Glycogenolytic and haemodynamic responses to bovine serum albumin in isolated perfused livers from sensitized rats

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Infusion of BSA into isolated perfused livers of rats sensitized by intraperitoneal injection of BSA led to rapid increases in portal-vein pressure, glucose output and the lactate/pyruvate ratio in the effluent perfusate, with concomitant decreases in oxygen consumption and lactate + pyruvate efflux. The responses were attenuated at low (~ 7 μM) perfusate Ca^{2+}, but were restored on re-addition of normal Ca^{2+} concentration. Co-infusion of the cyclo-oxygenase inhibitor ibuprofen (50 μM) or of the platelet-activating factor receptor antagonist WEB 2170 (1.2 μM) inhibited haemodynamic responses to BSA (5 μg/ml) by 48% and 59% respectively. Responses to BSA were also attenuated by prior infusion of the β-adrenergic agonist isoprenaline. Glycogen phosphorylase *a* activity was increased by 26% in livers freeze-clamped 2 min after onset of BSA infusion; tissue prostaglandin E_2 content was increased at 2 min, but returned to control levels at 5 min. Homologous desensitization of hepatic responses to BSA was observed, but heterologous desensitization with heat-aggregated IgG did not take place. It is concluded that livers from rats sensitized to antigen respond directly to subsequent antigen administration by vasoconstriction and glycogenolysis, and that autacoid mediators are involved in these responses.

INTRODUCTION

The sensitization of rats by intraperitoneal injection of antigen leads to anaphylactic responses when the rats are challenged by subsequent intravenous injection of antigen. Anaphylactic responses of sensitized animals to antigen were recognized as early as 1910 [1], and the importance of the liver in anaphylactic responses was also established early; exclusion of the liver from the circulation of dogs sensitized to foreign serum abolished the immune response to re-injection of the serum [1,2]. Hepatic vasoconstriction during anaphylaxis has been demonstrated in a wide variety of species, including the rat [3]. Whereas in rats the intestine is considered to be the primary organ involved in mediation of anaphylaxis [4], a role for the liver is supported by the finding that, during anaphylaxis induced by soluble immune complexes, extravasation was abolished and lysosomal hydrolase release was greatly decreased by blocking the liver circulation [5]. Mediators implicated in systemic responses to antigen include histamine, thromboxane A_2 and platelet-activating factor (PAF).

Systemic anaphylaxis is accompanied by increases in plasma glucose [6,7], a response that may reflect several factors. First, systemic production of mediators may have direct effects on the liver, which responds haemodynamically and metabolically to a number of mediators, including PAF [8,9], thromboxane A_2 [10] and prostaglandins [11–13]. Secondly, increases in plasma catecholamines in response to anaphylaxis may act to increase hepatic glycogenolysis via α- and β-adrenergic mechanisms. A third possibility is that the liver may respond directly to circulating antigen; since the liver contains mast cells and reticuloendothelial cells, in addition to the parenchymal cells, it is possible that membrane-bound IgE may respond directly to antigen. The aim of this study was to investigate the direct effects of antigen on isolated perfused livers from rats sensitized to BSA.

MATERIALS AND METHODS

Materials

BSA (fatty-acid- and immunoglobulin-free) was obtained from Boehringer Mannheim (Indianapolis, IN, U.S.A.). Indomethacin, ibuprofen and human γ-globulin were obtained from Sigma (St. Louis, MO, U.S.A.). WEB 2170 was kindly provided by Boehringer Mannheim.

Sensitization of rats

Male Sprague–Dawley rats (75–100 g) were injected intraperitoneally with BSA [1 mg, dissolved in 0.25 ml of saline/Freund's complete adjuvant (1:2, v/v)]. Then 2 weeks later, a second injection was performed, identical with the first except that Freund's incomplete adjuvant was substituted for the complete adjuvant. Sham-immunized rats received saline/Freund's adjuvant only. Rats were fed on standard laboratory chow *ad libitum*.

Liver perfusion

Then, 1–2 weeks later, rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (15 mg). The livers were perfused *in situ* by using a non-recirculating constant-flow haemoglobin-free perfusion medium. The perfusion medium was Krebs–Henseleit bicarbonate buffer [14], pH 7.4, saturated with O_2/CO_2 (19:1), and maintained at 37 °C. The $CaCl_2$ concentration of the perfusion medium was 1.25 mM unless stated otherwise. A Clark-type oxygen electrode, placed immediately after the liver in the perfusion circuit, was used to monitor continuously hepatic oxygen consumption. Portal-vein pressure, which can be used as an index of intrahepatic pressure [15], was

Abbreviations used: PAF, platelet-activating factor; PGE_2, prostaglandin E_2; PGD_2, prostaglandin D_2; TXB_2, thromboxane B_2.

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measured with a Gould pressure transducer and an oscillographic recorder.

Effluent perfusate was collected for 30 s intervals for assay of effluent metabolites. Glucose, lactate and pyruvate were measured by standard enzymic methods as described previously [8]. Livers were perfused for 30 min before commencement of sample collection to wash out endogenous hormones and to stabilize glucose output.

For measurement of glycogen phosphorylase *a* activity, livers were rapidly clamped between aluminium tongs cooled in liquid nitrogen. The frozen livers were ground to a powder with a porcelain pestle and mortar cooled with solid CO_2, and phosphorylase *a* activity was determined essentially as described by Stalmans and Hers [16].

For measurement of prostaglandin E_2 (PGE_2), powdered freeze-clamped liver tissue was extracted essentially as described by Powell [17]. PGE_2 was measured by radioimmunoassay (Advanced Magnetics Inc., Cambridge, MA, U.S.A.). PAF was extracted and separated by t.l.c. and h.p.l.c. as described previously [18]. PAF activity was assayed with a [^{3}H]5-hydroxytryptamine bioassay in washed rabbit platelets [18].

Preparation of heat-aggregated IgG

This was done essentially as described previously [19,20], except that human γ-globulin was used as the starting material, and BSA was omitted from the elution buffer for the column chromatography.

Presentation of results

Results are presented as means $\pm$ S.E.M. Significance of differences between means of two groups was assessed by using Student's *t*-test (two-tailed), and that between more than two groups by using analysis of variance followed by Dunnett's modified *t*-test, accepting a P value of 0.05 or less as significant.

RESULTS

The effect of infusion of BSA (50 μg/ml) on hepatic haemodynamics and metabolism are shown in Figure 1. BSA caused a rapid increase in portal-vein pressure, with maximal increase in portal pressure at 1.6 $\pm$ 0.5 min after initiation of BSA infusion. The haemodynamic response was accompanied by a biphasic increase in glucose output; glucose output initially increased significantly within 60 s of initiation of BSA infusion, then returned towards baseline, followed by a second, more sustained, increase. Oxygen consumption showed a small initial increase, followed by a large transient decrease in oxygen uptake. Output of lactate + pyruvate initially showed a rapid transient decrease, followed by a slower increase. A rapid transient increase in the cytosolic redox state, as indicated by the effluent lactate/pyruvate ratio, was also observed. The peak pressure response coincided temporally with the transient decreases in oxygen consumption and lactate + pyruvate, and with the maximal increase in the effluent lactate/pyruvate ratio. The maximal decrease in glucose output between the two stimulatory phases tended to lag behind the maximal decrease in lactate + pyruvate. Sham-immunized rats did not respond to infusion of BSA with changes in metabolism or haemodynamics (results not shown).

The concentration-dependence of the responses of portal-vein pressure and glycogenolysis to BSA is shown in Figure 2. The haemodynamic responses to BSA increased with the concentration of infused BSA, becoming maximal at 0.5 μg/ml. At higher BSA concentrations, the peak pressure response was unchanged, but the time taken to reach maximal portal pressure was decreased. Glucose output was biphasic in almost all experiments; at lower concentrations, some livers showed no response, e.g. two of four at 0.4 μg/ml BSA. There was considerable variability from liver to liver in the relative sizes of the two phases of increased glucose output. Because of the variable shape of the glycogenolytic responses to BSA, the increase in glucose output above baseline was summed for the 10 min period of BSA infusion. In view of the variability in the shape of the response, comparisons of summed glucose outputs should be treated with caution. The glycogenolytic responses to BSA were maximal at intermediate BSA concentrations, but there was no significant difference between responses at intermediate and higher concentrations.

Removal of perfusate Ca^{2+} 5 min before infusion of BSA markedly attenuated the hepatic responses to BSA (Figure 3). The increase in portal-vein pressure was decreased from 6.7 $\pm$ 0.4 to 2.4 $\pm$ 1.2 mmHg ($P < 0.001$), and became more transient. The increase in glucose output in response to BSA (50 μg/ml) was decreased in the absence of perfusate Ca^{2+}, and was monophasic, in contrast with the biphasic response observed at normal perfusate Ca^{2+}, with a maximal increase in glucose output of

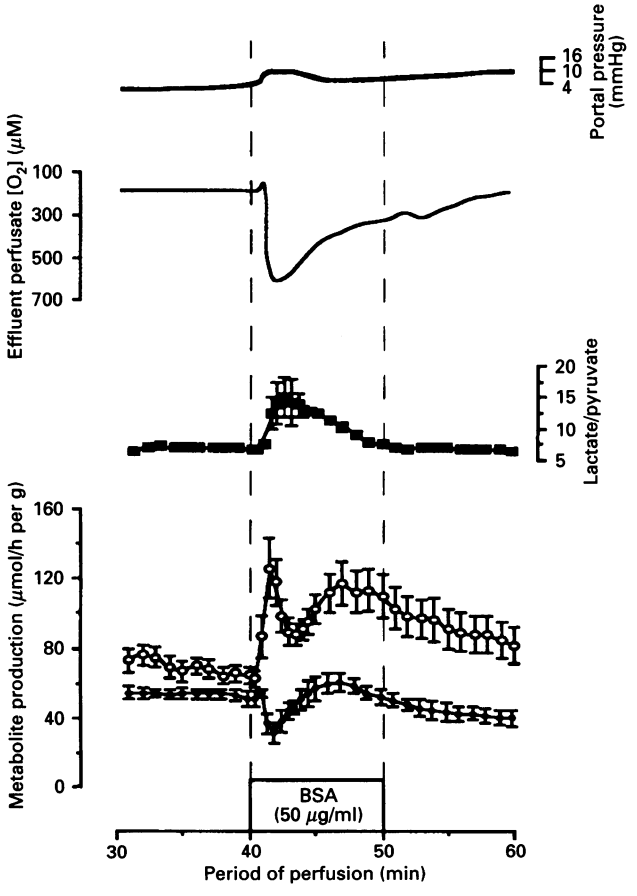

Figure 1 Effects of infusion of BSA (50 μg/ml) on glucose output, lactate + pyruvate output, effluent [lactate]/[pyruvate] ratio, oxygen consumption and portal-vein pressure in isolated perfused livers of sensitized rats

Glucose output (○) values are means $\pm$ S.E.M. for nine experiments, and lactate + pyruvate (●) and lactate/pyruvate (■) values are means $\pm$ S.E.M. for three experiments. Portal pressure and perfusate oxygen concentration traces are from representative experiments. Lactate + pyruvate output is expressed in glucose equivalents.

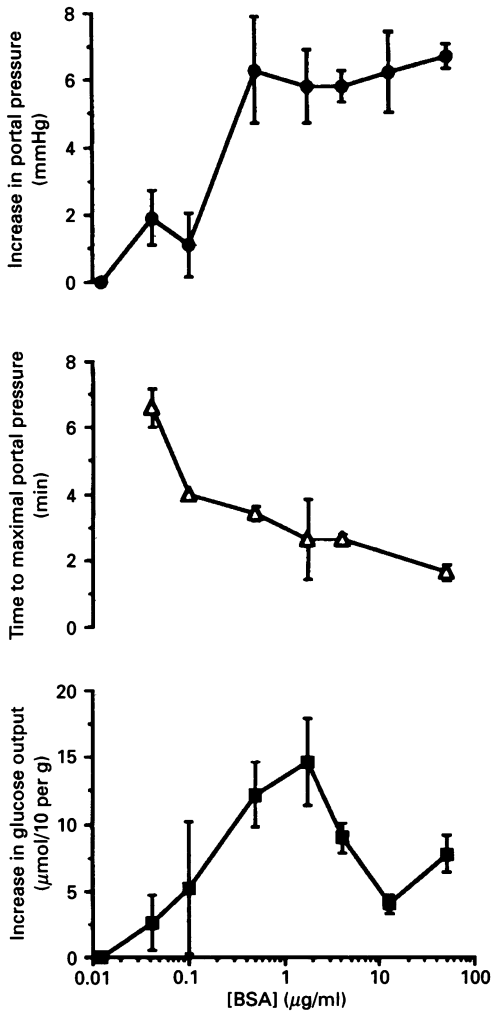

Figure 2 Dose–response curves for hepatic responses to BSA

Each liver was perfused with a single concentration of BSA, and the maximal increase in portal pressure, the time to maximal increase in portal pressure, and the summed increase in glucose output over 10 min of BSA infusion were determined. Results are means ± S.E.M. for 3–17 livers.

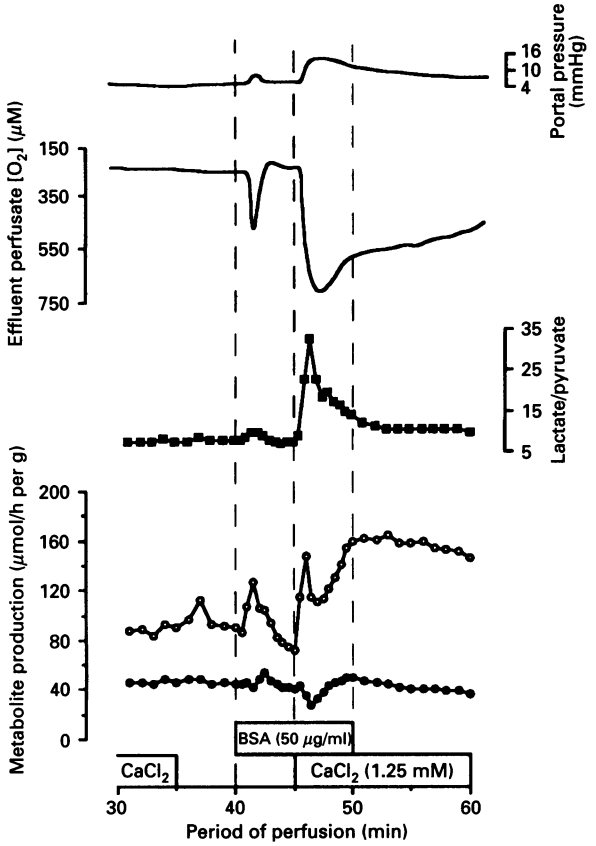

Figure 3 Effects of perfusate Ca^{2+} on responses to BSA (50 μg/ml) in livers of sensitized rats

Ca^{2+} was removed from the perfusate 5 min before infusion of BSA, and returned 5 min after initiation of BSA infusion. Results are the means of 4 experiments. Outputs of glucose (○) and lactate + pyruvate (●) are expressed in glucose equivalents. Error bars are omitted for clarity.

38 ± 15 μmol/h per g ($n = 7$) versus 86 ± 14 μmol/h per g ($n = 8$) at normal perfusate Ca^{2+} ($P < 0.05$). When sensitized livers were perfused with BSA for 15 min in the absence of perfusate Ca^{2+}, glucose output remained monophasic, indicating that the absence of a biphasic response in Figure 3 did not merely represent a delay in the response (results not shown). Similarly, lactate + pyruvate output showed only a small transient increase of 25 ± 12 %, which was not significantly elevated above baseline, in contrast with the large transient decrease found in the presence of Ca^{2+}. The increase in the lactate/pyruvate ratio was decreased to 46 ± 16 %, compared with 150 ± 41 % in the presence of Ca^{2+} ($P < 0.05$). When Ca^{2+} was re-introduced, a second, more pronounced, response was observed. The increase in portal-vein pressure in response to re-introduction of Ca^{2+} was 9.6 ± 1.1 mmHg, significantly greater than that in the absence of Ca^{2+} ($P < 0.001$). Glucose uptake again showed a biphasic increase, with an initial rapid increase which was significant within 30 s of Ca^{2+} infusion, and a maximal increase of 102 ± 17 μmol/h per g ($n = 4$; $P < 0.025$ versus initial response in absence of Ca^{2+}). The lactate/pyruvate ratio also showed a large increase, from 7.4 ± 0.7 to 32.7 ± 13.4 ($P < 0.01$), and the biphasic lactate + pyruvate output response observed in Figure 1 was restored. The large standard error for the peak lactate/pyruvate reflects the very low values obtained for effluent pyruvate, making accurate determination of the ratio problematical. Re-introduction of Ca^{2+} in control livers perfused at low Ca^{2+} concentration for short periods has been shown to cause only minor effects on portal pressure and glucose output [10].

Cyclo-oxygenase products have been demonstrated to cause vasoconstriction and glycogenolysis in perfused livers. To determine whether prostanoid mediators play a role in the responses of sensitized rats to antigen, cyclo-oxygenase inhibitors were employed. The effects of ibuprofen (50 μM), which abolished hepatic production of cyclo-oxygenase-derived mediators in response to PAF [21], on responses of sensitized livers to BSA (5 μg/ml) are shown in Figure 4. The summed increase in glucose output during the 10 min of BSA infusion was decreased by 46 % (Table 1). Ibuprofen also inhibited the hepatic portal-pressure responses to BSA, by 59 %.

Infusion of indomethacin at a concentration (5 μM) which attenuated hepatic responses to heat-aggregated IgG by 90 % without significant effects on responses to PAF [20] led to a similar inhibition of the haemodynamic response to BSA (Table 1). In this case, the increase in glucose output was not significantly decreased from control experiments.

WEB 2170, a PAF-receptor antagonist, afforded complete

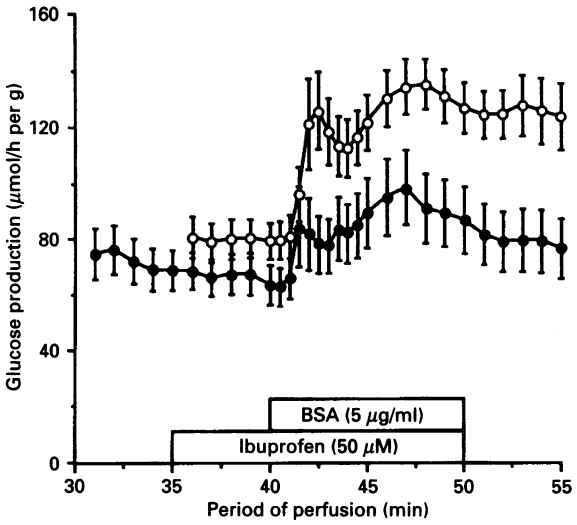

Figure 4 Inhibition of hepatic responses to BSA (5 μg/ml) by ibuprofen (50 μM)

Results obtained during co-infusion of ibuprofen are shown by (●), and control experiments in the absence of ibuprofen by (○). Results are means ± S.E.M. for 12 (●) and 13 (○) livers respectively.

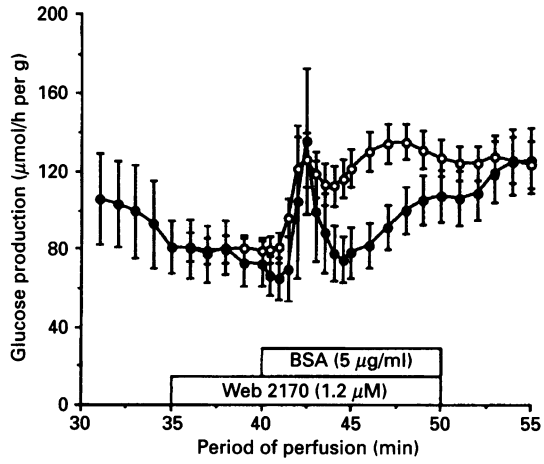

Figure 5 Inhibition of responses to BSA (5 μg/ml) by the PAF antagonist WEB 2170 (1.2 μM) in perfused livers of sensitized rats

Results obtained during co-infusion of WEB 2170 are shown by (●), and control experiments in the absence of WEB 2170 are shown by (○). Results are means ± S.E.M. for 7 and 13 livers respectively.

Table 1 Effect of inhibitors on haemodynamic and glycogenolytic responses to BSA (5 μg/ml) in livers of sensitized rats

Infusion of inhibitors was initiated 5 min before the start of BSA infusion and continued throughout BSA infusion, except for isoprenaline, which was infused for 5 min and then washed out for 10 min before BSA infusion. The increase in glucose output represents the summed increase relative to the pre-BSA value during 10 min of BSA infusion. Numbers in parentheses refer to the number of livers: $^{*}P < 0.01$, $^{**}P < 0.05$ versus no addition.

Inhibitor	Increase in portal pressure (mmHg)	Increase in glucose output (μmol/10 min per g)
None	5.8 ± 0.5 (17)	7.5 ± 0.9 (12)
Ibuprofen (50 μM)	2.4 ± 0.5 (12)*	4.0 ± 0.9 (12)**
Indomethacin (5 μM)	3.0 ± 1.2 (4)**	6.9 ± 0.4 (4)
WEB 2170 (1.2 μM)	3.0 ± 0.6 (5)**	4.4 ± 0.9 (5)
Ibuprofen + WEB 2170	3.6 ± 0.7 (4)**	3.5 ± 1.6 (4)**
Isoprenaline (10 μM)	3.0 ± 0.7 (5)**	5.0 ± 0.6 (5)

inhibition of haemodynamic and glycogenolytic responses to PAF (5 nM) at a concentration of 1.2 μM WEB 2170 (results not shown). The results of co-infusion of WEB 2170 on hepatic responses to BSA (5 μg/ml) in sensitized rats are shown in Figure 5. The overall increase in glucose output during the 10 min of BSA infusion was not significantly decreased by infusion of WEB 2170 (Table 1), although glucose output was significantly decreased relative to control measurements between 5 and 8 min of BSA infusion. Infusion of the inhibitor 5 min before administration of antigen decreased the portal-pressure response to BSA by 48% (Table 1). Co-infusion of ibuprofen and WEB 2170 did not have any further inhibitory effect on hepatic responses to BSA than separate infusion of the agents (Table 1).

To probe more directly the role of autacoid mediators in hepatic responses to BSA, livers from sensitized rats were freeze-clamped 2 min after infusion of BSA (50 μg/ml) and compared with livers perfused without BSA (Table 2). The tissue content of PGE_2 was significantly higher in BSA-perfused livers relative to control livers. PAF levels were also higher in BSA-perfused livers, but the difference was not significant. These alterations in tissue autacoid content were accompanied by a significant increase in glycogen phosphorylase *a* activity, which was elevated by 26% in the livers perfused with BSA. In a second series of rats, BSA-perfused livers freeze-clamped 5 min after onset of BSA infusion, PGE_2 content had returned to control levels.

Stimulation of β-adrenergic receptors by prior infusion of isoprenaline has been shown to inhibit subsequent responses in perfused rat liver [22,23]. The results of infusion of isoprenaline before BSA in sensitized rats are shown in Table 1. The portal-pressure response to BSA was decreased when BSA was infused 5 min after a 5 min infusion of isoprenaline. The glucose output also tended to be lower, but was not decreased significantly.

Homologous desensitization of increases in portal pressure and glycogenolysis has also been demonstrated for a number of bioactive substances. Figure 6 demonstrates that the glycogenolytic response to a second infusion of BSA in livers from sensitized rats was markedly attenuated. Owing to the short infusions of BSA used, most of the response to the first BSA infusion took place in the 5 min period after BSA infusion was terminated. Hepatic output of glucose was not significantly lower during the second 5 min BSA infusion (0.4 ± 0.6 μmol/5 min per g, compared with 1.3 ± 0.3 μmol/5 min per g for the first infusion); however, glucose output over the 5 min after the first BSA infusions was 5.1 ± 1.5 μmol/5 min per g, whereas glucose output declined towards baseline after termination of the second infusion. The haemodynamic response to BSA (5 μg/ml) was decreased from 4.1 ± 0.8 to 1.2 ± 0.3 mmHg ($n = 4$; $P < 0.02$) (results not shown). The increase in the lactate/pyruvate ratio was also attenuated, increasing by 28 ± 4% and 10 ± 9% for the first and second infusions respectively (results not shown).

Heat-aggregated IgG has been shown to increase hepatic glucose production and portal-vein pressure in perfused livers [11,20]. Since hepatic responses to heat-aggregated IgG undergo almost complete homologous desensitization, heterologous desensitization between the two agonists could occur if they shared a common path of action. To test this possibility, sequential

Table 2 Glycogen phosphorylase *a* activity and autacoid content in freeze-clamped perfused livers

Control livers were freeze-clamped after 30 min of perfusion. BSA-perfused livers were perfused for 30 min, and then with BSA (50 μg/ml) for 2 or 5 min, and freeze-clamped. Results are means $\pm$ S.E.M. for 7 livers (ND, not determined): $^{*}P < 0.02$, $^{**}P < 0.05$ versus control.

	Control	BSA 2 min	BSA 5 min
Phosphorylase *a* activity (μmol/min per g of protein)	96 $\pm$ 17	121 $\pm$ 18*	ND
PGE_2 (ng/g wet wt.)	5.2 $\pm$ 3.2	14.5 $\pm$ 7.5**	5.1 $\pm$ 0.7
PAF (ng/g wet wt.)	0.27 $\pm$ 0.18	0.57 $\pm$ 0.43	ND

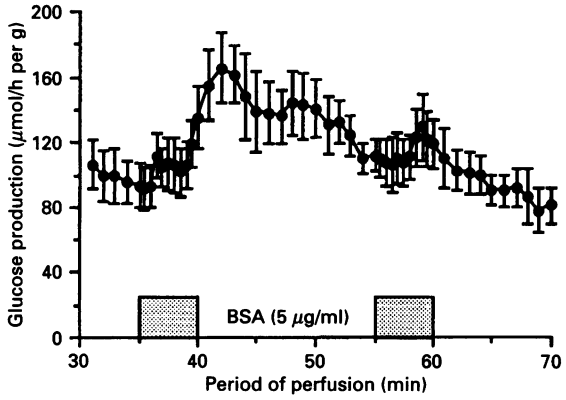

Figure 6 Homologous desensitization of the glycogenolytic response to BSA in perfused livers of sensitized rats

Results are means $\pm$ S.E.M. for 4 livers.

infusions of BSA and heat-aggregated IgG were performed. After infusion of BSA (5 μg/ml) for 5 min, livers were perfused for a further 10 min before infusion of heat-aggregated IgG (5 μg/ml) for 2 min. Results obtained in these livers were compared with those obtained by infusing heat-aggregated IgG first for 2 min, and then infusing BSA 8 min later. Portal pressure increased by 5.4 $\pm$ 1.4 mmHg in response to BSA (5 μg/ml) infused before heat-aggregated IgG ($n = 4$), and by 5.8 $\pm$ 0.9 mmHg when BSA was infused after heat-aggregated IgG ($n = 4$). For heat-aggregated IgG, the responses were 1.1 $\pm$ 0.1 and 3.3 $\pm$ 1.5 mmHg before and after BSA respectively. Glucose output was also unaffected; for BSA, glucose output, summed over 10 min from the onset of BSA infusion, was 7.1 $\pm$ 2.9 μmol/10 min per g when BSA was infused first, compared with 6.7 $\pm$ 2.2 μmol/10 min per g when BSA was infused after heat-aggregated IgG. For heat-aggregated IgG, values obtained for glucose output were 2.5 $\pm$ 1.4 and 2.8 $\pm$ 0.9 μmol/10 min per g for infusion before and after BSA respectively.

DISCUSSION

Recent studies from a number of groups have demonstrated that a wide variety of stimuli can lead to metabolic and haemodynamic changes in the liver [24]. Stimulation of the reticuloendothelial system with materials such as heat-aggregated IgG, zymosan and bacterial endotoxin leads to metabolic changes in the parenchymal cells which are believed to be the result of cell–cell communication with sinusoidal cells rather than a primary effect on the parenchymal cell itself. The present study has demonstrated that infusion of antigen into the isolated livers of rats sensitized to BSA causes hepatic vasoconstriction and increased glycogenolysis, in agreement with data obtained in rats sensitized to ovalbumin [7]. It is thus likely that increases in plasma glucose concentration occurring during antigen-induced anaphylaxis [6,7] represent, at least in part, a direct response of hepatic cells to antigen, rather than systemic responses via catecholamines and other mediators.

The hepatic responses to antigen are likely to be mediated by IgE, which is tightly bound to cell-surface Fc_ϵ receptors [25]. IgG, which can also mediate anaphylactic responses, binds with low affinity, and is readily washed from the cell surface [26]. The cell type(s) involved in the hepatic responses to antigen are as yet undefined. Since parenchymal cells do not carry Fc receptors, the glycogenolytic response is likely to be an indirect response to stimulation of another cell type. Rat liver has been shown to contain mast cells [27,28]. The number of mast cells is small in the liver itself, with a greater number associated with the liver capsule [27]. It is noteworthy that proliferation of connective tissue in the abdomen is observed in the immunized rats, much of it forming attachments to the liver, leading to the possibility that increased numbers of mast cells may be associated with the liver in these rats. Alternative candidates are the hepatic sinusoidal cells, since both Kupffer and endothelial cells have been shown to possess Fc receptors [29].

The mechanism by which the proposed binding of antigen to non-parenchymal cells stimulates glycogenolysis in parenchymal cells is also incompletely understood at this time. WEB 2170, a PAF antagonist, decreased hepatic responses to antigen by approx. 50%. A similar attenuation of increased portal pressure in response to antigen was found by using the cyclo-oxygenase inhibitors ibuprofen and indomethacin, although a significant decrease in glucose output was found only with ibuprofen; this difference may be due to the greater variability in glucose-output responses compared with portal-pressure increases. These results are consistent with a model in which binding of antigen to non-parenchymal cells leads to production of mediators, including PAF and cyclo-oxygenase products, which then act to increase glycogenolysis in parenchymal cells. Significant increases in PGE_2 were found 2 min after infusion of BSA into perfused livers, consistent with a role for these mediators. PAF content also tended to be higher, although the difference did not reach statistical significance. The finding that eicosanoid production was transient, with PGE_2 levels no longer increased significantly by 5 min of BSA infusion, suggests that other mediators are responsible for the second phase of glucose output; the shape of the response is very similar to those found with PAF [8,9], and the PAF-receptor antagonist WEB 2170 decreased glucose output significantly during the second phase, consistent with a role for PAF. However, since co-infusion of ibuprofen and WEB 2170 was unable to suppress either stimulation of glucose production or portal-pressure increases completely, the involvement of additional mediators is likely, leading to a complex system of interactions between parenchymal and non-parenchymal cells. Alternatively, interactions between cells may take place which are not fully accessible to infused inhibitors.

Hepatic formation of cyclo-oxygenase products occurs in response to various stimuli, including PAF, zymosan, bacterial lipopolysaccharide and heat-aggregated IgG [12,23,30–33], with the Kupffer cells representing the major site of formation [24]. Direct stimulatory effects of prostaglandins on hepatocyte glycogenolysis have been observed, although requiring high concentrations of prostaglandins [12]. The thromboxane A_2 analogue

U46619 was without effect on glycogenolysis in isolated hepatocytes [10]. Whereas prostanoid mediators have been proposed to be responsible for the metabolic effects of PAF [30,32,33], ibuprofen, under conditions where production of prostaglandin D_2 and thromboxane B_2 was inhibited almost completely, was without significant effect on haemodynamic or glycogenolytic responses to PAF [21]. Eicosanoids have also been implicated in transiently increasing the catalytic efficiency of phosphorylase *a* during stimulation of glycogenolysis by adenosine in livers where phosphorylase was fully activated to the *a*-form [34].

Haemodynamic responses to antigen in ovalbumin-sensitized rats were greatly attenuated by co-infusion of the vasodilator NO [7]. A concomitant inhibition of the increases in glucose output and decreases in oxygen consumption in response to these mediators was also found, supporting a causal link between the haemodynamic and metabolic effects. PAF does not increase glycogenolysis in isolated hepatocytes [35], but causes hepatic vasoconstriction [9], and decreases both the estimated intracellular volume of distribution of small freely permeable substances (V_1) and the ratio of intracellular to extracellular space, Q' [36]. These haemodynamic changes have been proposed to lead to local hypoxia via inhomogeneous redistribution of perfusate flow, with resultant stimulation of glycogenolysis [9,36].

Although the lactate/pyruvate ratio was increased in response to BSA in sensitized rats, the hepatic output of lactate + pyruvate was initially decreased. This differs from other mediators which increase portal-vein pressure and glycogenolysis, such as PAF, heat-aggregated IgG and the thromboxane A_2 analogue U44619, which lead to increases in both lactate/pyruvate and lactate + pyruvate output. An increase in anaerobic glycolysis would be expected during local hypoxia rather than a decrease in lactate + pyruvate output, unless glycolytic flux was also transiently inhibited during antigen infusion by some other mechanism. The decreased flux of glucose through the glycolytic pathway will make more glucose available to leave the cell, and thus contribute to the initial peak of glucose production. However, the amount of glucose diverted from glycolysis is insufficient to explain all the increase in glucose output.

Hepatic responses to BSA were attenuated when Ca^{2+} was removed from the perfusate 5 min before administration of antigen. Subsequent re-introduction of Ca^{2+} to the perfusate led to a very rapid and pronounced vasoconstrictive response. Similar results have been obtained with a number of agents that cause vasoconstriction and glycogenolysis in perfused liver, including PAF, heat-aggregated IgG and the thromboxane analogue U46619 [9–11]. Since the period of low-Ca^{2+} perfusion was brief, depletion of intracellular Ca^{2+} stores is unlikely to explain the decreased responses to BSA. Kupffer cells require extracellular Ca^{2+} for normal responses to phagocytic stimuli, with Ca^{2+} influx representing an initial event in response to the stimulus [37,38]. Extracellular Ca^{2+} could also be required for vasoconstriction in response to BSA and other agents.

No heterologous desensitization was observed between BSA and heat-aggregated IgG. Soluble IgG complexes are likely to interact with Kupffer cells via Fc_γ receptors, whereas, in perfused livers of sensitized rats, antigen interaction via IgE bound to Fc_ϵ receptors would be more likely. In mast cells, desensitization to antigen is antigen-specific, and mast cells prepared from animals sensitized to more than one antigen are still able to respond to a second antigen after becoming desensitized to one antigen. Thus it is likely that desensitization in the liver may be a receptor-specific event.

The haemodynamic and metabolic responses to BSA in sensitized rats were attenuated by prior infusion of isoprenaline. A similar attenuation of responses both to PAF and to heat-aggregated IgG by the β-adrenergic agonist has been demonstrated [22,23]. The mechanism for this heterologous desensitization is unclear, but appears to be cyclic-AMP-independent [22,23]. Attenuation of responses to heat-aggregated IgG was accompanied by attenuated production of autacoid mediators, PAF, prostaglandins and thromboxane B_2 [23].

In summary, infusion of antigen into perfused livers from sensitized rats led to Ca^{2+}-dependent increases in portal pressure and stimulation of glycogenolysis. Studies with inhibitors suggested the involvement of autacoid mediators, including cyclo-oxygenase products and PAF, and measurement of PGE_2 in freeze-clamped tissue from BSA-perfused livers demonstrated a significant transient increase. Although a transient decrease in oxygen consumption and increase in the effluent lactate/pyruvate ratio were consistent with local hypoxia as a possible mechanism for glycogenolytic stimulation, a transient decrease in lactate + pyruvate output appears inconsistent with this mechanism. We conclude that increases in plasma glucose concentration occurring during antigen-induced anaphylaxis are likely to represent, at least in part, a direct response of hepatic cells to antigen.

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