Stimulation by anaphylatoxin C5a of glycogen phosphorylase in rat hepatocytes via prostanoid release from hepatic stellate cells but not sinusoidal endothelial cells

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Abstract In the perfused rat liver, the anaphylatoxin C5a has been shown to enhance glucose output. Since hepatocytes lack C5a receptor mRNA, the metabolic effect of C5a must be elicited indirectly via C5a receptor expressing non-parenchymal liver cells. Kupffer cells were found to be able to mediate the C5a action via release of prostanoids. However, elimination of the Kupffer cells by pretreatment of the animals with gadolinium chloride reduced the metabolic effect of C5a to only about 40%. Therefore, it was investigated whether not only Kupffer cells but in addition also hepatic stellate cells or sinusoidal endothelial cells released prostanoids in response to C5a. In isolated hepatic stellate cells but not in sinusoidal endothelial cells, recombinant rat C5a induced a time- and dose-dependent release of thromboxane B_2 and prostaglandins D_2, E_2 and $F_{2\alpha}$. The rate of prostanoid release was maximal within the first two minutes and then declined again. C5a-induced prostanoid release from hepatic stellate cells was smaller than that from Kupffer cells and it differed in the prostanoid ratios ($PGE_2/PGD_2/PGF_{2\alpha}/TXB_2$ = 1:1:0.1:0.6 and 1:4:1:3, respectively). RrC5a activated hepatocellular glycogen phosphorylase via prostanoid release in cocultures of hepatocytes with hepatic stellate cells but not with sinusoidal endothelial cells. Thus, the part of the rrC5a-induced glucose output in the perfused rat liver, which was not abrogated by elimination of the Kupffer cells with gadolinium chloride, most likely was mediated by prostanoids released from hepatic stellate cells.

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1. Introduction

The anaphylatoxins C3a and C5a are generated during complement activation via the classical or the alternative pathway. The peptides are derived from the N-terminal 74–77 amino acid residues of the α-chains of the complement proteins C3 and C5 [1–4]. Various actions of anaphylatoxins on myeloid blood cells have long been known such as the release of histamine from mast cells or the chemotaxis and activation of neutrophils. The latter is characterized by invasion of the cells into the inflamed tissue and the local release of reactive oxygen species, cytokines and eicosanoids. Moreover, anaphylatoxins induce an enhanced permeability of blood vessels and the contraction of smooth muscle cells [5–7].

In the perfused rat liver, recombinant rat C5a (rrC5a) enhanced glucose release and reduced flow [8,9]. The enhanced glucose release cannot be explained by a direct action of rrC5a on the hepatocytes (HC), since these cells do not express C5a receptor (C5aR) mRNA [10]. Thus, this effect must be mediated indirectly by action of rrC5a on the C5aR mRNA expressing non-parenchymal liver cells, i.e. Kupffer cells (KC), hepatic stellate cells (HSC) or sinusoidal endothelial cells (SEC) [10], which then release a mediator capable of activating glycogen phosphorylase in hepatocytes. Two observations were in line with this assumption: (i) the metabolic and hemodynamic effects of rrC5a in the perfused rat liver were inhibited by the prostanoid synthesis inhibitor indomethacin [9]; (ii) rrC5a induced a prostanoid-dependent increase in glycogen phosphorylase activity in cocultures of Kupffer cells with hepatocytes but not in hepatocytes alone [11]. These results indicated that the metabolic and hemodynamic effects of rrC5a were mainly mediated by prostanoids released from Kupffer cells. Nevertheless, in perfused rat livers in which the Kupffer cells had been eliminated by pretreatment of the animals with gadolinium chloride, the rrC5a-induced metabolic and hemodynamic changes were inhibited to only about 40% [8]. Thus, most likely another cell type besides Kupffer cells was involved in rrC5a-induced glucose output and flow reduction.

Besides the Kupffer cells, also hepatic stellate cells strongly and sinusoidal endothelial cells weakly expressed C5aR mRNA [10]. Hepatic stellate cells and sinusoidal endothelial cells both have been shown to release prostaglandins after stimulation with various stimuli such as the neurotransmitters noradrenaline and ATP [12] or LPS [13] and ATP [14], respectively.

It was the aim of this study to investigate whether besides Kupffer cells also hepatic stellate cells or sinusoidal endothelial cells are involved in the anaphylatoxin-induced glucose output in the perfused rat liver by releasing prostanoids. It was shown that rrC5a induced the release of prostaglandins and thromboxane from hepatic stellate cells but not from sinusoidal endothelial cells and that the amounts of prostaglandins released were sufficient for the activation of glycogen phosphorylase in cocultures of hepatic stellate cells with hepatocytes.

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Abbreviations: rrC5a, recombinant rat C5a; C5aR, C5a receptor; PG, prostaglandins; TX, thromboxane; NPC, non-parenchymal liver cells; HC, hepatocytes; KC, Kupffer cells; HSC, hepatic stellate cells; SEC, sinusoidal endothelial cells; GPH, glycogen phosphorylase; HBSS, Hanks' balanced salt solution

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2. Materials and methods

2.1. Animals

Male Wistar rats (Winkelmann, Borchen, Germany, 150–200 g for the perfusion experiments, 350–450 g for the isolation of KC, HSC and SEC, 200–250 g for HC) were kept on a 12-h day/night rhythm with free access to water and a standard rat diet (Ssniff, Soest) for at least 2 weeks before the experiments. Treatment of the animals followed the German Law on the Protection of Animals and was performed with permission of the state animal welfare committee.

2.2. Chemicals

All chemicals were of analytical grade and from commercial sources. Radio-immunoassays for PGD_2 and $PGF_{2\alpha}$ were from Amersham (Braunschweig, Germany), enzyme immunoassays for TXB_2 (the stable biologically inactive metabolite of TXA_2) and PGE_2 were from R&D Systems (Wiesbaden-Nordenstadt, Germany). Pronase was obtained from Merck (Darmstadt, Germany), collagenase H and DNase from Boehringer Mannheim (Mannheim, Germany), Nycodenz from Life Technologies (Eggenstein, Germany) and Percoll from Pharmacia (Freiburg, Germany). RPMI 1640 was purchased from Biochrom (Berlin, Germany) and M199 from AppliChem (Darmstadt, Germany). Newborn calf serum (NCS) was from PAA Laboratories (Cölbe, Germany), insulin and noradrenaline from Serva (Heidelberg, Germany). Bis-benzimide, indomethacin and gadolinium chloride were purchased from Sigma-Aldrich (Deisenhofen, Germany) and tissue culture dishes were obtained from Nunc (Wiesbaden, Germany).

2.3. Preparation of rrC5a

RrC5a was prepared by synthesis of a cDNA from rat liver RNA and a subsequent PCR using degenerate 5′ and 3′ primers that were designed according to sequence data published in the Swiss-Prot protein sequence data bank (accession no. P08650, ID: C05A_RAT) [15] as described previously [4,11]. RrC5a contained in addition to the original sequence of amino acids 1–77 the N-terminal sequence MRGJHHHHHHGI used for its purification from bacterial lysates by Ni^{2+}-chelate chromatography and was depleted of endotoxins by affinity chromatography on polymyxin B agarose.

2.4. Cell preparation

Hepatocytes were prepared by collagenase perfusion of rat liver. Viable hepatocytes were obtained by removing detritus in two subsequent washing steps with centrifugation at $50\times g$ and a final centrifugation through 58% Percoll. Kupffer cells and sinusoidal endothelial cells were isolated by combined pronase/collagenase perfusion and purified by Nycodenz density gradient centrifugation and subsequent counterflow elutriation using a Beckman JE-6 elutriation rotor in a J-21 Beckman centrifuge [16,17]. HSC were obtained by enzymatic digestion of the liver with pronase and collagenase essentially as described in [18] with some minor modifications (N. Kawada, personal communication). The resulting cell suspension, subsequently handled at 4°C, was filtered through nylon gauze (mesh diameter 60 μm) and centrifuged for 10 min at $450\times g$. After 2 washing steps, cells were resuspended in Hanks' balanced salt solution (HBSS), mixed with Nycodenz to a final concentration of 8.13% and overlaid with HBSS. After centrifugation for 20 min at $1400\times g$ HSC were obtained from the interphase. Purity of HSC as identified on the basis of their typical light microscopic appearance and their vitamin A-specific autofluorescence was about 95% after 24 h and >98% after 48 h.

2.5. Non-parenchymal liver cell culture

Kupffer cells were plated at 4×10^6 cells/plate, sinusoidal endothelial cells at 8×10^6 cells/plate and hepatic stellate cells at 2.5×10^6 cells/plate on 3.5-cm diameter tissue culture plates in RPMI 1640 (KC and HSC) or M199 (SEC), each supplemented with 30% NCS and 1% penicillin/streptomycin. For SEC cultures and cocultures (see below) plates were coated with rat tail collagen prepared as in [19]. Cells were cultured for 48 h (SEC and HSC) or 72 h (KC) before the experiments unless indicated otherwise with medium changes every 24 h. For elimination of possibly remaining KC in HSC, cultures were treated with 10 μM gadolinium chloride for 24 h before the start of the experiments where indicated.

2.6. Hepatocyte culture

Hepatocytes were plated at 5×10^5 cells/plate on 3.5-cm diameter collagenated (see Section 2.5) tissue culture plates in M199 supplemented with 10% NCS, 0.5 nM insulin and 1% penicillin/streptomycin for 24 h before the experiment with one medium change after 4 h.

2.7. Cocultures

For cocultures with hepatocytes, Kupffer cells were seeded at 3×10^6 cells/plate in RPMI 1640, sinusoidal endothelial cells at 6×10^6 cells/plate in M199 and hepatic stellate cells at 2×10^6 cells/plate in RPMI 1640 on 3.5-cm diameter collagenated (see Section 2.5) tissue culture plates. All media were supplemeted with 30% NCS and 1% penicillin/streptomycin. Medium was changed after 24 h. After 24 h (HSC and SEC) or 48 h (KC) freshly prepared hepatocytes (5×10^5 cells/plate) were plated on top of the HSC, SEC or KC. Cocultures were incubated in M199 supplemented with 10% NCS, 0.5 nM insulin and 1% penicillin/streptomycin for another 24 h with one medium change after 4 h.

2.8. Detection of C5aR mRNA in hepatic stellate cells by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA from freshly isolated and cultured HSC was isolated by an RNeasy Kit provided by Qiagen (Hilden, Germany). Six μg total RNA were preincubated for 10 min at 68°C with 500 ng oligo-$d(T)_{12-18}$ and transcribed with reverse transcriptase (Superscript II, Gibco, Eggenstein, Germany) at 45°C into cDNA. cDNAs thus generated were amplified in a 50-μl reaction mix with foreward and reverse oligonucleotide primers rC5aR-F and rC5aR-R (positions 267–287 and 777–756 according to the sequence published under GenBank accession no. Y09613) or r-beta-actin-F and r-beta-actin-R (positions 1251–1271 and 2570–2553, GenBank accession no. J00691) in the presence or absence of an internal standard for quantification as described in detail in [10].

2.9. Determination of prostanoid formation in hepatic stellate cells and sinusoidal endothelial cells

At the indicated times, cells were washed three times with HBSS, supplemented with 20 mM HEPES, pH 7.4, and preincubated at 37°C for 5 min in HEPES-buffered HBSS and for another 5 min in HEPES-buffered HBSS with or without 20 μM indomethacin in 0.1% DMSO. After 10 min preincubation rrC5a was added to the final concentration indicated, normally 100 nM (= 1 μg/ml). Immediately after addition and at the time points indicated, samples of the medium were taken from the supernatant and shock frozen in liquid nitrogen for the later determination of prostanoid concentrations. Prostanoids were determined by radio- or enzyme immunoassays without further purification according to the instructions of the manufacturers. At the end of the experiment, cells were scraped off the dish for DNA determination in a fluorescence assay based on the intercalation of bis-benzimide into DNA [20].

2.10. Determination of glycogen phosphorylase activity in mono- and cocultures

Mono- and cocultures were washed three times with HEPES-buffered saline (20 mM HEPES, 120 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 5 mM glucose, 2 mM lactate and 0.2 mM pyruvate, pH 7.4). Cells were incubated for 5 min at 37°C in the same buffer and for another 5 min in the same buffer with or without 20 μM indomethacin in 0.1% DMSO. The medium was then discarded and replaced with HEPES-buffered saline containing rrC5a (final concentration 100 nM = 1 μg/ml) or noradrenaline (1 μM). After 2 min of incubation the buffer was removed and the plates were frozen in liquid nitrogen. Cells were scraped off the dishes and glycogen phosphorylase activity was determined by a standard assay [21].

3. Results and discussion

3.1. C5a receptor mRNA expression in cultured hepatic stellate cells and sinusoidal endothelial cells

The possible C5a-elicited release of prostanoids from hepatic stellate cells or sinusoidal endothelial cells cannot be investigated in freshly isolated cells, since during the isolation of

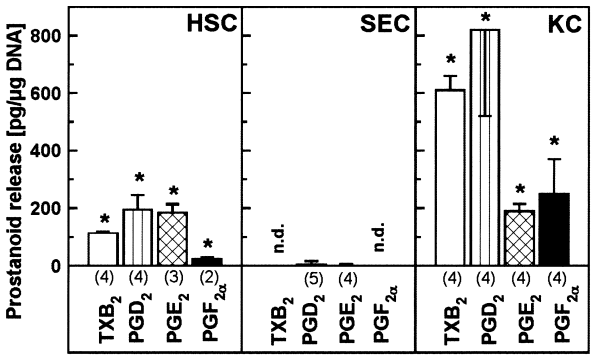

Fig. 1. Increase by rrC5a of TXB_2, PGD_2, PGE_2 and $PGF_{2\alpha}$ release from cultured hepatic stellate cells and Kupffer cells but not sinusoidal endothelial cells. Hepatic stellate cells, sinusoidal endothelial cells and Kupffer cells were isolated and cultured for 48 h (HSC and SEC) or 72 h (KC) as described in Section 2. After three washes in HEPES-buffered HBSS, cells were preincubated for 10 min in the same buffer and then stimulated with rrC5a at a final concentration of 100 nM (= 1 µg/ml). Supernatants were removed after 10 min and increases in prostanoid concentrations over basal levels at 0 min were determined by radio- or enzyme immunoassays. Values are means ± S.E.M. of the number of experiments given in parentheses. $*P \leq 0.01$, significant differences compared with controls (Student's *t*-test for unpaired samples). n.d. = not determined. Values for KC are taken from [11] and shown for easier comparison.

non-parenchymal cells by enzymatic digestion of the liver tissue ectocellular receptors can be damaged to varying degrees. The problem can be studied in cultured cells, because the receptors are normally re-expressed during culture. Since so far C5aR mRNA expression has only been studied in freshly isolated cells [10], it was first investigated whether hepatic stellate cells or sinusoidal endothelial cells modulated C5aR mRNA expression during the culture period of up to 72 h. This control investigation was also mandatory, because especially hepatic stellate cells might change their state of differentiation [22] and their state of receptor expression during culture. With quantitative RT-PCR it was demonstrated that the expression of C5aR mRNA did not vary between freshly isolated hepatic stellate cells and cells cultured for 24 h, 48 h and 72 h (not shown). Also the low level C5aR mRNA expression in sinusoidal endothelial cells cultured for 48 h did not differ from that found in freshly isolated cells (not shown). Moreover, Kupffer cells, which in contrast to hepatic stellate cells and sinusoidal endothelial cells can easily be detached from the tissue culture plates, have been shown by flow cytometry to fully re-express C5aR protein after 48 h of culture (unpublished observations). Similar kinetics of C5aR protein re-expression can be expected for hepatic stellate cells and sinusoidal endothelial cells.

3.2. Increase by rrC5a of prostanoid release from cultured hepatic stellate cells but not sinusoidal endothelial cells

RrC5a (100 nM) induced the release of TXB_2 (the stable product of the actually released but unstable TXA_2), PGD_2, PGE_2 and $PGF_{2\alpha}$ within 10 min from hepatic stellate cells cultured for 48 h (Fig. 1). With cells cultured for 72 h the rate of rrC5a-elicited prostanoid formation did not differ significantly from that in cells cultured for 48 h (not shown). This was in contrast to prostaglandin release from hepatic stellate cells elicited by noradrenaline or ATP, which was found to be maximal after 24 h and not detectable after 72 h [12]. These differences might be due to differences in the turnover rates of the respective receptors and/or differences in the modulation of receptor expression during cell culture.

RrC5a failed to induce a release of PGD_2 and PGE_2 from sinusoidal endothelial cells cultured for 48 h (Fig. 1). Thus, sinusoidal endothelial cells do not seem to be involved as a source of prostanoids in short-term defense reactions of the liver in response to C5a.

Hepatic stellate cells released less prostanoids upon stimulation with rrC5a than Kupffer cells. Moreover, the ratios of prostanoid output were different: while hepatic stellate cells and Kupffer cells released similar absolute amounts of PGE_2, the relative amounts of $PGE_2/PGD_2/PGF_{2\alpha}/TXB_2$ were 1:1:0.1:0.6 with hepatic stellate cells (Fig. 1) and 1:4:1:3 with Kupffer cells [11]. Thromboxane and the various prostaglandins have distinct functions within the liver tissue and with each liver cell type, since Kupffer cells, hepatic stellate cells, sinusoidal endothelial cells and hepatocytes express characteristic sets of prostanoid receptors [23]. Therefore, the different ratios of prostanoids released from Kupffer cells and hepatic stellate cells after stimulation with rrC5a might reflect different functions of these cells in the regulation of defense reactions of the liver during inflammation (see below).

After a single application of 100 nM rrC5a the levels of TXB_2, PGD_2 and PGE_2 increased over 5 min and then remained almost constant for another 5 min. As has been described for Kupffer cells [11], the rate of prostanoid release was maximal within the first 2 min (Fig. 2). Thus, rrC5a-induced prostanoid release from hepatic stellate cells was rapid enough for the mediation of anaphylatoxin-induced glucose output in the perfused rat liver, which was maximal already 2–3 min after infusion of rrC5a [8].

Low levels of rrC5a (1 nM and 10 nM) increased prostanoid production only slightly, but medium levels of rrC5a (100 nM) elicited a significant release of TXB_2, PGD_2 and

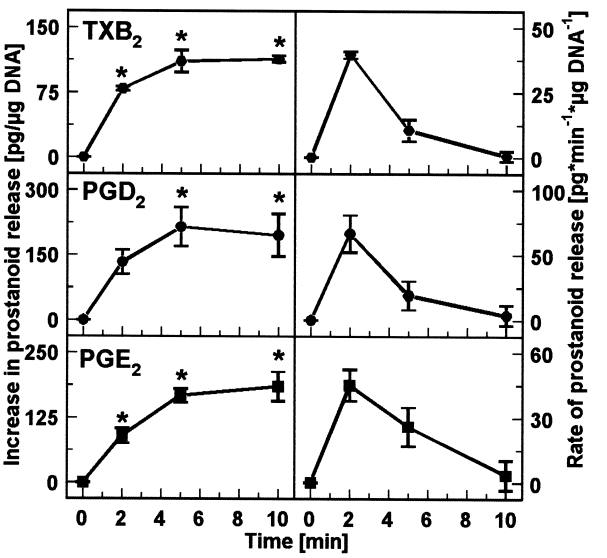

Fig. 2. Time dependence of rrC5a-induced prostanoid release from cultured hepatic stellate cells. Experiments were performed essentially as described in Fig. 1. Aliquots of the supernatant were taken at the times indicated and increases in prostanoid concentrations over basal levels at 0 min were determined by radio- or enzyme immunoassays. Values are means ± S.E.M. of 3 (TXB_2) or 4 (PGD_2 and PGE_2) independent experiments. $*P \leq 0.05$, significant differences compared with controls (Student's *t*-test for unpaired samples).

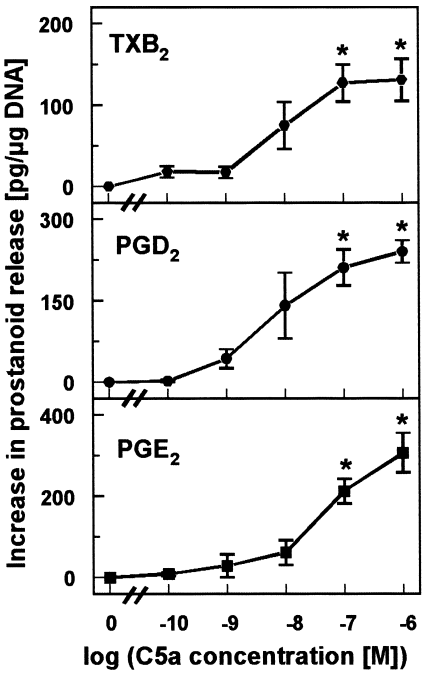

Fig. 3. Dose-dependence of rrC5a-induced prostanoid release from cultured hepatic stellate cells. Experiments were performed essentially as described in Fig. 1. Cells were stimulated with the concentrations of rrC5a indicated. After 10 min supernatants were removed and increases in prostanoid concentrations over basal levels at 0 min were determined by radio- or enzyme immunoassays. Values are means ± S.E.M. of 4 independent experiments. $*P \leq 0.05$, significant differences compared with controls (Student's *t*-test for unpaired samples).

PGE_2. This stimulation was almost maximal, since it could not be enhanced significantly by high doses of rrC5a (1 μM) (Fig. 3). Again, the dose dependence of rrC5a-induced prostanoid release from hepatic stellate cells resembled that described for Kupffer cells [11]. Thus, also for hepatic stellate cells, effective rrC5a concentrations were similar to those of about 100 nM reached in human serum after complete activation of the complement system [24]. As has been discussed previously [11], the structural differences between the recombinant C5a used in this study and native C5a (12 additional N-terminal amino acids (see Section 2) and lack of glycosylation with the recombinant protein) did not cause major changes in efficiency.

The rrC5a-induced release of PGD_2 and PGE_2 from hepatic stellate cells was completely inhibited to control levels by the prostanoid synthesis inhibitor indomethacin (Table 1). It was not influenced by pretreatment of the hepatic stellate cell cultures with gadolinium chloride for 24 h (Table 1). This indicates that prostanoid release from hepatic stellate cells could not be ascribed to contaminating Kupffer cells, since gadolinium chloride, which was found to cause a functional depletion of Kupffer cells also in culture [25], inhibited the rrC5a-elicited prostanoid release in Kupffer cell cultures to about 45% (not shown). Three further observations corroborate that prostanoid release from the hepatic stellate cell cultures was not due to contaminating Kupffer cells: (i) Contamination of hepatic stellate cell cultures with Kupffer cells after 48 h was less than 2%, whereas the average prostanoid release was 20% of that described for Kupffer cells (Fig. 1). (ii) Sinusoidal endothelial cell cultures, which were contaminated with Kupffer cells to a similar degree as hepatic stellate cell cultures, did not release significant amounts of PGD_2 or PGE_2 after stimulation with rrC5a (Fig. 1). (iii) The prostanoid ratios released after stimulation with rrC5a clearly differed between hepatic stellate cells and Kupffer cells (see above).

3.3. *Activation by rrC5a of glycogen phosphorylase in cocultures of hepatic stellate cells with hepatocytes but not in monocultures of hepatocytes*

Since hepatocytes lack C5aR mRNA, it was assumed that rrC5a might enhance glucose output in the perfused rat liver indirectly via the release of prostanoids or of a 'factor X' from the C5aR mRNA expressing non-parenchymal liver cells (see Section 1). Therefore, it was investigated next whether rrC5a activated glycogen phosphorylase not only in cocultures of Kupffer cells with hepatocytes as shown previously [11] but also in cocultures of hepatic stellate cells or sinusoidal endothelial cells with hepatocytes. In hepatic stellate cell/hepatocyte cocultures, rrC5a significantly activated glycogen phosphorylase. This activation was completely inhibited by pretreatment of the cocultures with indomethacin (Fig. 4), indicating that it was mediated by prostanoids only.

In a recent study it was shown that hepatocytes express mRNA for the Gq-linked PGE_2 receptor subtype 1 (EP1R) and $PGF_{2\alpha}$ receptor (FPR) but not TXA_2 receptor (TPR) [23]. Thus, PGE_2 via the EP1R and $PGF_{2\alpha}$ and PGD_2 via the FPR but not TXA_2 can mediate an increase in inositol-1,4,5-trisphosphate and intracellular Ca^{2+} resulting in the activation of glycogen phosphorylase in these cells [26–28]. Even though Kupffer cells and hepatic stellate cells differed not only in the ratios but also in the maximal amounts of prostaglandins released (Fig. 1), rrC5a activated glycogen phosphorylase in hepatic stellate cell/hepatocyte cocultures to a similar extent as in Kupffer cell/hepatocyte cocultures (Fig. 4). This might be

Table 1
Inhibition of rrC5a-induced PGD_2 and PGE_2 release in isolated hepatic stellate cells by the prostanoid synthesis inhibitor indomethacin but not by functional depletion of Kupffer cells with gadolinium chloride

	PGD_2 release (% of rrC5a)	PGE_2 release (% of rrC5a)
	0.7 ± 1.4 (5)*	18.0 ± 6.8 (5)*
rrC5a	100 (5)	100 (5)
rrC5a+indomethacin	2.9 ± 2.9 (3)*	1.4 ± 1.4 (3)*
rrC5a+gadolinium chloride	91.2 ± 25.2 (3)	118.5 ± 12.4 (3)

Hepatic stellate cells were isolated and cultured for 48 h with or without 10 μM gadolinium chloride during the last 24 h as described in Section 2. Experiments were performed essentially as described in Fig. 1, except that cells were preincubated with 20 μM indomethacin where indicated. Values are means ± S.E.M. of the number of experiments given in parentheses. $*P \leq 0.001$, significant difference compared with rrC5a-stimulated cells (Student's *t*-test for unpaired samples). The effectiveness of the pretreatment of the cells with gadolinium chloride was checked with Kupffer cell cultures: here rrC5a-induced $PGDE_2$ and PGE_2 release was inhibited to 45%.

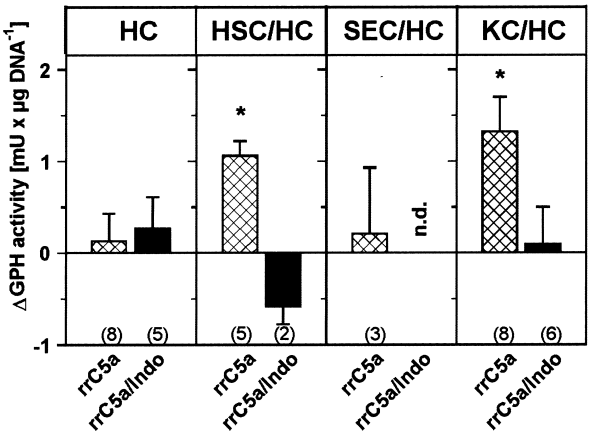

Fig. 4. Activation by rrC5a of glycogen phosphorylase in cocultures of hepatocytes with hepatic stellate cells or Kupffer cells but not sinusoidal endothelial cells. Non-parenchymal liver cells and hepatocytes were isolated and cultured as described in Section 2. After three washes in HEPES-buffered saline, cells were incubated in the same buffer in the absence or presence of 20 μM indomethacin (Indo) in 0.1% DMSO. After 10 min cells were stimulated with rrC5a (final concentration 100 nM = 1 μg/ml) or as a control with noradrenaline (1 μM). Supernatants were removed after 2 min and glycogen phosphorylase (GPH) activity was determined in cell homogenates. Basal GPH activity was about 5 mU/μg hepatocyte DNA. In the cocultures noradrenaline elicited an enhancement of about 5 mU/μg hepatocyte DNA (not shown); rrC5a caused the increases in GPH activity shown. Values of the increases in GPH activity are means ± S.E.M. of the number of experiments indicated. $^*P \leq 0.05$ (Student's *t*-test for unpaired samples).

explained by a stronger activation of glycogen phosphorylase by PGE_2, which is released in similar amounts by both cell types, rather than by PGD_2 or $PGF_{2\alpha}$. However, in the perfused rat liver $PGF_{2\alpha}$ enhanced glucose output more strongly than PGE_2 or PGD_2 [29] and in isolated hepatocytes PGE_2 and $PGF_{2\alpha}$ activated glycogen phosphorylase to similar extents [28]. Thus, more likely, the relatively small amounts of prostaglandins released from hepatic stellate cells after stimulation with rrC5a induced a maximal prostaglandin-dependent activation of glycogen phosphorylase due to the paracrine nature of their action.

RrC5a failed to induce glycogen phosphorylase activity in sinusoidal endothelial cell/hepatocyte cocultures as well as in hepatocyte monocultures (Fig. 4). This finding demonstrates that sinusoidal endothelial cells were not involved in rrC5a-induced activation of glycogen phosphorylase neither by prostaglandins nor by any other 'factor X'. Noradrenaline (1 μM), which acts directly on hepatocytes, activated glycogen phosphorylase in all cocultures (not shown).

The finding that rrC5a activated glycogen phosphorylase in hepatic stellate cell/hepatocyte and Kupffer cell/hepatocyte cocultures equally well supports the assumption that the remaining C5a-induced metabolic and hemodynamic effects in gadolinium-treated rats [8] were due to non-parenchymal cells other than Kupffer cells and not to an incomplete functional elimination of Kupffer cells as has also been discussed in detail previously [8]. Nevertheless, the experiments with isolated cells do not allow a conclusion on the relative contribution of Kupffer cells and hepatic stellate cells to the anaphylatoxin-induced glucose output in vivo. Differences in (i) the accessibility of anaphylatoxins to the Kupffer cells and the hepatic stellate cells within the liver tissue, (ii) the absolute amounts of the various cells and (iii) the proximity of Kupffer cells or hepatic stellate cells to the hepatocytes must be taken into account. The discrepancy that in gadolinium chloride-treated rats the rrC5a-induced metabolic and hemodynamic effects were inhibited to only about 40%, whereas the prostanoid overflow was almost completely abrogated [8], might indicate the involvement of other factors besides prostanoids released from non-parenchymal liver cells in rrC5a-induced glucose output. However, several observations rule out this possibility: (i) The activation of glycogen phosphorylase in cocultures of Kupffer cells or hepatic stellate cells with hepatocytes was completely inhibited by the prostanoid synthesis inhibitor indomethacin. (ii) rrC5a did not induce glycogen phosphorylase activity in sinusoidal endothelial cell/hepatocyte cocultures (Fig. 4). (iii) The rrC5a-induced metabolic and hemodynamic effects in the perfused rat liver were completely inhibited by indomethacin together with the thromboxane receptor antagonist daltroban (unpublished observations). Thus, the action of rrC5a on glucose output was completely mediated by prostanoids. Apparently, the overflow of prostanoids into the hepatic vein did not reflect the availability of prostanoids in the Space of Disse for the hepatocytes. Taken together, the present findings suggest that besides Kupffer cells also hepatic stellate cells are involved in C5a-induced glucose output in the perfused rat liver. Final evidence that hepatic stellate cells participate in the action of C5a on the liver would be provided by functional elimination of these cells analogously to that of Kupffer cells with gadolinium chloride. However, so far such a method is not available.

4. Conclusions

Besides Kupffer cells hepatic stellate cells also released prostaglandins and thromboxane in response to the anaphylatoxin rrC5a in a time- and dose-dependent manner. Even though hepatic stellate cells differed from Kupffer cells in the absolute amounts and in the ratios of prostanoids released, glycogen phosphorylase was activated to similar extents in cocultures of hepatic stellate cells or Kupffer cells with hepatocytes after stimulation with rrC5a. Thus, the part of rrC5a-induced glucose output in the perfused rat liver which was not inhibitable by elimination of the Kupffer cells with gadolinium chloride most probably was due to rrC5a-induced prostanoid release from hepatic stellate cells.

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