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Glycogenolytic and haemodynamic responses to heat-aggregated immunoglobulin G and prostaglandin E_2 in the perfused rat liver

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Infusion of heat-aggregated immunoglobulin G (HAG) into perfused livers from fed rats caused transient increases in hepatic glycogenolysis and portal-vein pressure, accompanied by a transient increase in hepatic glycogen phosphorylase *a* content. The hepatic responses to HAG were inhibited by indomethacin (2 μM). In contrast, HAG was without effect on phosphorylase *a* content and glucose output in isolated hepatocytes. HAG infusion caused a transient decrease in hepatic cyclic AMP. Lowering the extracellular Ca^{2+} concentration to 6 or 50 μM attenuated markedly the glycogenolytic and haemodynamic responses to HAG; efflux of Ca^{2+} from the liver was not observed in response to HAG. Co-infusion of the specific platelet-activating-factor antagonist U-66985 (1-*O*-octadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphoric acid 6′-trimethylammoniumhexyl ester) did not attenuate the glycogenolytic response to HAG. Infusion of prostaglandin E_2 caused increases in glucose output, portal-vein pressure and the reduction state of the cytosolic NAD(H) redox couple similar to those seen with HAG. The present study suggests that the glycogenolytic activation after HAG infusion may be an indirect consequence of the haemodynamic response of the hepatic vasculature to stimulation of the reticuloendothelial cells of the liver.

INTRODUCTION

Soluble immune complexes are removed from the circulation by the reticuloendothelial system via an Fc-receptor-mediated process, with the liver comprising the major site of uptake [1–4]. Infusion of heat-aggregated immunoglobulin G (HAG) into the isolated perfused rat liver leads to a transient stimulation of hepatic glycogenolysis and O_2 consumption, with a concomitant synthesis and release of platelet-activating factor from the liver [5]. Synthetic platelet-activating factor (acetyl-glyceroether phosphocholine) has been demonstrated to activate hepatic glycogenolysis in the perfused rat liver [6,7] and in the rat *in vivo* [8]. The purpose of the present study was to characterize further the process by which hepatic glycogenolysis is stimulated by HAG, and to elucidate the role of potential autacoid mediators in the stimulatory process.

EXPERIMENTAL

Liver perfusion studies

Non-recirculating perfusion of livers *in situ* from male Sprague–Dawley rats (Harlan, Houston, TX, or Benton and Kingman, Vermont, CA, U.S.A.), weighing 160–200 g and fed on a standard laboratory chow *ad libitum*, was performed as described previously [9] at a constant flow rate of 35 ml/min. The perfusion medium was Krebs–Henseleit bicarbonate buffer, pH 7.4 [10], saturated with O_2/CO_2 (19:1) and maintained at 37 °C. The $CaCl_2$ concentration was 1.25 mM unless stated otherwise in the Figure legend. O_2 consumption was monitored continuously with a Clark-type oxygen electrode placed in the perfusion circuit immediately after the liver. Portal-vein pressure, a measure of intrahepatic pressure [11], was measured by using a Statham P23 pressure transducer in conjunction with an oscillographic recorder. Livers were perfused for 30 min before collection of samples to ensure removal of endogenous hormones, and to stabilize glucose output. Effluent perfusate samples were collected for 30 s intervals for assay of metabolites. Glucose was assayed by the method of Bergmeyer *et al.* [12]. Lactate and pyruvate were measured as described by Gutmann & Wahlefeld [13] and Passoneau & Lowry [14] respectively. Ca^{2+} was measured by atomic-absorption spectroscopy, with a Varian AA6 spectrometer. Metabolite values are presented as μmol/h per g wet wt.

For measurement of cyclic AMP, phosphorylase *a* and adenine nucleotides, livers were freeze-clamped, stored and assayed as described previously [15].

Hepatocyte studies

Preparation of hepatocytes, incubations and measurements of phosphorylase *a* and glucose output were described previously [16].

Preparation of HAG

This was done from normal human serum as described previously [7].

Prostaglandin E_2 and indomethacin were obtained from Sigma; U-66985 was generously given by D. E. Ayer, The Upjohn Co. Prostaglandin E_2 and U-66985 were dissolved in saline containing bovine serum albumin (0.25%, w/v) before infusion. Bovine serum albumin (fatty acid- and immunoglobulin-free)

Abbreviation used: HAG, heat-aggregated immunoglobulin G.

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was obtained from Calbiochem. All other reagents were of analytical grade and obtained from commercial sources.

Numerical results presented in this paper are expressed as means ± S.E.M. Statistical analysis was by Student's *t* test. Perfusion experiments illustrated in the various Figures are representative experiments performed at least three times with essentially identical results.

RESULTS

Conflicting results have appeared in the literature concerning the primary hepatic cell type involved in the uptake of soluble immune complexes from the circulation. Although several studies have indicated that Kupffer cells, the resident macrophage cells of the liver, are the predominant cell type involved [17,18], others have proposed that the parenchymal cell is the major target for soluble immune complexes [19,20]. Hepatic responses to infused immune complexes could represent either a direct effect of the immune complex on the parenchymal cell, or an indirect effect exerted on the parenchymal cells via another cell type. Fig. 1 shows that HAG had no effect on the glycogen phosphorylase *a* content of isolated hepatic parenchymal cells (hepatocytes). In contrast, adrenaline increased the phosphorylase *a* content of hepatocytes by approx. 250%. A similar increase in hepatocyte glucose output was observed in response to adrenaline, but again HAG was without effect (results not shown).

In contrast with the lack of effect of HAG on hepatocyte phosphorylase *a* content, extracts of livers perfused with HAG (10 μg/ml) contained significantly elevated phosphorylase *a* activity (Fig. 2). Phosphorylase *a* activity was increased by approx. 60% ($P < 0.001$) 2 min after onset of HAG infusion, before returning to control values at 5 min. After 1 min phosphorylase *a* was increased by approx. 20%, but was not significantly different from control values. Indomethacin (2 μM), which inhibited the increase in glucose output in response to HAG [5], also inhibited the increase in phosphorylase *a* content by approx. 40%, with no effect on basal phosphorylase *a* activity. In a parallel series of experiments, indomethacin (2 μM) decreased the maximal increase in glucose output in response to HAG (10 μg/ml) from 90 ± 9 ($n = 20$) to 34 ± 8 μmol/h per g ($n = 9$; $P < 0.001$). Basal glucose output was 84 ± 5 μmol/h per g ($n = 20$).

Measurement of hepatic cyclic AMP in the freeze-clamped liver tissue demonstrated that the HAG infusion did not lead to significant increase in cyclic AMP, but caused a small transient decrease in the cyclic nucleotide (Fig. 2). In contrast, glucagon (10 nM) increased hepatic cyclic AMP approx. 50-fold (results not shown).

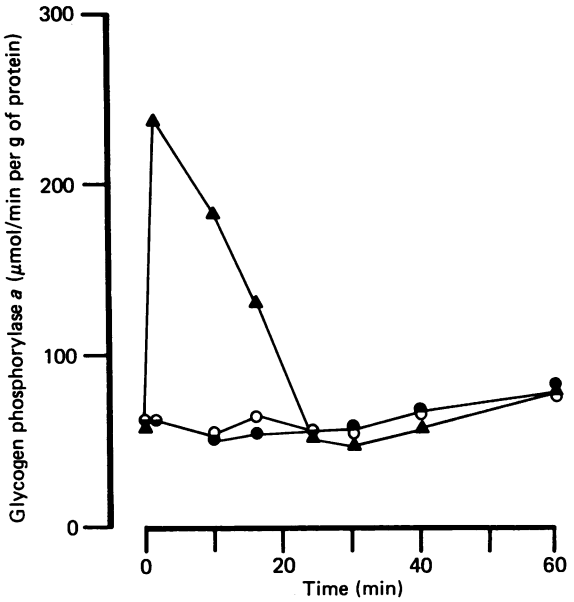

Fig. 1. Effect of HAG and adrenaline on the glycogen phosphorylase *a* content of isolated rat hepatocytes

Hepatocytes were isolated by collagenase perfusion of rat liver as described in the Experimental section. Isolated hepatocytes were incubated at 37 °C in a shaking water bath, and samples removed at intervals for assay of phosphorylase *a* as described previously [16]. ○, No addition; ●, +HAG (20 μg/ml); ▲, +adrenaline (10 μM). The experiment shown is representative of three essentially identical experiments.

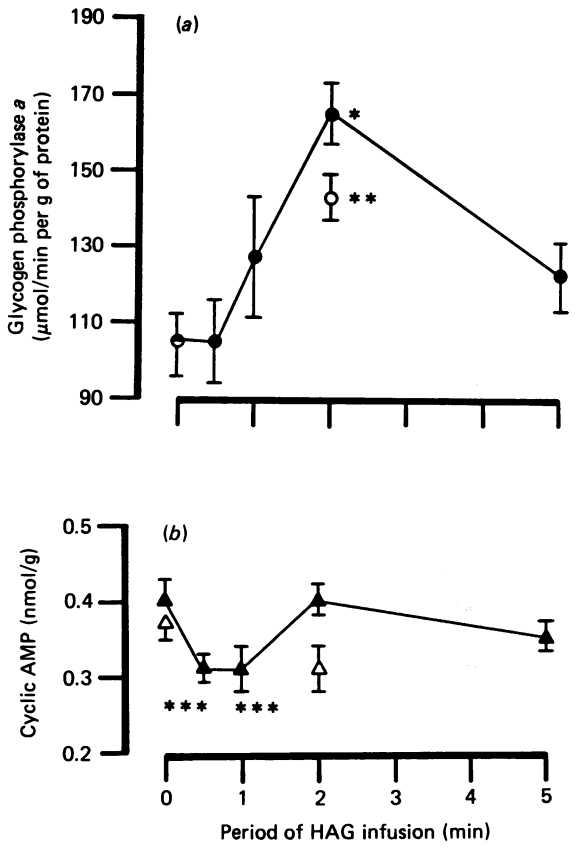

Fig. 2. Effect of HAG on phosphorylase *a* and cyclic AMP content of extracts from perfused rat liver

Livers were rapidly freeze-clamped between liquid-N_2-cooled aluminium tongs at the times indicated after onset of infusion of HAG (10 μg/ml) (●, ▲). In some experiments infusion of indomethacin (2 μM) was initiated 5 min before infusion of HAG (10 μg/ml) (○, △). Each point represents the mean ± S.E.M. for four to seven livers. *$P < 0.001$ versus basal; **$P < 0.001$ versus basal + indomethacin, $P < 0.05$ versus HAG; ***$P < 0.05$ versus basal.

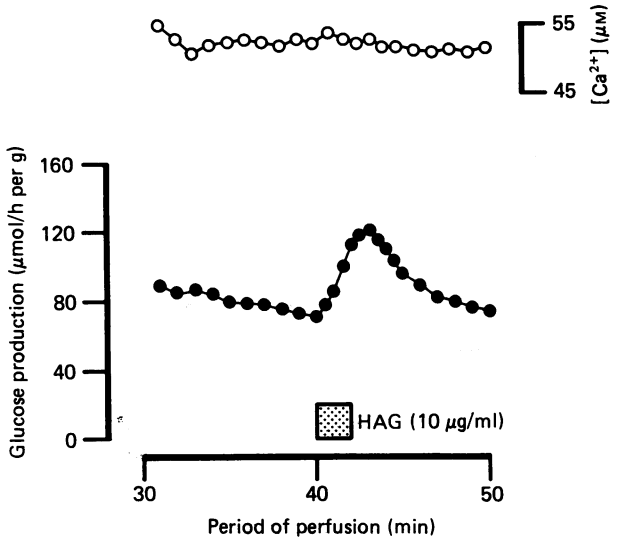

Fig. 3. Effects of HAG on hepatic glucose output and effluent perfusate Ca^{2+} concentration

Livers were perfused as described in the Experimental section with Krebs–Henseleit buffer with the Ca^{2+} concentration decreased to 50 μM. Ca^{2+} was measured by atomic-absorption spectrophotometry. Results are means from four experiments.

A number of substances, including α-adrenergic agonists, vasopressin and adenine nucleotides, stimulate hepatic glycogenolysis via Ca^{2+}-dependent mechanisms involving mobilization of intracellular Ca^{2+} [21–25]. In experiments in which the perfusate Ca^{2+} concentration was decreased to 50 μM to facilitate measurement of changes in effluent perfusate Ca^{2+}, infusion of HAG had no effect on the effluent Ca^{2+} concentration (Fig. 3). In similar experiments, phenylephrine (10 μM) caused a transient increase in effluent Ca^{2+} of 27 ± 2 μM ($n = 3$). However, Ca^{2+} appears to play some part in the response to HAG, since the maximal glycogenolytic response to HAG was decreased by 40%, to 53 ± 14 μmol/h per g ($n = 4$; $P < 0.05$), when the Ca^{2+} concentration was lowered from 1.25 to 0.05 mM.

The role of Ca^{2+} was investigated further in the experiments shown in Fig. 4. Removal of added Ca^{2+}, which lowered the perfusate Ca^{2+} concentration to approx. 7 μM (as measured by atomic-absorption spectroscopy) 5 min before infusion of HAG, markedly attenuated the glycogenolytic response to HAG, decreasing it from 90 ± 9 ($n = 20$) to 30 ± 5 μmol/h per g ($n = 10$; $P < 0.001$); re-infusion of Ca^{2+} during HAG infusion led to a large increase in glucose output, 123 ± 3 μmol/h per g ($n = 3$), whereas in control experiments addition of Ca^{2+} to livers perfused without Ca^{2+} for the same length of time in the absence of HAG caused only a modest increase in glucose output, 27 ± 4 μmol/h per g ($n = 12$; $P < 0.001$). Addition of HAG to these control livers 5 min after addition of Ca^{2+} restored nearly completely the glycogenolytic response, to 73 ± 16 μmol/h per g ($n = 9$). Interestingly, in livers perfused with Ca^{2+}-free media for 5 min before addition of HAG, addition of Ca^{2+} 8 min after cessation of HAG infusion still enhanced glucose output, e.g. to 75 ± 11 μmol/h per g ($n = 3$; $P < 0.001$ versus Ca^{2+} re-addition without HAG infusion), suggesting that a cellular process remains activated for several minutes.

Since infusion of synthetic platelet-activating factor into perfused livers has been demonstrated to cause hepatic vasoconstriction, which may be causally related to the glycogenolytic effect of this lipid mediator [15,26], the effect of HAG infusion on portal-vein pressure was determined. Fig. 5 shows that infusion of HAG led to a transient increase in portal-vein pressure, which was attenuated by co-infusion of indomethacin (2 μM). The increase in portal-vein pressure was also attenuated at lower perfusate Ca^{2+} concentration (Table 1).

In order to investigate the role of platelet-activating factor in the glycogenolytic response to HAG, the specific platelet-activating-factor antagonist U-66985 [27,28] was employed. U-66985 (1 μM) has been shown to inhibit hepatic responses to infused synthetic platelet-

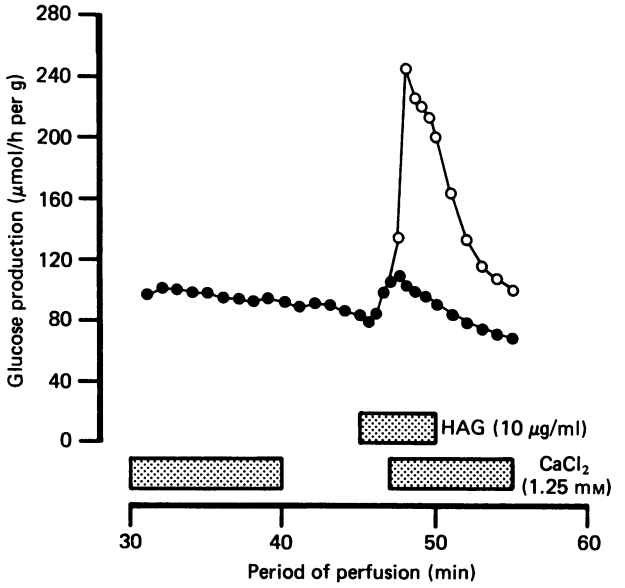

Fig. 4. Effect of short-term removal of perfusate Ca^{2+} on the glycogenolytic response to HAG

Livers were perfused as described in the Experimental section with Ca^{2+}-free buffer, and Ca^{2+} (1.25 mM) was infused in a syringe pump. Ca^{2+} infusion was stopped at 40 min; ●, perfusion continued without Ca^{2+} ($n = 10$); ○, Ca^{2+} re-infused after 47 min ($n = 3$). Results are means from the numbers of experiments in parentheses.

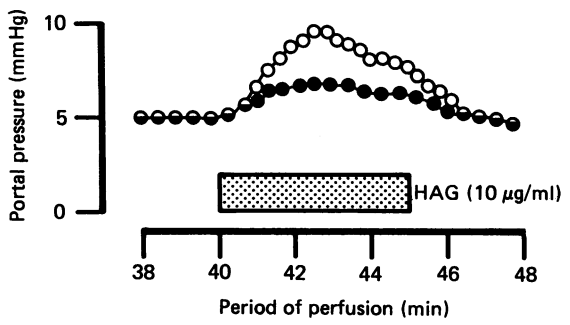

Fig. 5. Effect of HAG on hepatic portal pressure

Livers were perfused as described in the Experimental section. Traces shown are representative recordings from livers perfused in the absence (○) or presence (●) of indomethacin (2 μM). Infusion of HAG (10 μg/ml) was initiated 5 min after infusion of indomethacin.

Table 1. Effect of HAG on hepatic portal-vein pressure in the isolated perfused rat liver

Livers were perfused as described in the Experimental section, at the Ca^{2+} concentration indicated. The maximal increase in portal-vein pressure in response to HAG was determined by continuous monitoring of portal pressure with a pressure transducer connected to the portal cannula perfusion tubing. $*P < 0.001$ versus HAG alone (1.25 mM-Ca^{2+}). Basal portal-vein pressure was 5.3 ± 0.15 mmHg.

Perfusion condition	$[Ca^{2+}]$ (mM)	Maximal increase in portal-vein pressure (mmHg)
No addition, 40 min; +HAG (10 μg/ml), 5 min	0.05	1.1 ± 0.2 ($n = 7$)
No addition, 40 min; +HAG (10 μg/ml), 5 min	1.25	3.6 ± 0.5 ($n = 12$)
No addition, 40 min +indomethacin (2 μM), 5 min; +indomethacin (2 μM) and HAG (10 μg/ml), 5 min	1.25	1.0 ± 0.1* ($n = 8$)

activating factor (0.2 nM) almost completely [28]. However, infusion of U-66985 into livers 5 min before co-infusion of HAG (10 μg/ml) did not affect the glycogenolytic response to HAG; the maximal increase in glucose output in the presence of U-66985 (1 μM) was 81 ± 17 μmol/h per g ($n = 7$), and in a parallel control series without U-66985 the maximal increase was 80 ± 14 μmol/h per g ($n = 7$).

The inhibition of hepatic responses to HAG by indomethacin [5] suggested the possibility that a prostanoid mediator may be involved in the glycogenolytic and haemodynamic consequences of HAG infusion. Since hepatic Kupffer cells and other macrophage-type cells have been shown to release prostaglandins, including E_2 and F, in response to phagocytic stimuli [29–31], the effect of prostaglandin E_2 infusion on hepatic parameters was investigated. The effects of prostaglandin E_2 (10 μM) on hepatic glucose output, effluent lactate/pyruvate ratio and portal-vein pressure are shown in Fig. 6(*a*); it caused increases in these metabolic and haemodynamic indices similar to those observed in response to HAG. Total lactate+pyruvate output was also increased transiently by approx. 60%. Increases in all parameters were statistically significant ($P < 0.01$). Fig. 6(*b*) shows a partial dose/response for the stimulation of glycogenolysis and hepatic vasoconstriction by prostaglandin E_2. Higher concentrations of prostaglandin E_2 were not tested, owing to the prohibitive amount of material required.

Hepatic responses to prostaglandin E_2 also were markedly attenuated at 7 μM perfusate calcium; the glycogenolytic response to prostaglandin E_2 (10 μM) was decreased from 55 ± 8 ($n = 6$) to 12 μmol/h per g ($n = 2$) and the change in the portal-vein pressure was similarly attenuated (results not shown).

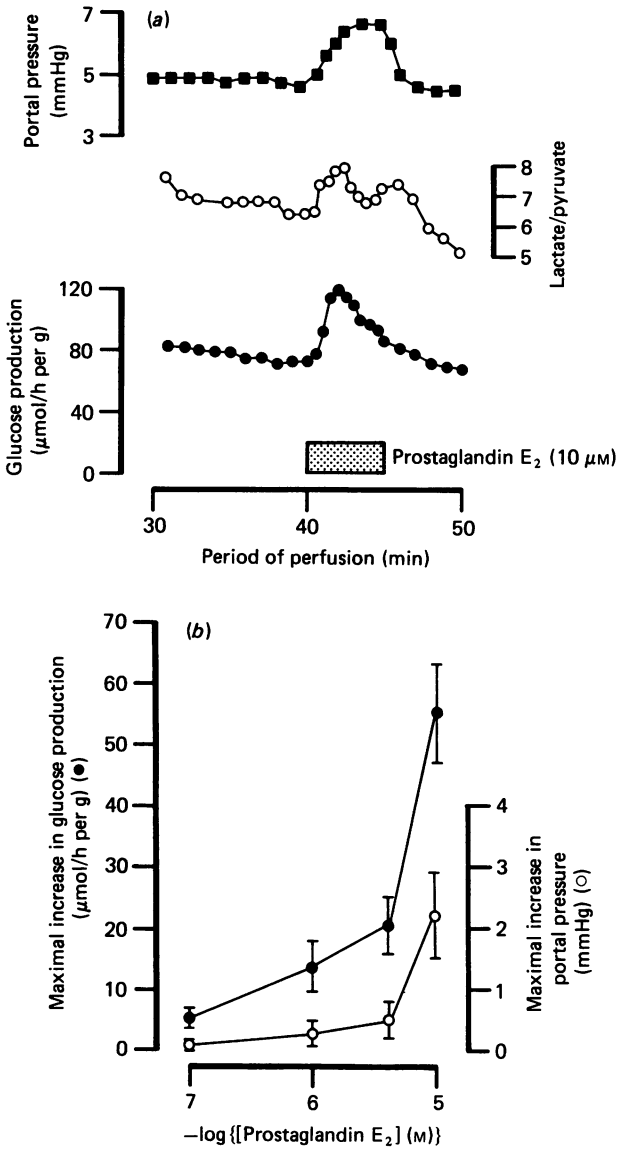

Fig. 6. Glycogenolytic and haemodynamic responses to prostaglandin E_2 in the perfused rat liver

Livers were perfused as described in the Experimental section. (*a*) Effect of prostaglandin E_2 (10 μM) on hepatic portal pressure, glucose output and effluent lactate/pyruvate ratio. (*b*) Partial dose/response curve for glycogenolytic and haemodynamic responses to prostaglandin E_2, which was infused for 5 min, and the maximal increases in glucose output and portal pressure were determined.

DISCUSSION

The present study has demonstrated that the increases in glucose output and glycogen phosphorylase *a* content of perfused rat liver in response to HAG are mediated neither by a cyclic-AMP-dependent mechanism, analogous to glucagon and β-adrenergic agonists, nor by release of intracellular Ca^{2+}, analogous to stimulation by α-adrenergic agonists, vasopressin and adenine nucleotides. However, Ca^{2+} clearly is important for full expression of the hepatic responses to HAG. Since removal of Ca^{2+} for only 5 min markedly attenuated hepatic responses to HAG, it is likely that extracellular Ca^{2+} is important, and that inhibition of HAG-mediated glucose output and the elevated portal-vein pressure by Ca^{2+} removal do not reflect depletion of intracellular Ca^{2+}.

The lack of sensitivity of isolated parenchymal cells to HAG suggests that a mediator produced by another cell type may be involved, with Kupffer cells and/or the endothelial cells representing the logical candidates. An alternative possibility, that parenchymal cells possess a receptor for HAG which is lost or modified during enzymic preparation of the cells, cannot be discounted at present. Potential mediators of the hepatic responses to HAG include platelet-activating factor and prostaglandins. The finding that U-66985 (1 μM), a specific platelet-activating-factor antagonist, does not attenuate hepatic responses to HAG argues against involvement of platelet-activating factor in the mechanism of action of HAG, although it is possible that the factor's action at a cell–cell junction would be inaccessible to U-66985 in the perfusate. It is interesting to compare the metabolic responses of livers to HAG with similar responses of livers from rats sensitized *in vivo* by immunization with bovine serum albumin when the antigen (albumin) is infused. When isolated livers from sensitized rats are perfused with albumin, stimulation of glycogenolytic activity occurs, which can be blocked by prior infusion of U-66985 (1 μM) (O. Halvorsen, D. B. Buxton, D. L. Briseno & M. S. Olson, unpublished work). This observation suggests that in livers from sensitized rats, in contrast with the HAG response in non-sensitized livers, the glycogenolytic action of antigen is mediated via platelet-activating factor.

Although several studies have demonstrated hyperglycaemic effects of prostaglandins *in vivo* [32–34], studies *in vitro* have furnished apparently conflicting results. Whereas some studies have demonstrated glycogenolytic responses to prostaglandins in isolated hepatic systems [34–36], others have shown no effect [37–40] or inhibition [41] of hepatic glycogenolysis by E-series prostaglandins, leading to the suggestion [40] that the hyperglycaemia observed in response to prostaglandins *in vivo* may be secondary to changes in hormonal status [42–44]. It is clear from the present study that prostaglandin E_2 has glycogenolytic effects in the perfused rat liver. The haemodynamic changes observed in response to both prostaglandin E_2 and HAG, and the concomitant transient increase in the effluent lactate/pyruvate ratio, indicative of a more reduced state for the cytosolic NADH/NAD^+ couple [45], are consistent with transient hypoxia caused by changes in the hepatic microcirculation. Hypoxia has been demonstrated to stimulate hepatic glycogenolysis rapidly, possibly by increases in AMP and ADP leading to activation of glycogen phosphorylase [46,47]. Measurement of adenine nucleotides in freeze-clamped liver extracts did not show any significant differences between control and HAG-treated livers (results not shown); however, in view of the complex microcirculation of the liver [48–50], it is likely that rearrangements in hepatic flow may cause local hypoxia without causing measurable changes in global tissue adenine nucleotide content. An indirect mechanism for prostaglandin E_2 stimulation of glycogenolysis would explain the lack of effect of prostaglandin E_2 in isolated parenchymal cells [40].

The requirement for extracellular Ca^{2+} for full expression of the glycogenolytic response to HAG may be related to the demonstration that Ca^{2+} influx is an initial event in responses of Kupffer cells to phagocytic stimuli [31,51]; superoxide release by primary cultures of Kupffer cells in response to zymosan was inhibited in the absence of Ca^{2+} [51], and addition of the Ca^{2+} ionophore A23187 to Kupffer-cell cultures mimicked the effect of zymosan in stimulating prostaglandin release [31]. An alternative or additional role for Ca^{2+} may lie in a requirement of Ca^{2+} in the vasoconstrictive response. If the suggestion that prostaglandin E_2 may be involved in mediating the hepatic responses to HAG is correct, the inhibition of hepatic responses to prostaglandin E_2 by Ca^{2+} removal would be consistent with such a role for Ca^{2+}.

Although the prostaglandin concentration required to stimulate hepatic glycogenolysis is rather high, it should be noted that the liver removes from the circulation and metabolizes prostaglandins rapidly, the perfused liver clearing 90% of infused prostaglandin in a single pass [52], and thus there will be a steep prostaglandin concentration gradient across the liver. Additionally, treatment of isolated macrophages with immune complexes can elevate the prostaglandin concentration in the incubation medium to micromolar values [30], demonstrating that high local concentrations of prostaglandin in the perfused liver are feasible.

In summary, the present study suggests that HAG stimulates glycogenolysis in the perfused liver by an indirect mechanism involving mediator production and hepatic vasoconstriction. Moreover, a potential role for prostaglandin E_2 in mediating these hepatic responses to HAG infusion is suggested.

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