Hormonal Control of Glycogenolysis in Parenchymal Liver Cells by Kupffer and Endothelial Liver Cells*

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Conditioned media of isolated Kupffer and endothelial liver cells were added to incubations of parenchymal liver cells, in order to test whether secretory products of Kupffer and endothelial liver cells could influence parenchymal liver cell metabolism. With Kupffer cell medium an average stimulation of glucose production by parenchymal liver cells of 140% was obtained, while endothelial liver cell medium stimulated with an average of 127%. The separation of the secretory products of Kupffer and endothelial liver cells in a low and a high molecular weight fraction indicated that the active factor(s) had a low molecular weight. Media, obtained from aspirin-pretreated Kupffer and endothelial liver cells, had no effect on the glucose production by parenchymal liver cells. Because aspirin blocks prostaglandin synthesis, it was tested if prostaglandins could be responsible for the effect of media on parenchymal liver cells. It was found that prostaglandin (PG) E1, E2, and D2 all stimulated the glucose production by parenchymal liver cells, PGD2 being the most potent. Kupffer and endothelial liver cell media as well as prostaglandins E1, E2, and D2 stimulated the activity of phosphorylase, the regulatory enzyme in glycogenolysis. The data indicate that prostaglandins, present in media from Kupffer and endothelial liver cells, may stimulate glycogenolysis in parenchymal liver cells. This implies that products of Kupffer and endothelial liver cells may play a role in the regulation of glucose homeostasis by the liver.

The liver is a major site of glycogen storage and plays a crucial role in the homeostasis of blood glucose. Glycogen synthesis and breakdown are under strict hormonal regulation. Besides the well-known stimulators of glycogenolysis, i.e. glucagon and epinephrine, whose mode of action is well defined, other factors stimulate glycogenolysis. Recently, the effect of the tumor-promoting phorbol ester, phorbol-12-myristate 13-acetate (PMA)1 on glucose release by the liver was studied, to test the possible involvement of protein kinase C (Ca2+/phospholipid-dependent enzyme) in the regulation of glycogenolysis. PMA stimulated glycogenolysis in the perfused liver (1) but failed to stimulate isolated parenchymal liver cells, the cellular site of glycogen storage (6–8). With platelet-activating factor (PAF), similar observations have been reported (2–5) although the mechanism of action of platelet-activating factor might not involve activation of protein kinase C.

Besides parenchymal cells other cell types are present in the liver, i.e. Kupffer cells, endothelial liver cells, fat-storing cells, and pit cells (9). Since PMA and PAF both act on intact liver but fail to affect glycogenolysis in isolated parenchymal liver cells, the possibility was raised that non-parenchymal liver cells may mediate the regulatory effects of PMA and PAF on glycogenolysis in the liver. The present study was undertaken to test whether Kupffer or endothelial liver cells secrete factors that may influence parenchymal liver cell metabolism.

In recent years, techniques have been developed which enable the isolation and purification of parenchymal liver cells, Kupffer cells, and endothelial liver cells (10). The isolated cells have been used to study, e.g. the relative importance of the different cell types in the receptor-mediated uptake of lipoproteins (10–12). These isolation techniques were applied to assess the relationship between various cell types in the regulation of glycogenolysis in the liver.

MATERIALS AND METHODS

Prostaglandins and collagenase type I and IV were from Sigma, 0-acetylsalicylic acid (aspirin) was from BDH, and other chemicals were of Pro Analyse quality.

Male Wistar rats, fed ad libitum, weighing 200–220 g were used. 18 mg of nembutal was given intraperitoneally for anaesthesia, usually performed between 9.00–10.00 a.m.

Parenchymal liver cells were isolated after 20 min of collagenase (type IV, 0.1%) perfusion by the method of Seglen (13), modified as previously described (14). Parenchymal liver cells were incubated at 37 °C under constant shaking at 5 mg of protein/ml in Krebs-Ringer bicarbonate buffer, saturated with O2/CO2 (95%/5%), pH 7.4, keeping the viability of the cells >95%. At 10-min intervals aliquots of cell suspensions were withdrawn, rapidly cooled to 0 °C, centrifuged at 500 x g for 5 min, and subsequently glucose was determined in the supernatant by the glucose oxidase-ABTS method (15). Zero time values were determined as follows: aliquots of the cell suspension were withdrawn and after cooling, stimuli (conditioned media or prostaglandins) were added and samples were prepared for glucose determination similar as with the other time points.

Kupffer and endothelial liver cells were isolated by collagenase (type I) perfusion at 37 °C. After collagenase digestion the liver was excised, cut into pieces, and filtered through a nylon gauze. Parenchymal and non-parenchymal liver cells were separated by differential centrifugation. In a subsequent centrifugation on a metrizamide gradient, cell debris was removed from the non-parenchymal cell fraction. Finally, Kupffer and endothelial liver cells were purified by counterflow centrifugation in a Beckman elutriation rotor. The method has been described in detail elsewhere (10) and was employed.
Liver Glycogenolysis by Non-parenchymal Liver Cells

RESULTS

In Fig. 1 it is shown that conditioned media of Kupffer and endothelial liver cells stimulate the glucose production of isolated parenchymal liver cells. For endothelial liver cell media, the increase in glucose production after 10 min was 127 ± 44%, for Kupffer cell media an increase of 140 ± 59% was found. The zero time values for the assay performed in the presence of endothelial- or Kupffer-conditioned media were not different from the control, thus indicating that no glucose was present or formed in these media. The time course of the percentual stimulation by Kupffer and endothelial liver cell media is shown in Fig. 2. The increase in glucose production is maximal at 10 min after addition of media and then the stimulation declines. A similar time course is seen for the effect of glucagon on the glucose production by parenchymal liver cells. The maximal effect of glucagon is about twice as high as the effect of the conditioned media.

In order to investigate the nature of the stimulatory factor(s) present in conditioned media of Kupffer and endothelial liver cells, low molecular weight components of the conditioned media were removed by gel filtration on Sephadex G-25. After removal of low molecular weight components, the stimulatory effect of endothelial liver cell and Kupffer cell media was mostly abolished (Fig. 3). These data suggest the involvement of low molecular weight factors in the stimulatory effect of non-parenchymal liver cell media on the glucose production by parenchymal liver cells. Since prostaglandins may act as intercellular messengers, the effect of individual prostaglandins on the glucose production by isolated parenchymal liver cells was studied. It appeared that prostaglandin E₃, E₇, and D₇ stimulate the glucose production by isolated parenchymal liver cells (Fig. 4). The stimulation at 10 min...
after addition of prostaglandin E₁ (21 ± 4%, n = 4) and prostaglandin E₂ (28 ± 14, n = 4) was significantly smaller than the stimulation by prostaglandin D₂ (68 ± 12%, n = 5) and a mixture of prostaglandins E₁, E₂, and D₂ (63 ± 11%, n = 4).

The time course of stimulation of glucose production by parenchymal liver cells by prostaglandin D₂ was similar to that by Kupffer and endothelial liver cell media (Fig. 5). Prostaglandins E₁ and E₂ also had a similar time course of stimulation (data not shown).

Since it is known that Kupffer cells can produce several prostaglandins (20–22), it seems possible that prostaglandins are the active factor(s) in the conditioned media of Kupffer and endothelial liver cells. To test this hypothesis, Kupffer and endothelial liver cells were preincubated in the presence of aspirin, a well-known irreversible inhibitor of cyclooxygenase, to obtain prostanoid-free conditioned media. These were obtained from cells preincubated for 1 h with aspirin, the cells were washed in order to remove aspirin and subsequently incubated for 1 h in order to obtain the conditioned media.

Conditioned media of endothelial liver cells gave a stimulation at 10 min of 208 ± 61% (n = 4), aspirin preincubation reduced the stimulation by endothelial liver cell media completely. The stimulation at 10 min by conditioned Kupffer cell media of 149 ± 39% was also reduced completely by pretreatment of Kupffer cells with aspirin (Fig. 6).

In order to verify the intracellular target in parenchymal liver cells of Kupffer and endothelial cell media and prostaglandins, we measured the activity of phosphorylase, the enzyme responsible for glycogen breakdown and considered to be the regulatory site for this process.

In Table I it is shown that at 10 min after addition of Kupffer or endothelial cell media or prostaglandin E₁, E₂, or D₂, the phosphorylase activity, measured in parenchymal liver cell extracts, is stimulated. If measured 30 min after addition, this stimulation had disappeared (data not shown), resembling the time course shown in Figs. 2 and 5 for glucose production.
Stimulation of Liver Glycogenolysis by Non-parenchymal Liver Cells

FIG. 5. Time course of prostaglandin D2 stimulation of glucose production by parenchymal liver cells. Data are mean ± S.E. of four experiments.

DISCUSSION

Products of Kupffer and endothelial liver cells were shown to enhance the glucose production of isolated parenchymal liver cells. Because of limiting experimental conditions, e.g. the yield of Kupffer and endothelial liver cells obtained during isolation, it was not possible to exactly define the maximal extent of stimulation. With different amounts of medium of the same batch of cells, we achieved a near-linear dose response relationship (data not shown), indicating that maximal stimulation had not yet been reached.

Non-parenchymal liver cells synthesize and secrete several products including various proteins (27, 28). Gel filtration indicated that the factor(s) in non-parenchymal liver cell media responsible for the effect on glucose production by parenchymal liver cells, was (were) not of high molecular weight nature. In the low molecular weight region, prostaglandins E1 and E2 were shown earlier to be important products of Kupffer cells (20-22). Results from our laboratory (30) show that endothelial liver cells also produce several prostaglandins and that the main prostaglandin present in conditioned media of both Kupffer and endothelial liver cells is prostaglandin D2. For this reason we further explored the possibility that prostaglandins are the stimulating factor(s) in non-parenchymal cell media.

Prostaglandin D2 added to parenchymal liver cells proved to be the most effective prostaglandin in stimulating glucose production and since it is the most abundant eicosanoid product of both Kupffer and endothelial liver cells (30), it seems to be a good candidate for the putative factor(s) in non-parenchymal cell media. The stimulation obtained by high concentrations (10^-6 M) of prostaglandins should be expected to be larger than that of non-parenchymal liver cell media. It seems possible, however, that a physiological combination of prostanoids is needed for the full expression of their effect. Furthermore, it should be realized that prostaglandins are rapidly metabolized by parenchymal liver cells (23-26), resulting in a decline in prostaglandin concentration during the incubation. The prostanoid nature of the non-parenchymal liver cell media factor(s) which is (are) responsible for the stimulation of glucose production in parenchymal liver cells is also strongly suggested by the finding that preincubation of Kupffer and endothelial liver cells with aspirin fully depresses the stimulatory effect of non-parenchymal liver cell media. Since aspirin is an irreversible inhibitor of cyclooxygenase, it blocks the formation of prostaglandins.

The intracellular mediator, which mediates the increase in glucose production, is shown to be phosphorylase, and its activity was found to be increased under conditions stimulatory for glucose production. The possible involvement of factors of non-parenchymal liver cell types in the regulation of glycogenolysis has been suggested on the basis of experiments with the perfused liver (1-8). PMA and PAF were shown to stimulate glycogenolysis in the perfused liver but failed to act on isolated parenchymal liver cells. Furthermore, the glycogenolytic effect of PMA and

TABLE I

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

Results are expressed as means ± S.D. of 4-5 experiments, and the level of significant difference from control is indicated. Parenchymal liver cells were challenged for 10 min with additives. 50 µl of media was added to 450 µl of parenchymal cell suspension.

| Media                        | Phosphorylase activity (nmol glucose produced/mg protein/min) | % stimulation
|------------------------------|-------------------------------------------------------------|------------------
| Control                      | 19 ± 4                                                     | 100              |
| Endothelial cell medium      | 29 ± 4 (p<0.001)                                          | 55               |
| Kupffer cell medium          | 38 ± 5 (p<0.005)                                          | 97               |
| Prostaglandin E1, 10^-6 M    | 31 ± 4 (p<0.001)                                          | 60               |
| Prostaglandin E2, 10^-5 M    | 29 ± 15 (p<0.05)                                          | 55               |
| Prostaglandin D2, 10^-5 M    | 33 ± 2 (p<0.001)                                          | 71               |
| Prostaglandin E1, E2, D2, 10^-4 M | 33 ± 5 (p<0.001)                                      | 71               |

FIG. 6. Effect of aspirin preincubation of Kupffer (KC) and endothelial (EC) liver cells on the stimulatory effect of Kupffer and endothelial cell media on the glucose production by parenchymal cells. 50 µl conditioned medium of untreated (○) or aspirin-preincubated (●) Kupffer or endothelial liver cells were added to 450 µl of a parenchymal liver cell suspension and compared to control incubations. Zero time values were determined as described under “Materials and Methods.” Data are expressed as mean stimulation (%) ± S.E. of four experiments.
Stimulation of Liver Glycogenolysis by Non-parenchymal Liver Cells

PAF in perfused liver can be abolished by the cyclooxygenase inhibitor indomethacin and the phospholipase A\(_2\) inhibitor bromophenacyl-bromide (1, 4, 29), suggesting that prostaglandins may mediate the PMA and PAF stimulation of glycogenolysis. Our experiments with the reconstituted liver cell system directly prove that both Kupffer and endothelial liver cells can secrete factors, probably prostaglandins, that can modulate parenchymal cell metabolism. The physiological involvement of the non-parenchymal liver cell types in the regulation of the glucose homeostasis maintained by parenchymal liver cells clearly extends the regulatory potential of this process and introduces the concept of cellular communication as an additional system for metabolic regulation in the liver.

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REFERENCES