Conditioned media of Kupffer and endothelial liver cells influence protein phosphorylation in parenchymal liver cells

Involvement of prostaglandins

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The possible role of Kupffer and endothelial liver cells in the regulation of parenchymal-liver-cell function was assessed by studying the influence of conditioned media of isolated Kupffer and endothelial cells on protein phosphorylation in isolated parenchymal cells. The phosphorylation state of three proteins was selectively influenced by the conditioned media. The phosphorylation state of an M_r -63000 protein was decreased and the phosphorylation state of an M_r -47000 and an M_r -97000 protein was enhanced by these media. These effects could be mimicked by adding either prostaglandin E_1 , E_2 or D_2 . Both conditioned media and prostaglandins stimulated the phosphorylase activity in parenchymal liver cells, suggesting that the M_r -97000 phosphoprotein might be phosphorylase. Parenchymal liver cells secrete a phosphoprotein of M_r -63000 and pI 5.0–5.5. The phosphorylation of this protein is inhibited by Kupffer- and endothelial-liver-cell media, and prostaglandins E_1 , E_2 and D_2 had a similar effect. The data indicate that Kupffer and endothelial liver cells secrete factors which influence the protein phosphorylation in parenchymal liver cells. This forms further evidence that products from non-parenchymal liver cells, in particular prostaglandin D_2 , might regulate glucose homeostasis and/or other specific metabolic processes inside parenchymal cells. This stresses the concept of cellular communication inside the liver as a way by which the liver can rapidly respond to extrahepatic signals.

INTRODUCTION

Protein phosphorylation is a well-studied regulatory phenomenon in the liver. Many key enzymes of major metabolic routes in the liver are shown to be regulated by phosphorylation/dephosphorylation. So far, attention has been focused on the major liver cell type, parenchymal cells, and several hormones have been shown to regulate the phosphorylation/dephosphorylation status in these cells [1,2].

However, besides parenchymal cells, the liver contains other cell types, i.e. Kupffer, endothelial, fat-storing and pit cells [3]. Although knowledge of the secretory response of the non-parenchymal liver cells is limited, it is known that Kupffer cells are able to produce various prostaglandins [4–6]. It has even been suggested, on the basis of studies with perfused liver [7–10], that phorbol esters and platelet-activating factor might exert a regulatory effect on parenchymal liver cells mediated by non-parenchymal cells. This hypothesis was based on the observation that these agents do not stimulate glycogenolysis in isolated parenchymal liver cells [10–14], whereas in the perfused liver system a stimulation of glycogenolysis [7–10] by these agents could be blocked by indomethacin [7,10,15,16], a cyclo-oxygenase inhibitor. Previously we described that prostaglandin D_2 concentrations in liver perfusates are increased by phorbol ester, and it was shown that prostaglandin D_2 could stimulate glucose

release from the liver [17]. However, no direct evidence with isolated cells was obtained, which showed that secretory products of non-parenchymal cells can exert a regulatory response in parenchymal liver cells. In order to test directly the possibility of cellular communication in the liver, we isolated the various cell types and studied the influence of conditioned media of Kupffer and endothelial cells on the phosphorylation state of parenchymal-cell proteins. The effects of these media were compared with the effect of prostaglandins.

Parenchymal liver cells have been shown to synthesize and secrete a phosphorylated acute-phase protein [18]. This protein has an M_r of 63000 and a pI of 4.8–5.3, and its synthesis is depressed during acute-phase response [19]. The influence of conditioned media of Kupffer and endothelial liver cells on the phosphorylation of this secretory phosphoprotein was also studied.

MATERIALS AND METHODS

Prostaglandins E_1 , E_2 and D_2 , collagenase type I and IV were from Sigma; carrier-free [32 P]P_i was from Amersham; other chemicals were of P.A. quality.

Male Wistar rats, fed *ad libitum*, weighing 200–220 g were used. Nembutal (18 mg) was given intraperitoneally for anaesthesia, usually performed between 09:00 and 10:00 h.

Parenchymal liver cells were isolated after perfusion

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for 20 min with collagenase (type IV, 0.1%) by the method of Seglen [20], modified as previously described [21]. Parenchymal liver cells were incubated at 37 °C under constant shaking at 5 mg of cell protein/ml in phosphate-free Krebs-Ringer bicarbonate buffer, saturated with O_2/CO_2 (19:1), pH 7.4, keeping the viability of the cells > 95%. At zero time 1 mCi of [^{32}P]P_i/ml was added, and the cells were preincubated for 1 h. During the preincubation time the [^{32}P]P_i equilibrated and the specific radioactivity of [^{32}P]ATP reached a steady state [22]. Cells were challenged for 5 min with non-parenchymal-cell media or prostaglandins. Subsequently 50 μ l of cell suspension was added to 200 μ l of a digitonin (2 mg/ml)-containing buffer as described in [22]. After 20 s the samples were centrifuged (30 s, 10000 g) and 50 μ l of the resulting supernatant was mixed with 200 μ l of sample buffer [23] and heated at 95 °C for 5 min.

In the experiments to study the secretory phosphoprotein, the parenchymal cells were challenged with non-parenchymal-liver-cell media or prostaglandins during 1 h. Subsequently the samples were rapidly cooled to 0 °C and centrifuged (1 min, 500 g), and the supernatant was centrifuged again (5 min at 20000 g); 50 μ l of the resulting supernatant was mixed with 200 μ l of sample buffer [22] and heated for 5 min at 95 °C.

The resulting samples were separated by one- [23] and two- [24] dimensional gel electrophoresis. Dried gels were exposed to Kodak SB-5 autoradiography films, and exposure times were checked to give a linear response [25]. Autoradiographs of one-dimensional gels were quantified with a Vitatron TLD 100 spectrophotometer. Spots from two-dimensional gels were cut out, and radioactivity was measured by liquid-scintillation counting.

Kupffer and endothelial liver cells were isolated by collagenase (type I) perfusion at 37 °C and subsequent counterflow centrifugation as described in [26], except for the first elutriation step, which was replaced by a centrifugation step (2 min, 75 g). Kupffer cells were > 90% pure; endothelial liver cells were 99% pure. The cells were incubated at 37 °C with constant shaking at 0.5–2 mg of protein/ml in RPMI 1640 medium, saturated with O_2/CO_2 (19:1), pH 7.4, keeping the viability > 95%. After a 10 min preincubation, the cells were washed and incubated again. After 1–2 h conditioned media were collected.

Phosphorylase *a* activity was determined in parenchymal-liver-cell extracts prepared as described in [13] with an assay described in [27] in the presence of 0.5 mM-caffeine.

RESULTS AND DISCUSSION

The influence of Kupffer- and endothelial-liver-cell media on the phosphorylation state of parenchymal-liver-cell proteins was studied. Fig. 1 shows that the phosphorylation of an M_r -63000 band is inhibited by both types of conditioned media, whereas the phosphorylation of an M_r -47000 and an M_r -97000 band is enhanced by both media. These data clearly show that media of both non-parenchymal-liver-cell types, i.e. Kupffer and endothelial cells, can influence the phosphorylation state of some specific parenchymal-cell proteins, whereas the phosphorylation state of most of the protein is unaffected. Since Kupffer cells can produce

Fig. 1. Influence of conditioned Kupffer- (KC) and endothelial-liver-cell (EC) media on the phosphorylation state of parenchymal-liver-cell proteins

Parenchymal-cell suspension (150 μ l) was challenged with 50 μ l of conditioned media for 5 min. A longer-exposed film is also shown for the M_r -97000 band.

several prostaglandins [4–6], and endothelial liver cells were recently also shown to possess this capacity [28], we added prostaglandins in order to determine if prostaglandins could mimic the effect of non-parenchymal-liver-cell media.

As shown in Fig. 2 and Table 1, prostaglandins D₂ and E₂ have the same effect as the non-parenchymal-cell media on the phosphorylation state of parenchymal-cell proteins, i.e. inhibition of the phosphorylation of an M_r -63000 protein and enhanced phosphorylation of proteins of M_r 47000 and 97000. The facts that prostaglandins mimic the effect of Kupffer- and endothelial-liver-cell media, and are also produced by these cells [28], suggest that prostaglandins are the active factor in these media.

Since conditions media from Kupffer and endothelial liver cells and prostaglandins bring about both enhanced and decreased phosphorylation of specific proteins in parenchymal liver cells, they are likely to act on specific intracellular targets which might involve both protein kinase and phosphatase activity. Further studies are, however, needed to establish the intracellular mechanism of action.

Fig. 2. Influence of prostaglandin (PG) D_2 and E_2 on the phosphorylation state of parenchymal liver cell proteins

Parenchymal liver cells were challenged with $1 \mu\text{M}$ -prostaglandins for 5 min.

It has been suggested [7–10] that in the regulation of glycogenolysis by phorbol ester and platelet-activating factor in parenchymal cells the effect is mediated by non-parenchymal cells. This suggestion is based on studies with perfused liver in which it is shown that the effect of phorbol ester and platelet-activating factor can be blocked by indomethacin [7–12,15,16]. More recently this hypothesis was substantiated in experiments showing that in the perfused liver phorbol ester stimulates production of prostaglandin D_2 [17]. Prostaglandin D_2 is the major eicosanoid product of both Kupffer and endothelial liver cells [28], and it stimulates glycogenolysis both in perfused liver and in isolated parenchymal liver cells [17]. Our present experiments with purified cells show that Kupffer and endothelial liver cells form prostaglandins in such quantities that effective specific changes in protein phosphorylation in parenchymal liver cells can be induced. The enzyme which regulates glycogenolysis in parenchymal liver cells is phosphorylase, and its activity depends completely on its phosphorylation state. It is likely that this enzyme

Table 1. Effect of conditioned Kupffer- and endothelial-liver-cell media and prostaglandins E_1 , E_2 and D_2 on the phosphorylation state of the M_r -97 000, M_r -68 000 and M_r -47 000 proteins

Data are expressed as means $\pm$ s.d. of four experiments (all values are significantly different from control, $P < 0.05$).

Protein ...	Change in phosphorylation (fold over control)		
	M_r 97 000	M_r 68 000	M_r 47 000
Kupffer-cell medium	2.7 ± 0.4	0.62 ± 0.04	2.3 ± 0.3
Endothelial-cell medium	2.4 ± 0.3	0.55 ± 0.07	1.8 ± 0.2
Prostaglandin E_1	5.8 ± 0.7	0.61 ± 0.04	1.9 ± 0.3
Prostaglandin E_2	6.4 ± 0.5	0.66 ± 0.06	1.7 ± 0.2
Prostaglandin D_2	9.3 ± 0.6	0.74 ± 0.05	1.8 ± 0.1

Fig. 3. Influence of conditioned Kupffer- (KC) and endothelial-liver-cell (EC) media on the amount of $[^{32}\text{P}]$ phosphate in the M_r -63 000 secreted phosphoprotein

Parenchymal-liver-cell suspension ($350 \mu\text{l}$) was challenged with $50 \mu\text{l}$ of conditioned media or $50 \mu\text{l}$ of RPMI medium (control) during 60 min.

Table 2. Effect of conditioned Kupffer- and endothelial-cell media and prostaglandins E₁, E₂ and D₂ on the activity of phosphorylase α in parenchymal-cell extracts

Results are expressed as means $\pm$ s.d. of four or five experiments; the level of significant difference from control is indicated. Parenchymal cells were challenged for 10 min with additives; 50 μ l of medium was added to 450 μ l of parenchymal-cell suspension.

	Phosphorylase α activity (pmol/s per mg of protein)	Stimulation factor
Control	316 $\pm$ 67	
Endothelial-cell medium	483 $\pm$ 67 ($P < 0.001$)	1.53
Kupffer-cell medium	633 $\pm$ 83 ($P < 0.005$)	2.00
Prostaglandin D ₂ (1 μ M)	550 $\pm$ 33 ($P < 0.001$)	1.74
Prostaglandin E ₁ (1 μ M)	517 $\pm$ 67 ($P < 0.001$)	1.63
Prostaglandin E ₂ (1 μ M)	483 $\pm$ 217 ($P < 0.05$)	1.53
Prostaglandins E ₁ , E ₂ and D ₂ (each 1 μ M)	550 $\pm$ 83 ($P < 0.001$)	1.74

Table 3. Effect of conditioned Kupffer- and endothelial-liver-cell media and prostaglandins E₁, E₂ and D₂ on the amounts of [³²P]phosphate and [³H]fucose detected in the M_r-63000 secreted phosphoprotein

Data are expressed as means $\pm$ s.d. of four experiments;
*significantly different from control ($P < 0.05$); n.d., not
determined.

	Change in incorporation (fold over control)	
	[³² P]Phosphate	[³ H]Fucose
Kupffer-cell medium	0.79 $\pm$ 0.04*	0.97 $\pm$ 0.03
Endothelial-cell medium	0.81 $\pm$ 0.05*	0.95 $\pm$ 0.04
Prostaglandin E ₁	0.78 $\pm$ 0.05*	n.d.
Prostaglandin E ₂	0.68 $\pm$ 0.97*	n.d.
Prostaglandin D ₂	0.68 $\pm$ 0.06*	1.01 $\pm$ 0.02

correlates with the phosphorylation band of M_r 97000 [29]. We therefore studied the effect of Kupffer- and endothelial-liver-cell media and prostaglandins on the activity of phosphorylase in parenchymal-liver-cell extracts, as shown in Table 2. Both Kupffer- and endothelial-cell media and prostaglandins D₂, E₂ and E₁ stimulated phosphorylase activity. These data are consistent with the assumption that the M_r-97000 phosphoprotein is phosphorylase.

Parenchymal liver cells secrete a single phosphoprotein, of M_r 63000 and pI 5.0–5.5, which has been shown to be a negatively regulated acute-phase protein [18,19]. The influence of Kupffer- and endothelial-liver-

Fig. 4. Influence of prostaglandin D₂ on the amount of [³²P]phosphate in the M_r-63000 secreted phosphoprotein

Parenchymal liver cells were challenged with 1 μ M-prostaglandin D₂ (PGD₂) for 60 min.

cell media and prostaglandins D₂, E₂ and E₁ on the phosphorylation of this protein was studied. Fig. 3 shows that both Kupffer- and endothelial-cell media decrease the amount of [³²P]phosphate detected in the secreted phosphoprotein. The amount of [³²P]phosphate was significantly decreased by about 20% (Table 3). The inhibitory influence of prostaglandin D₂ on the amount of [³²P]phosphate detected in the secreted phosphoprotein is shown in Fig. 4. The amount of [³²P]phosphate in the secreted phosphoprotein was also inhibited by prostaglandins E₂ and E₁ (Table 3). To investigate the specificity of the decrease in [³²P]phosphate in the secretory phosphoprotein, experiments were performed in which the [³H]fucose incorporation in the secreted M_r-63000 protein was quantified (previous data [18,19] showed a high content of carbohydrate residues). The incorporation of [³H]fucose into the secreted phosphoprotein was not influenced by non-parenchymal-liver-cell media or prostaglandins, indicating that the phosphorylation was influenced selectively (Table 3).

In conclusion, the present data show that Kupffer and endothelial liver cells secrete products which can influence the phosphorylation state of some specific proteins in parenchymal liver cells. Since the same specific effects can be brought about by prostaglandins, it is likely that prostaglandins are the active components present in conditioned media of Kupffer and endothelial cells. Since

prostaglandin D₂ is the most prominent prostaglandin produced by these cells, it is likely that the effects resulted from the action of prostaglandin D₂. These data sustain the concept of cellular communication between the various liver cell types, and it can be concluded that the products from non-parenchymal liver cells do not exert a general effect on parenchymal cells, but influence specific intracellular targets. These specific changes might form an additional possibility for the liver to adapt hepatic metabolism to extra-hepatic signals.

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