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# Feedback-Inhibition of Glucagon-Stimulated Glycogenolysis in Hepatocyte/Kupffer Cell Cocultures by Glucagon-Elicited Prostaglandin Production in Kupffer Cells

URSULA HESPELING, KURT JUNGERMANN, AND GERHARD P. PÜSCHEL

**Prostaglandins, released from Kupffer cells, have been shown to mediate the increase in hepatic glycogenolysis by various stimuli such as zymosan, endotoxin, immune complexes, and anaphylotoxin C3a involving prostaglandin (PG) receptors coupled to phospholipase C via a $G_0$ protein. PGs also decreased glucagon-stimulated glycogenolysis in hepatocytes by a different signal chain involving $PGE_2$ receptors coupled to adenylate cyclase via a $G_i$ protein ($EP_3$ receptors). The source of the prostaglandins for this latter glucagon-antagonistic action is so far unknown. This study provides evidence that Kupffer cells may be one source: in Kupffer cells, maintained in primary culture for 72 hours, glucagon (0.1 to 10 nmol/L) increased $PGE_2$, $PGF_{2\alpha}$, and $PGD_2$ synthesis rapidly and transiently. Maximal prostaglandin concentrations were reached after 5 minutes. Glucagon (1 nmol/L) elevated the cyclic adenosine monophosphate (cAMP) and inositol triphosphate ($InsP_3$) levels in Kupffer cells about fivefold and twofold, respectively. The increase in glycogen phosphorylase activity elicited by 1 nmol/L glucagon was about twice as large in monocultures of hepatocytes than in cocultures of hepatocytes and Kupffer cells with the same hepatocyte density. Treatment of cocultures with 500 μmol/L acetylsalicylic acid (ASA) to irreversibly inhibit cyclooxygenase (PGH-synthase) 30 minutes before addition of glucagon abolished this difference. These data support the hypothesis that PGs produced by Kupffer cells in response to glucagon might participate in a feedback loop inhibiting glucagon-stimulated glycogenolysis in hepatocytes. (HEPATOLOGY 1995;22: 1577-1583.)**

Abbreviations: PG, prostaglandin; cAMP, cyclic adenosine monophosphate; NCS, newborn calf serum; IBMX, isobutyl-methyl-xanthine; ASA, acetylsalicylic acid; $cPLA_2$, cytosolic phosphilase $A_2$; $InsP_3$, inositol trisphosphate.

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Prostaglandins (PGs) produced by nonparenchymal liver cells[1-3] are involved in the regulation of liver carbohydrate metabolism. They mediate the mobilization of glucose from the liver in response to inflammatory stimuli,[4] e.g., zymosan,[5] endotoxins,[6] immune complexes,[7] platelet activating factor,[8] and anaphylotoxins.[9] PGs increased inositol triphosphate ($InsP_3$) formation, glycogen phosphorylase activity, and glucose output in hepatocyte cultures and suspensions.[10-12] This glycogenolytic effect was mediated by $PGF_{2\alpha}$ receptors and $PGE_2$ receptors of the $EP_1$ subtype that are linked to phospholipase C by a pertussis toxin-insensitive G-protein.[12] In addition, primarily $PGE_2$ and to a lesser extent also $PGF_{2\alpha}$ and $PGD_2$ inhibited the glucagon-stimulated cyclic adenosine monophosphate (cAMP) formation,[13] glycogen phosphorylase activity,[12] glucose mobilization from glycogen,[14,15] and gluconeogenesis[12] in hepatocytes. This glucagon-antagonistic effect was mediated by $PGE_2$ receptors of the $EP_3$ subtype,[12] that are linked to adenylate cyclase by a pertussis toxin-sensitive $G_i$-protein.[16-18] Therefore, PGs especially $PGE_2$, may be involved in a feedback inhibition of glucagon-stimulated glucose mobilization from hepatocytes, if PGs were formed in a glucagon-dependent manner in the liver.

Because glucagon receptors have been detected on Kupffer cells in binding studies,[19] it appeared possible that they might be involved in a glucagon-stimulated PG formation and thus the proposed feedback loop. The current study provides evidence for such a mechanism: glucagon stimulated PG formation in Kupffer cells and caused a greater increase in glycogenolysis in hepatocyte monocultures than in hepatocyte/Kupffer cell cocultures.

## MATERIALS AND METHODS

***Materials.*** All chemicals were of analytical grade and from commercial sources. [$^3$H]Myoinositol and the radioimmunoassays for $PGE_2$, $PGF_{2\alpha}$, $PGD_2$, and cAMP were bought from Amersham (38110 Braunschweig, Germany), Dowex-formate from Serva (69115 Heidelberg, Germany). The scintillation cocktail Hydroluma was purchased from Baker (Deventer, The Netherlands), pronase from Merck AG (64293 Darmstadt, Germany) and collagenase, and deoxyribonuclease DNase from Boehringer (68305 Mannheim, Germany). Tis-

sue culture dishes were obtained from Nunc (65203 Wiesbaden, Germany), Nycodenz from Life Technologies GmbH (76344 Eggenstein, Germany), Percoll from Pharmacia Biotech GmbH (79021 Freiburg, Germany), newborn calf serum (NCS) and bis-benzimide from Sigma Chemical Co. (82041 Deisenhofen, Germany), RPMI 1640 from Biochrom KG (12247 Berlin, Germany), and M199 from Gibco BRL (76344 Eggenstein, Germany).

***Animals.*** Male Wistar rats (350 to 450 g for the preparation of Kupffer cells and 200 to 250 g for the isolation of hepatocytes) were bought from Winkelmann (33178 Borchen, Germany) and kept on a 12-hour day-night rhythm (light from 7 AM to 7 PM) with free access to water and a rat standard diet from Ssniff (59494 Soest, Germany) at least 2 weeks before the experiments. Animal care and the use of the animals in this study were in compliance with the German Law on the Protection of Animals.

***Kupffer Cell Preparation.*** Nonparenchymal cells were prepared by a combined collagenase/pronase perfusion.[20,21] Kupffer cells were then purified by Nycodenz density-gradient centrifugation and subsequent centrifugal elutriation using a Beckman JE-6 elutriation rotor in a J-21 Beckman centrifuge.

***Hepatocyte Preparation.*** Hepatocytes were isolated according to Meredith[22] without the use of collagenase as described previously.[23] The liver was perfused without recirculation via the portal vein with a $Ca^{2+}$-free Krebs-Henseleit buffer containing 15 mmol/L glucose, 2 mmol/L lactic acid, 0.2 mmol/L sodium pyruvate and 2 mmol/L ethylenediaminetetraacetic acid. Detritus was removed by two subsequent washing steps, sedimenting the viable hepatocytes at 50*g*. Viable hepatocytes were further purified by centrifugation through a gradient with 58% Percoll.

***Kupffer Cell Culture.*** Kupffer cells were plated ($5 \times 10^6$/plate) on 3.5-cm tissue culture plates with or without collagen coating in RPMI 1640 in the presence of 1% penicillin/streptomycin and 30% NCS for 72 hours before the experiment. Medium was changed every 24 hours.

***Hepatocyte Culture.*** Hepatocytes were plated ($7 \times 10^5$/plate) on collagen-coated 3.5-cm tissue culture plates in M199 with 0.5 nmol/L insulin, 1% penicillin/streptomycin and 30% NCS for 24 hours before the experiment with a medium change after 4 hours.

***Coculture.*** For coculture with hepatocytes, Kupffer cells were seeded ($4 \times 10^6$/plate) on collagen-coated, 3.5-cm culture plates in RPMI 1640 in the presence of 1% penicillin/streptomycin and 30% NCS for the first 48 hours. Medium was changed every 24 hours. After 48 hours, freshly prepared hepatocytes ($7 \times 10^5$/plate) were plated on top of the Kupffer cells. Cocultures were incubated in M199 with 0.5 nmol/L insulin, 1% penicillin/streptomycin, and 30% NCS for another 24 hours with one medium change after 4 hours.

***Determination of Prostanoid and cAMP Formation in Kupffer Cells.*** After 72 h cells were washed three times with Hank's balanced salt solution (137 mmol/L NaCl, 5.4 mmol/L KCl, 1.3 mmol/L $CaCl_2$, 0.8 mmol/L $MgCl_2$, 4.2 mmol/L $NaHCO_3$, 0.34 mmol/L $Na_2PO_4$, 0.44 mmol/L $KH_2PO_4$, 20 mmol/L HEPES, and 5 mmol/L glucose, pH 7.4) to remove all residual serum and medium and then preincubated for 10 minutes in the same buffer. Then glucagon was added to the final concentrations indicated. Samples of the supernatant were taken at different times and frozen immediately in liquid nitrogen for the later determination of prostanoid concentrations. $PGF_{2\alpha}$, $PGD_2$, and $PGE_2$ were determined in the cell supernatants without further purification by radioimmunoassay according to the instructions of the manufacturer. At the end of the experiment, Kupffer cells were scraped off the dish for DNA determination in a fluorescence assay based on the intercalation of bis-benzimide into DNA.[24] For determination of cAMP formation, Kupffer cell cultures were treated similarly except that at the end of a 2-minute incubation period, after addition of glucagon or vehicle, medium was removed, and cells were shock-frozen in liquid nitrogen. cAMP was extracted from these cells with 500 $\mu$L 10 mmol/L HCl containing 1 mmol/L isobutyl-methyl-xanthine (IBMX). cAMP was determined with a $^{125}$I-radioimmunoassay according to the instructions of the supplier.

***Determination of $InsP_3$ Formation in Kupffer Cells.*** After 48 hours of culture, medium was replaced by inositol-free RPMI medium supplemented as described above containing in addition 10 $\mu$Ci/mL [$^3$H]myoinositol. Culture was continued for another 24 hours to label inositol-phospholipids. Medium was removed, cells were washed five times with Hank's balanced salt solution, and then incubated for 10 minutes at 37°C in the same buffer containing in addition 10 mmol/L LiCl to inhibit degradation of inositol phosphates. Glucagon (1 nmol/L final concentration) was added. After 3 minutes, medium was removed and cells were frozen in liquid nitrogen. Inositol phosphates were extracted with 350 $\mu$L 7.5% TCA. Inositol phosphates were separated on Dowex formate $1 \times 8$ as described previously[25]; briefly, TCA was removed from the samples by 3 extractions with a 10-fold volume of ethylether. The water phase was diluted fourfold and applied to 1.5 mL (moist volume) Dowex formate in a small column. Inositol and inositol phosphates were eluted subsequently with 6 mL $H_2O$ (inositol), 4 mL 0.18 mol/L ammonium formate (uncharacterized inositol-containing compounds), 3 mL 0.4 mol/L ammonium formate/0.1 mol/L formic acid (InsP), 3 mL 0.9 mol/L ammonium formate/0.1 mol/L formic acid ($InsP_2$), and 1.5 mol/L ammonium formate/0.1 mol/L formic acid ($InsP_3$); 1 mL eluate fractions were collected and counted in a scintillation counter with 10 mL Hydroluma. Columns were calibrated with authentic inositol phosphate standards.

***Determination of Glycogen Phosphorylase Activity in Monocultures and Cocultures.*** Hepatocyte, Kupffer cell, and hepatocyte/Kupffer cell cultures were washed three times with HEPES-buffered saline (20 mmol/L Hepes, 120 mmol/L NaCl, 4.8 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L $KH_2PO_4$, 2.5 mmol/L $CaCl_2$, 5 mmol/L glucose, 2 mmol/L lactate, and 0.2 mmol/L pyruvate, pH 7.4). Cells were incubated for 10 minutes at 37°C in the same buffer. Then the medium was discarded and replaced by 200 $\mu$L HEPES-buffered saline with glucagon (final concentration 1 nmol/L), $PGE_2$ (final concentration 10 $\mu$mol/L), or both. After 2 minutes of incubation the buffer was removed, and the plates were frozen in liquid nitrogen. To inhibit the cyclooxygenase (PGH-synthase) cell cultures were pretreated with 500 $\mu$mol/L acetylsalicylic acid (ASA) in M199 30 minutes before the experiment. Control plates were incubated with M199 without ASA. Glycogen phosphorylase activity was determined with a standard assay.[26]

## RESULTS

***Stimulation by Glucagon of PG Formation in Kupffer Cells.*** In Kupffer cells that had been cultured for 72 hours on untreated plastic dishes, glucagon (10 nmol/L) increased the formation of $PGE_2$, $PGF_{2\alpha}$, and $PGD_2$. The increase was rapid in onset and transient, so that the PG concentrations in the medium remained increased over a period of 10 minutes (Fig. 1). The increase was dose-dependent; concentrations of glucagon

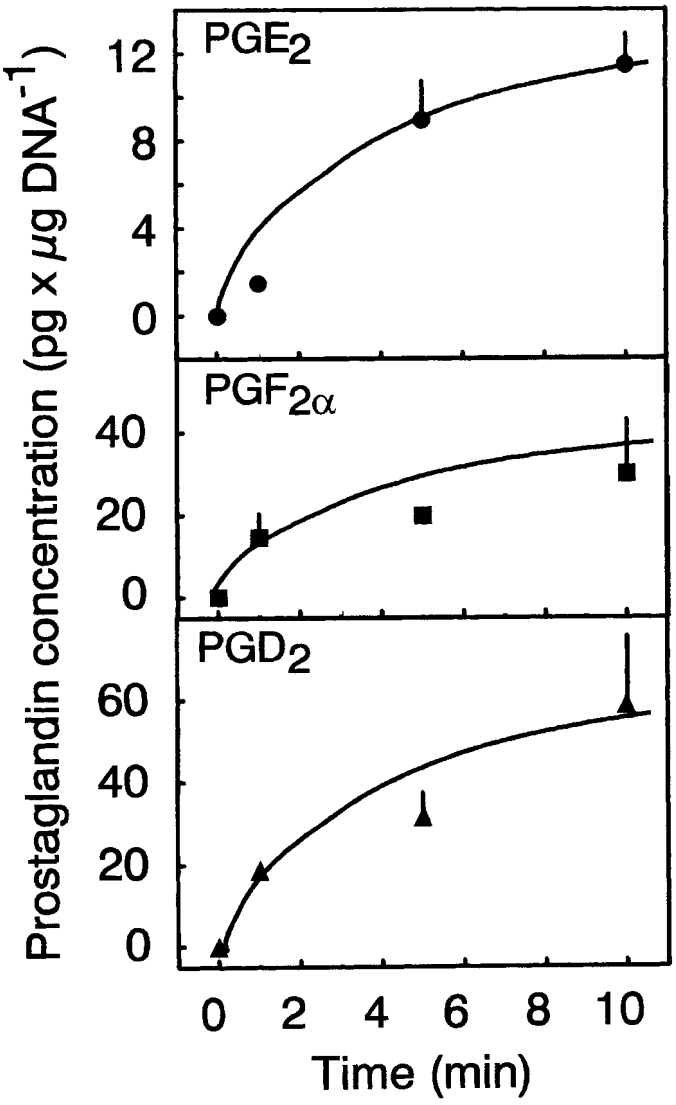


FIG. 1. Time dependence of glucagon-stimulated PG formation in Kupffer cells. Rat Kupffer cells were prepared by collagenase/pronase liver perfusion, Nycodenz gradient centrifugation, and subsequent centrifugal elutriation. They were cultured for 72 hours on uncoated tissue culture plates. Medium was changed every 24 hours. Medium was replaced by a HEPES-buffered balanced salt solution. After a preincubation period of 10 minutes, cells were stimulated with 1 nmol/L glucagon. At the times indicated, aliquots of the supernatant were taken and used without further purification for PG determination by radioimmunoassay. Values (increase over zero-time levels) are means ± SEM of 4 independent experiments.

that are known to stimulate glycogenolysis in hepatocytes and that can occur in portal blood, i.e., 0.1 to 1 nmol/L, already enhanced PG formation nearly to the maximum (Fig. 2). Glucagon also increased PG formation in Kupffer cells cultured on collagen-coated plates to the same extent as on uncoated tissue culture plates (not shown).

Kupffer cells were treated with acetylsalicylic acid (ASA) (500 μmol/L) for 30 minutes. ASA was then removed by three washes, and the cells incubated another 10 minutes without ASA. They were then stimulated with 1 nmol/L glucagon. Under these conditions basal PG formation was reduced to less than detection limit, and PG formation was no longer stimulated by glucagon (not shown).

Glucagon (1 nmol/L) increased cAMP formation in Kupffer cells fivefold over basal within 2 minutes (Table 1). In Kupffer cells that had been labelled with [$^3$H]myoinositol for 24 hours, glucagon (1 nmol/L) increased [$^3$H]inositol-trisphosphate formation in presence of 10 mmol/L LiCl twofold over basal within 3 minutes (Table 1). In contrast to zymosan, no further increase in $InsP_3$ formation was observed during prolonged incubation (not shown).

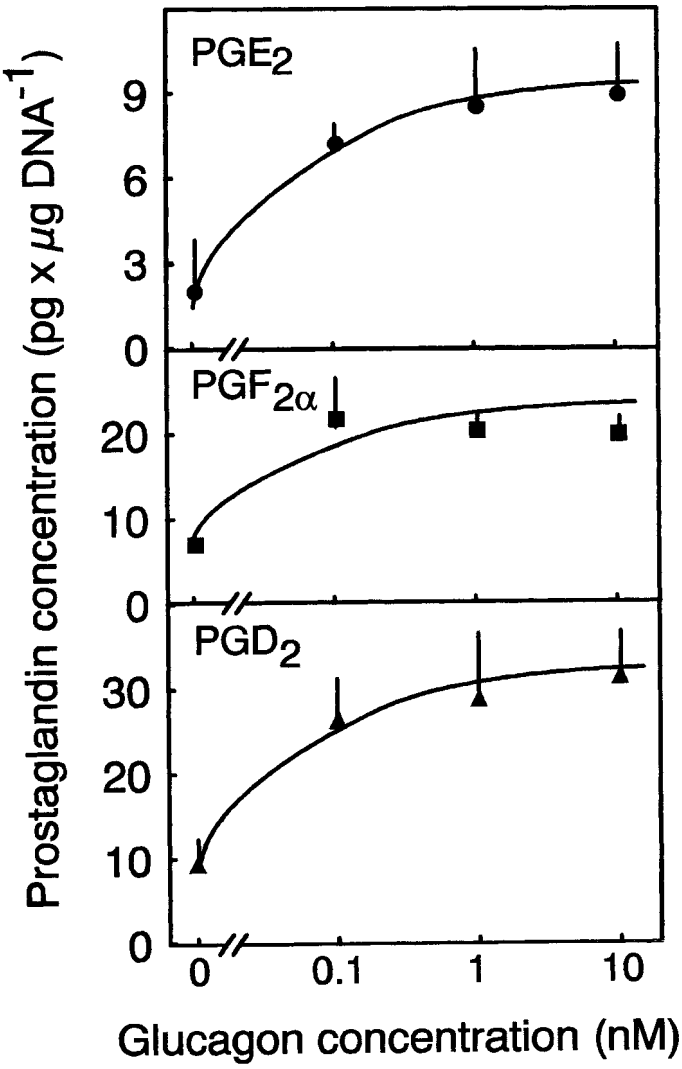


FIG. 2. Dose dependence of glucagon-stimulated PG formation in Kupffer cells. The experiments were performed as in Fig. 1. Five minutes after glucagon addition aliquots of the supernatant were obtained and used without further purification for PG determination by radioimmunoassay. Values (increase over zero-time levels) are means ± SEM of 4 independent experiments.

**TABLE 1. Glucagon-Stimulated Formation of cAMP and $InsP_3$ in Kupffer Cells**

| | cAMP (nM) | $InsP_3$ (CPM) |
|---|---|---|
| Control | 1.5 ± 0.3 | 173 ± 20 |
| Glucagon (1 nM) | 8.5 ± 1.6* | 334 ± 36* |

NOTE. Kupffer cells were cultured for a total of 72 hours. For determination of $InsP_3$ formation 10 μmol/L [$^3$H]-inositol was added to the culture medium. Medium was replaced by a HEPES-buffered Hank's balanced salt solution that contained in addition 10 mmol/L LiCl for $InsP_3$ determination. After 10 minutes preincubation cells were stimulated with glucagon, buffer was removed after 2 minutes (cAMP) or 3 minutes ($InsP_3$), and cells were frozen in liquid nitrogen. cAMP was determined in the cell lysates by radioimmunoassay, $InsP_3$ by separation of radioactively labeled inositol phosphates on Dowex formate columns. Values are means ± SEM of four (cAMP) and 3 ($InsP_3$) experiments. Statistics: Student's t-test for paired samples.

* $P < .05$.

***Stimulation by Glucagon of Glycogen Phosphorylase Activity in Hepatocyte/Kupffer Cell Cocultures.*** To test the working hypothesis that PGs released from Kupffer cells in response to glucagon might feedback inhibit glucagon-stimulated glycogen phosphorylase activity in hepatocytes, a coculture system of Kupffer cells and hepatocytes was established.

Kupffer cells were cultured on collagen-coated culture dishes for 48 hours. Then hepatocytes were seeded on top of the Kupffer cells. Culture was continued for another 24 hours before the experiment. Parallel to Kupffer cell/hepatocyte cocultures, Kupffer cell monocultures and hepatocyte monocultures were maintained on collagen-coated culture dishes (Fig. 3). There was no difference in morphology of hepatocytes in cocultures and monocultures. Kupffer cells in cocultures seemed to contain somewhat more phagocytosed material, probably debris of dead hepatocytes. Cocultures contained the same number of hepatocytes/dish as control hepatocyte monocultures and the same number of Kupffer cells/dish as control Kupffer cell monocultures. The DNA content in cocultures (13.5 μg/dish) was about the sum of the DNA content in Kupffer cell (3.7 μg/dish) and hepatocyte (8.5 μg/dish) monocultures (Fig. 3). Thus, it could be assumed that between 60% and 70% of the total DNA of cocultures were to be ascribed to hepatocyte DNA. For all further calculations the fraction of hepatocyte DNA in cocultures was set equal to 65%.

When related to hepatocyte DNA, basal glycogen phosphorylase activity was identical in hepatocyte monocultures and hepatocyte/Kupffer cell cocultures (1.86 ± 0.24 U × mg hepatocyte $DNA^{-1}$ vs. 1.89 ± 0.26 U × mg hepatocyte $DNA^{-1}$). Glucagon stimulated glycogen phosphorylase activity in hepatocyte monocultures by threefold over basal. Yet, in hepatocyte/Kupffer cell cocultures, glucagon increased glycogen phosphorylase activity only by twofold over basal (Fig. 4). There was no appreciable degradation of glucagon either in hepatocyte monocultures or hepatocyte/Kupffer cell cocultures during the course of the experiment (not shown). $PGE_2$ added concomitantly with glucagon, reduced the glucagon-stimulated glycogen phosphorylase activity in hepatocyte monocultures by 25%. However, addition of $PGE_2$ did not further reduce the glucagon effect in hepatocyte/Kupffer cell cocultures (Fig. 4). Thus, it seemed possible that endogenously released PGs attenuated the glucagon-stimulated glycogen phosphorylase activity in cocultures.

To corroborate this hypothesis, cell cultures were incubated with 500 μmol/L ASA for 30 minutes. This treatment completely and irreversibly inactivated PGH

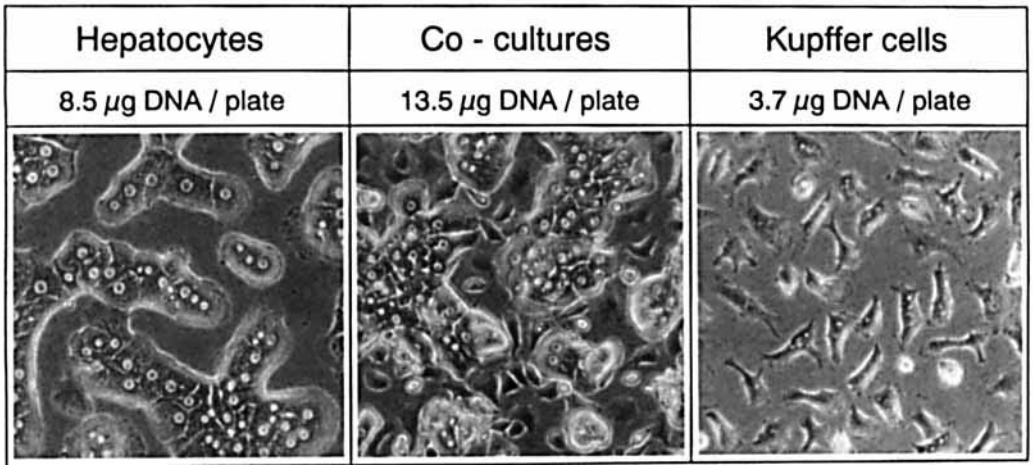


FIG. 3. Hepatocyte/Kupffer cell cocultures. Rat Kupffer cells were prepared as in Fig. 1 and cultured for 48 hours on collagen-coated tissue culture dishes. Medium was changed every 24 hours. Hepatocytes were prepared by calcium-free ethylenediaminetetraacetic acid liver perfusion without the use of collagenase. They were purified by Percoll gradient centrifugation and either seeded on top of Kupffer cell cultures or on separate collagen-coated culture dishes. Culture was continued for another 24 hours with a medium change after 4 hours. The DNA content was determined by a fluorometric assay.

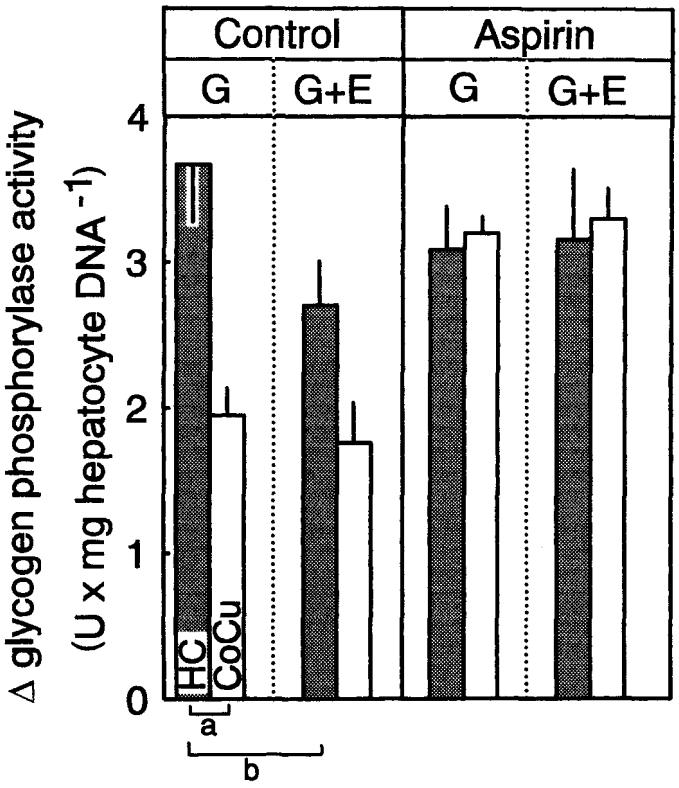


FIG. 4. Stimulation by glucagon of glycogen phosphorylase activity in hepatocyte cultures and hepatocyte/Kupffer cell cocultures. Hepatocyte and Kupffer cell cultures as well as hepatocyte/Kupffer cell cocultures were prepared and maintained for 72 hours as in Fig. 3. Medium was then replaced by fresh serum-free medium containing 500 μmol/L ASA when indicated. Cells were incubated for 30 minutes and medium replaced by a HEPES-buffered balanced salt solution. After a preincubation period of 10 minutes, cells were stimulated with 1 nmol/L glucagon or 10 μmol/L $PGE_2$ or both. After 2 minutes, buffer was removed and cells were frozen in liquid nitrogen for later determination of glycogen phosphorylase in a standard assay. Values are means ± SEM of four independent experiments. HC, hepatocyte mono-culture; Cocu, hepatocyte/Kupffer cell co-culture; G, glucagon; E, $PGE_2$. Statistics: Student's *t*-test for unpaired samples, (a) $P < .01$; (b) $P < .05$.

synthases in Kupffer cells (see previously). ASA was then removed, and after another 10 minutes, cell cultures were stimulated with glucagon. In ASA-treated hepatocyte monocultures and hepatocyte/Kupffer cell cocultures, basal glycogen phosphorylase activity was somewhat less than in untreated cultures (1.56 ± 0.26 U × mg hepatocyte $DNA^{-1}$ and 1.44 ± 0.21 U × mg hepatocyte $DNA^{-1}$). In ASA-treated, as in untreated hepatocyte monocultures, glucagon increased glycogen phosphorylase activity by threefold. However, in contrast to untreated cocultures, glucagon increased glycogen phosphorylase activity in ASA-treated hepatocyte/Kupffer cell cocultures also by threefold (Fig. 4). Thus, ASA treatment abolished the difference of glucagon-stimulated glycogen phosphorylase activity between hepatocyte monocultures and hepatocyte/Kupffer cell cocultures. Surprisingly, ASA treatment also abolished the attenuation by $PGE_2$ of glucagon-stimulated glycogen phosphorylase activity in hepatocyte monocultures. This effect is not understood and most likely cannot be ascribed to the inactivation of PGH synthase.

## DISCUSSION

***Functional Role of the Glucagon Receptor on Kupffer Cells.*** Glucagon receptors have previously been shown on Kupffer cells by electron microscopy using glucagon that had been cross-linked to gold particles via albumin and also in [$^{125}$I]glucagon-binding studies.[19] The electron microscopical studies showed that glucagon receptors were internalized via a clathrin-independent pathway and that the endocytotic vesicles were fused with lysosomes. Therefore, glucagon receptors on Kupffer cells have been implicated in intrahepatic glucagon degradation; however, only a minor fraction (20%) of [$^{125}$I]glucagon was degraded by Kupffer cells in suspension within 1 hour at 37°C.[19] Similarly, in the current study, no appreciable glucagon degradation could be observed in Kupffer cell cultures within 2 minutes. Therefore, the role of Kupffer cells in glucagon degradation is doubtful. Thus, the glucagon-elicited PG release shown in the current study (Figs. 1 and 2) appears to be the first example for a physiological role of glucagon receptors on Kupffer cells.

***Mechanism of the Glucagon-Induced PG Release From Kupffer Cells.*** The glucagon receptor appears to be coupled to two intracellular signalling pathways; the ligand occupied receptor stimulates the adenylate cyclase and also increases the cytosolic $Ca^{2+}$ concentration and $InsP_3$ formation.[27,28] Accordingly, glucagon increased both cAMP formation by fivefold and $InsP_3$ formation by twofold in Kupffer cells (Table 1). In previous studies glucagon was found to only slightly increase cAMP formation in Kupffer cells. This increase was very small compared with the increase in total liver plasma membranes.[29] In purified Kupffer cell plasma membranes, no increase in adenylate cyclase activity in response to glucagon could be detected.[29] In the older studies, it remained unclear what the physiological meaning of the glucagon-dependent increase in cAMP could be. Later, extracellularly applied dibutyryl cAMP was shown to attenuate zymosan-stimulated and phorbol ester–stimulated but not calcium ionophore–stimulated PG formation by about 50%.[30] The increase in cAMP formation that is also elicited by PGs in Kupffer cells has been implicated as a possible negative feedback loop of PG formation.[30] Glucagon would thus elicit an inhibitory signal for PG formation in Kupffer cells. However, protein kinase A–dependent phosphorylation of cytosolic phospholipase $A_2$ ($cPLA_2$) has also been shown to activate this key regulatory enzyme of arachidonic acid and thus PG synthesis,[31,32] even though the increase in activity was small compared with activation with MAP kinase.[32] Therefore, it seems to be possible that the glucagon-mediated increase in cAMP formation in Kupffer cells could also increase basal PG formation. In line with this hypothesis, dibutyl cAMP applied in high concentrations (1 mmol/L) to Kupffer cell cul-

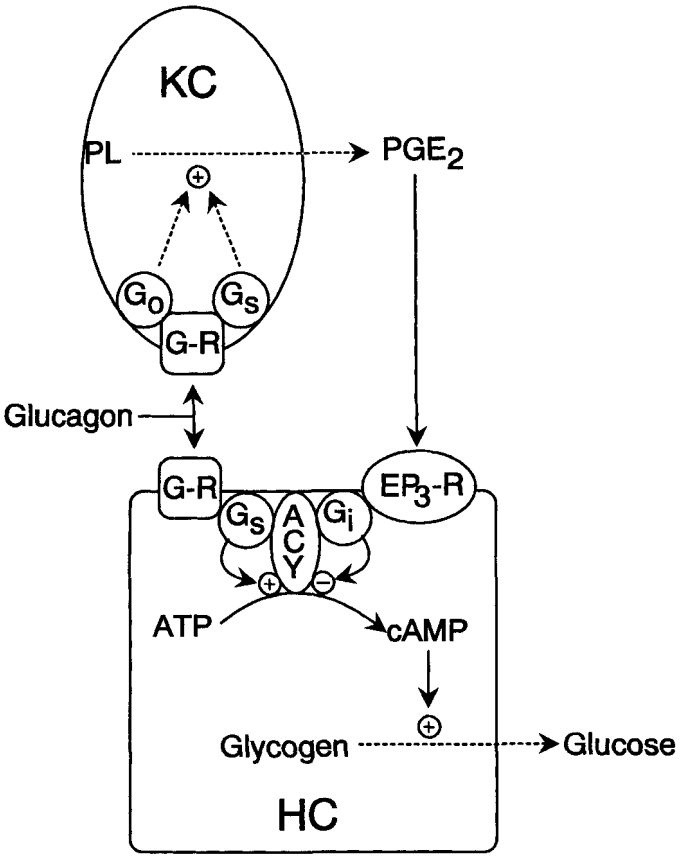


FIG. 5. Model of the glucagon-elicited feedback inhibition by PG production in Kupffer cells of the glucagon-stimulated glycogenolysis in hepatocytes. ACY, adenylate cyclase; dashed lines, indirect regulation or multistep reaction; $EP_3$-R, $PGE_2$ receptor of the $EP_3$ subtype; $G_i$, heterotrimeric inhibitory G protein; $G_o$, phospholipase C–linked heterotrimeric G protein; G-R = glucagon receptor; $G_s$ = heterotrimeric stimulatory G protein; HC, hepatocyte; KC, Kupffer cell; PL, phospholipid; solid lines, direct regulation or one step reaction.

tures increased PG formation (Hespeling, Unpublished observation, February 1995).

On the other hand, activation of phospholipase C with the generation of DAG and $InsP_3$ seems to be one mechanism by which PG formation can be enhanced in Kupffer cells[33] both directly and indirectly via the activation of $cPLA_2$. Glucagon could thus initiate a stimulatory signal for prostaglandin formation also by these pathways.

***Functional Significance of the Glucagon-Induced PG Formation in Kupffer Cells.*** PGs, primarily $PGE_2$, have been shown to attenuate glucagon-stimulated glycogenolysis in hepatocytes. The PG concentrations needed to inhibit glucagon-stimulated glycogen phosphorylase activity or glucose mobilization ranged from 20 to 200 nmol/L.[12-14,18] The prostaglandin concentration in portal and hepatovenous blood, however, is only in the range of 1 nmol/L. Thus, to be operative in a hypothetical feedback inhibition loop, prostaglandins must be formed locally in the vicinity of hepatocytes in response to glucagon and reach the neighboring hepatocytes through paracrine diffusion not via the circulation. The current study provides evidence for such a hypothesis: glucagon-stimulated prostaglandin formation in Kupffer cells (Figs. 1 and 2) may be one possible intrahepatic source for the prostaglandins needed in the feedback loop.

Moreover, the study shows that glucagon-stimulated glycogenolysis was partially attenuated in a hepatocyte/Kupffer cell coculture system and that this attenuation could be abolished by aspirin treatment of Kupffer cells/hepatocyte cocultures (Fig. 4). This indicates that prostaglandins produced by Kupffer cells may indeed act on nearby hepatocytes in a paracrine mechanism to attenuate glucagon-stimulated glycogenolysis.

In addition to the inhibition of PGH-synthase, aspirin inhibited the attenuation of glucagon-stimulated glycogenolysis by externally added $PGE_2$ (Fig. 4). The mechanism of the aspirin action is almost certainly not caused by the inhibition of PGH synthase (cyclooxygenase). It is possible that aspirin interferes with the signal chain of the $PGE_2$ receptor involved, but the site of interference is currently not known. Other PGH synthase inhibitors like indomethacin, ibuprofen, and piroxicam also attenuated the inhibition by $PGE_2$ of glucagon-stimulated glycogenolysis in hepatocytes (Hespeling, Unpublished data, February, 1995).[34] In addition, indomethacin and ibuprofen stimulated glycogenolysis in hepatocytes. Thus, the mechanism of abolishment by aspirin of the inhibitory action of exogenous $PGE_2$ on the glucagon-dependent glycogenolysis remains to be defined.

***Conclusion.*** The current study provides evidence for the following model (Fig. 5): glucagon stimulates glycogenolysis in hepatocytes. At the same time glucagon enhances PG formation in Kupffer cells. In turn, PGs, especially $PGE_2$, inhibit the glucagon-stimulated glycogenolysis in hepatocytes; thereby, glucagon can feedback inhibit its own stimulatory signal on hepatic glycogenolysis by an intercellular communication mechanism between nonparenchymal and parenchymal cells.

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