGEPC or increased $[K^+]_o$, the α -adrenergic agonist, phenylephrine, did not stimulate uric acid release, and its metabolic effects were not sensitive to allopurinol.

A number of explanations can be offered to describe the mechanism causing increased uric acid levels in the perfusate of the liver. These are: (1) increased metabolic flux through the adenine nucleotide catabolic pathway, (2) conversion of XDH to XOD and its subsequent activation, (3) inhibition of the uric acid cleavage step catalysed by uricase, and/or (4) stimulation of the secretion of uric acid from its intracellular site of synthesis. The results presented in this report do not permit conclusive identification of the involvement of any one or combination of these mechanisms. However, in combination with previously published data the first two explanations appear to be most plausible.

XOD catalyses the first irreversible step in the catabolism of purines [30]. Thus, any small changes in flux through this pathway will be reflected in increased levels of the products of the XOD reaction, uric acid and

Fig. 4. Effect of 50 mM- K^+ in the absence (—, $\square$, $\triangle$, $\circ$) or presence (---, $\blacksquare$, $\blacktriangle$, $\bullet$) of 5 mM-allopurinol on O_2 consumption, portal pressure, urate and glucose production in the perfused rat liver

superoxide. Increased catabolism of the purine nucleotide ATP has been shown to occur during experimental conditions which lead to decreased O_2 concentrations of the vasculature, such as in haemorrhagic shock or hypotension [9,10,13]. These conditions also lead to increased blood levels of the end products of purine metabolism, hypoxanthine, uric acid and allantoin, in the intact organism [10,11]. It has been suggested that only a very small fraction of the adenine nucleotide pool is lost due to increased XOD activity [11]. Similarly, after exposure of the perfused liver to AGEPC or increased $[K^+]_o$, uric acid levels increased transiently by up to 17-fold in the absence of significant changes in the tissue adenine nucleotide concentrations [18,23]. Moreover, these results also suggest that only a small fraction of the adenine nucleotide pool is sensitive to the haemodynamic effects of AGEPC or increased $[K^+]_o$.

Further evidence that suggests a role for XOD in the release of uric acid is the sensitivity of the latter to allopurinol, a specific inhibitor of XOD [31]. Allopurinol has been shown to inhibit the histological and biochemical changes produced by low-flow-induced ischaemia in a variety of tissues [1,8,10]. It has been proposed that allopurinol is able to protect the tissue from ischaemic injury by inhibiting the production of

Fig. 5. Effect of 1×10^{-5} M-phenylephrine in the absence (—, $\square$, $\triangle$, $\circ$) or presence (---, $\blacksquare$, $\blacktriangle$, $\bullet$) of 5 mM-allopurinol on O_2 consumption, portal pressure, urate and glucose production in the perfused rat liver

superoxide [32–34]. In addition to allopurinol, ischaemic injury [1,3,5,14], or generation of superoxide by vascular endothelial cells [34], also can be inhibited by exposure of the tissue to the superoxide scavenging enzyme, superoxide dismutase. It has been suggested that the majority of the responses to ischaemia result directly from the production of superoxide, possibly via the vascular endothelium, rather than simply a loss of purine metabolites [2]. Similarly, in the present experiments allopurinol inhibited the haemodynamic and metabolic responses to AGEPC or increased $[K^+]_o$ and blocked completely the release of uric acid, indicating that products of the XOD reaction are involved in the

evocation of the sequence of metabolic and haemodynamic events.

Evidence that a conversion from the NAD^+ - to the O_2 -utilizing form of XOD occurs during experimental ischaemia comes from assay of the enzyme in homogenized tissue extracts. Conversion from XDH to XOD is thought to occur during the hypoxic interval, possibly through the action of a Ca^{2+} -dependent protease [2,4,7]. However, the XDH/XOD ratio varies from one tissue to another, and shows different sensitivities to O_2 -limiting conditions. Whereas 10 min of complete perfusion stasis is required to convert XDH activity from 57% to 3% of the total activity (XDH + XOD) in extracts of heart, the rat liver enzyme requires at least 30 min for conversion from 90% in the XDH form to 55% in the XOD form [4]. In addition, conversion of XDH to XOD in isolated extracts from rat liver is highly sensitive to the conditions of extraction and assay [7,35,36]. Incubation of extracts in the presence of thiol and reducing agents results in 90% of the activity in the XDH form [36]. In the absence of these agents the enzyme activity is converted rapidly to the XOD form. Conversion to the XOD form occurs during incubation under anaerobic conditions at 37 °C, and this conversion is reversed by exposing the tissue extract to O_2 [7]. The present report shows that pre-treatment of the perfused liver with AGEPC or increased $[\text{K}^+]_o$ in the absence or presence of allopurinol did not affect significantly the XDH/XOD ratio under similar conditions of extraction and assay. Thus, in our hands, assay of the enzyme *in vitro* does not appear to reflect reliably the form of the enzyme *in vivo*. Alternatively, it is possible that only a small fraction of the total XDH was exposed to conditions which induced conversion to the XOD forms, and/or it was converted rapidly back to the XDH form either prior to or during measurement.

Elucidation of the mechanism by which AGEPC or increased $[\text{K}^+]_o$ stimulates glycogenolysis has thus far been difficult due to the multiphasic nature of the haemodynamic and metabolic responses [18,19,23]. In this report two observations are presented which suggest that two separate processes occur upon exposure of the liver to AGEPC or increased $[\text{K}^+]_o$. These observations are: (1) a distinction between two phases of responses (see the Results section for a detailed description of these phases), and (2) the responses of the liver to glycogenolytic hormones which are known to act at hepatocyte receptors differ significantly from those responses to AGEPC or increased $[\text{K}^+]_o$, indicating a dependence between haemodynamic and metabolic events within the liver for sensitivity to AGEPC or increased $[\text{K}^+]_o$ but not for α -adrenergic agonists.

Two phases of haemodynamic and metabolic responses of the liver to near-maximal concentrations of AGEPC or increased $[\text{K}^+]_o$ have been reported [18,19,23]. In contrast to the first phase of O_2 consumption and glucose release, the second phase is sensitive to allopurinol, and is accompanied by release of uric acid. It is suggested that the second phase results from localized stasis of perfusion and tissue ischaemia (as indicated by attainment of a peak increase in portal pressure, a peak decrease in O_2 uptake, and a transient decrease in glucose output). The resulting ischaemia-induced increase in membrane permeability and increase in cytosolic Ca^{2+} concentration [13,37] serve to activate XOD [2] (as indicated by the onset of uric acid release). The second portion of this phase represents reperfusion of the ischaemic regions

within the liver (as indicated by increased O_2 uptake and decreased portal pressure). During this interval it is suggested that O_2 toxicity occurs via XOD-generated superoxide (as indicated by increased uric acid release), which initiates membrane damage via lipid peroxidation [6,32–34,38]. The increase in glucose release, most notably present during exposure to high concentrations of AGEPC but also observed during exposure to high concentrations of $[\text{K}^+]_o$ [22,23], that occurs at this stage may result from a combination of mechanisms. These are a reperfusion-induced washout of glucose produced during the period of stasis, and further activation of glycogenolysis by Ca^{2+} influx through peroxidative damage of the hepatocyte membrane.

The primary target for the action of AGEPC or increased $[\text{K}^+]_o$ in the liver has been suggested to be the vascular endothelium [18,20,23] based on localization studies of specific binding of ^3H AGEPC (C. E. Hill, P. J. Sheridan, D. J. Hanahan & M. S. Olson, unpublished work), mobilization of a small pool of calcium compared with α -adrenergic agonists [39] and on lack of glycogenolytic effects of these agents in suspensions of isolated hepatocytes [20,23,24]. Further evidence for this conclusion derives from the observations that phenylephrine, an α -adrenergic agonist which has well-documented hepatocyte receptors, did not stimulate uric acid release although its vascular response was significantly inhibited by allopurinol. Inhibitors of phospholipase A_2 and lipoxygenase also have been observed to block the haemodynamic, but not the metabolic, effects of α -adrenergic agonists in the perfused liver [20]. Since lipoperoxide is required for the activation of the arachidonate-metabolizing enzymes, and this substrate can be generated rapidly and non-enzymically by superoxide attack of membrane lipids (see [30]), we suggest that the source of superoxide is an XOD present in the vascular endothelium. (This does not exclude a secondary increase in hepatocyte XOD activity, which is presumably the source of the uric acid release observed here.) In addition, it has been shown that uric acid itself may stimulate directly activity of enzymes involved in the metabolism of arachidonic acid [40]. It is possible that uric acid production in the liver also may contribute to hepatic prostanoid generation. Although it has been suggested that the effects of AGEPC may be mediated by an arachidonic acid metabolite [20,25], the present results suggest that at least the second phase of the hepatic response may be due to O_2 -derived free radicals generated by XOD.

In conclusion, the results in this report suggest that hepatic uric acid production stimulated by AGEPC or increased $[\text{K}^+]_o$ may be a sensitive indicator of local tissue ischaemia in the liver, especially under conditions where significant changes in adenine nucleotides cannot be measured. Further, these results provide a new parameter for distinguishing between glycogenolytic agents which act initially at different sites within the liver.

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