STUDIES ON THE STIMULATION OF GLUCONEOGENESIS AND LIPOLYSIS BY GLUCAGON AND EPINEPHRINE IN ISOLATED RAT KUPFFER CELLS*

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SUMMARY

Kupffer cells were isolated by collagenease-pronase treatment. Activity and leakage of GOT, GPT, LDH, GlDH and of nucleotide pyrophosphatase were measured and compared to parenchymal cells. In addition, the effects of glucagon and epinephrine on gluconeogenesis and lipolysis were studied. Both glucagon and epinephrine stimulated gluconeogenesis from lactate and alanine. The epinephrine response, however, was far greater than that of glucagon. Additional studies showed a 50% stimulation of lipolysis by epinephrine with triolein and tripalmitin as substrates. No stimulation of lipolysis was observed with glucagon.

Recently several methods have been developed to isolate parenchymal (1-4) and Kupffer cells (5-7) from rat liver. The isolated cells provide an unique opportunity for studying the chemical, enzymatic, hormonal and morphological characteristics of the two major cell types found in mammalian liver. Extensive studies have been reported on hormonal responses in parenchymal cells (8-12), but not in isolated Kupffer cells.

In this communication we wish to report a rapid method for obtaining large quantities of viable Kupffer cells with gluconeogenic and lipolytic activity which is stimulated by epinephrine.

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Abbreviations used: GlDH, glutamate dehydrogenase (E.C.1.4.1.2); GOT, aspartate aminotransferase (E.C.2.6.1.1); GTP, alanine aminotransferase (E.C.2.6.1.2); LDH, lactate dehydrogenase (E.C.1.1.1.27); NPPase, nucleotide pyrophosphatase (E.C.3.6.1.9).

MATERIALS AND METHODS

Female Wistar rats weighing 165-180 g (Ivanovas, Kisslegg, West Germany) were maintained on Altromin® diet (Altrogge GmbH, Lage/Lippe, West Germany). They were anaesthesized with pentobarbital, the liver removed and perfused with collagenase; hepatocytes were obtained as reported earlier (4). Kupffer cells were isolated by modifying the method of Mills and Zucker-Franklin (5) as follows: After filtration of the cell suspension containing hepatocytes and Kupffer cells, 25 ml portions (30 mg cells/ml) were incubated in Krebs-Henseleit buffer with 5.5 mM glucose and 0.25 % pronase for 90 min at 90 oscillations/min and 37°C. At the end of the incubation they were centrifuged (350 x g) for 3 min, washed three times, filtrated through a nylon filter (79 x 79 µm mesh) and resuspended in the same buffer containing 2.5% albumin, 5.5 mM glucose and an amino acid mixture according to Schimassek and Gerok (13). Experiments for gluconeogenesis were carried out with various substrates in the absence of glucose and amino acid mixture. For the studies on the incorporation of radioactivity into glucose 1.0 µCi of U-^{14}C lactate (spec. radioact. 174 µCi/µmole) or U-^{14}C alanine (spec. radioact. 50 µCi/µmole) was added. Cells were incubated under an atmosphere of 95% O_2 and 5% CO_2 at 37°C in 1 ml of medium containing 25 mg of cells. The gas phase was renewed every 60 min. Incubations were performed in stoppered plastic vials (Nalgene 2002) at 90 oscillations per min in a metabolic incubator. At the end of the incubation the vial contents were placed in Eppendorf cups and centrifuged. Aliquots of the supernatants were taken for analysis. Glucose was estimated by the glucose oxidase method (14). Radioactive glucose was isolated as phenylosazone as described previously (15). The rate of lipolysis was determined by titration of the liberated free fatty acids by the Dole method (16) as reported previously (17). The activities of GOT, GPT, LDH, GlDH and NPPase were assayed as described previously (18).

RESULTS AND DISCUSSION

The Kupffer cells shown in Figure 1 are typical for the cells isolated in the present study. Isolated Kupffer cells were completely dissociated from each other and demonstrated large distinct nuclei and intact cell membranes; only very few broken cells were seen. Less than 2% of the cells could be stained by trypan blue. They were also essentially free from red blood cells and hepatocytes. The average yield was 110 mg of Kupffer cells from a 180 g rat; approximately 1.63 x 10^9 cells were found to be equal to 1 g of wet weight.

The activities of GlDH, GOT, GPT, LDH and of NPPase are shown in Figure 2. Only GOT activity was higher in Kupffer cells than in hepatocytes. 70% of the NPPase activity found in Kupffer cells are localisized on the plasma membrane. This agrees with

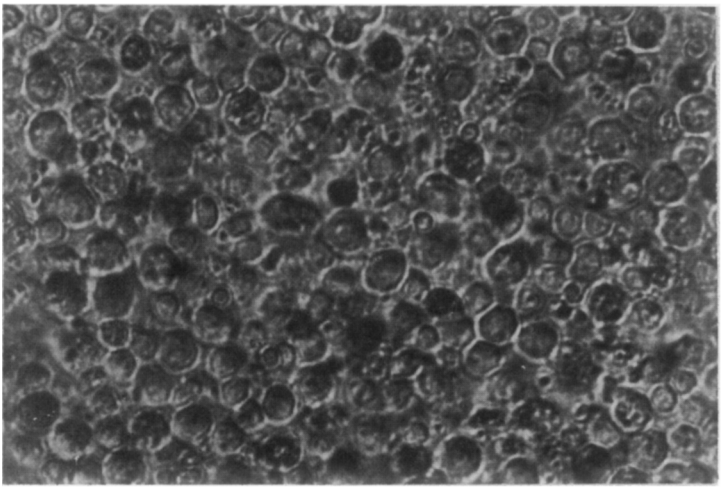

Figure 1: Light micrograph (360 x mag) of normal isolated rat Kupffer cells just prior to incubation. Kupffer cells are completely dissociated from each other and demonstrate intact cell membranes. Very few broken cells may be seen. They are almost free of red blood cells or hepatocytes.

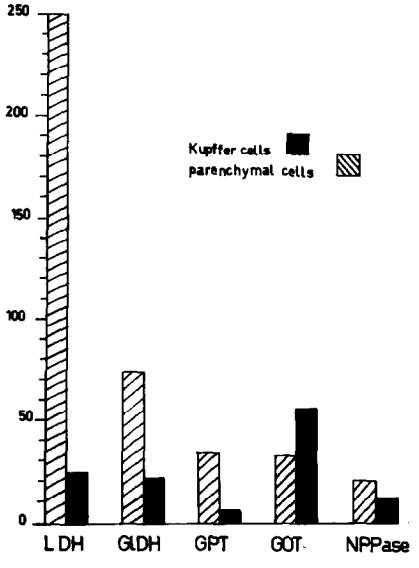

Figure 2: Comparative activity of lactate dehydrogenase (LDH), glutamate dehydrogenase (GlDH), aspartate aminotransferase (GOT), alanine aminotransferase (GPT) and nucleotide pyrophosphatase (NPPase) in isolated Kupffer and parenchymal cells. Values are given in units/g wet wt. They are the averages of five or more different preparations.

Table I

SYNTHESIS OF GLUCOSE FROM LACTATE, ALANINE, ASPARTATE, ARGININE, VALINE AND GLUTAMATE IN ISOLATED RAT KUPFFER CELLS

Substrates (2 mM)	µmoles glucose formed/g wet wt. during 30 min	60 min
lactate	1.70 ± 0.7 (4)	3.8 ± 1.0 (4)
alanine	1.6 ± 0.7 (5)	3.2 ± 0.9 (11)
aspartate	3.0 ± 1.0 (5)	8.1 ± 2.0 (5)
arginine	1.7 ± 0.4 (5)	4.2 ± 0.3 (5)
valine	1.0 ± 0.4 (5)	2.8 ± 1.0 (5)
glutamate	3.1 ± 1.0 (5)	4.0 ± 1.1 (4)

20-25 mg of Kupffer cells were incubated in 1 ml of Krebs-Henseleit buffer with 2.5% albumin. Values are given ± S.E.M. The number of observations is given in brackets.

data obtained with isolated hepatocytes where about 30% of this enzyme was found in the endoplasmic reticulum and the remainder on the plasma membrane (19). Further studies showed that the percentage of the activity of these enzymes released into the incubation medium did not increase significantly when Kupffer cells were incubated for 100 min at 37°C. Exclusion of trypan blue and low depletion of cytoplasmic enzymes into the incubation medium indicate that these isolated Kupffer cells have intact cell membranes.

Studies on gluconeogenesis from various substrates are summarized in Table I. Kupffer cells synthesized glucose from lactate, alanine, aspartate, arginine, valine and glutamate. The rate of glucose formation however, was only 10-20% of that produced by hepatocytes. This glucose synthesis was not due to glycogenolysis as no glycogen was detected in Kupffer cells. It was also not due to contamination with hepatocytes as only 12 ± 9 hepatocytes were present per 10,000 Kupffer cells in our preparation. Studies with radioactive U-^{14}C lactate showed that 1563 ± 350 and 2684 ± 540 cpm/g were incorporated into

Table II

STIMULATION OF GLUCONEOGENESIS FROM ALANINE AND LACTATE BY GLUCAGON AND EPINEPHRINE IN ISOLATED RAT KUPFFER CELLS*

Substrate and hormone additions	(mM)	µmoles glucose formed/g wet wt. during 1 h		2 h	
lactate	2	3.5 ± 1.2	(7)	4.4 ± 1.4	(7)
lactate + glucagon	2 10^{-5}	4.7 ± 1.3	(4)	5.6 ± 1.4	(4)
lactate + epinephrine	2 10^{-3}	9.3 ± 2.0	(4)	9.7 ± 2.3	(4)
alanine	5	2.9 ± 0.9	(7)	4.1 ± 1.6	(7)
alanine + glucagon	5 10^{-5}	4.1 ± 1.0	(4)	5.4 ± 1.4	(4)
alanine + epinephrine	5 10^{-3}	6.3 ± 1.0	(4)	6.8 ± 1.3	(4)

*Experimental conditions are the same as in Table I

glucose in 30 and 60 min, respectively; similarly, 2049 ± 460 and 3707 ± 380 cpm/g were incorporated from U-^{14}C alanine into glucose in 30 and 60 min, respectively, indicating de novo synthesis of glucose from these substrates. Both glucagon and epinephrine stimulated gluconeogenesis in isolated Kupffer cells (Table II). Epinephrine, however, was far more effective than glucagon in stimulating glucose synthesis from lactate and alanine. This is in contrast to hepatocytes where glucagon is more effective than epinephrine (8, 20).

Studies on lipolysis are summarized in Table III. Isolated Kupffer cells showed lipolytic activity with tributyrin, tripalmitin and triolein as substrates. The high activity observed with tributyrin may be due to an esterase activity since it was not stimulated by epinephrine or by glucagon. A stimula-

Table III

EFFECT OF GLUCAGON AND EPINEPHRINE ON LIPOLYSIS IN ISOLATED RAT KUPFFER CELLS*

Hormone addition	Substrates		
	tributyrin	tripalmitin	triolein
	Free fatty acids liberated (µmoles/g/h)		
none	1250 ± 100 (6)	95 ± 16 (6)	363 ± 78 (6)
glucagon (10^{-8}M)	1290 ± 77 (3)	95 ± 22 (3)	323 ± 68 (3)
epinephrine (10^{-6}M)	1120 ± 93 (3)	140 ± 14 (3)	530 ± 50 (3)

*20-30 mg of Kupffer cells were incubated in 1 ml of Krebs-Henseleit buffer containing 2.5% albumin, 5.5 mM glucose, an amino acid mixture (13) and 10 mM triglycerides. The number of observations is given in brackets

tion of lipolytic activity by epinephrine was, however, observed in Kupffer cells with triolein and tripalmitin as substrates. On the other hand, addition of glucagon had no effect on lipolysis in Kupffer cells.

The results presented here show that Kupffer cells differ in hormonal responses from hepatocytes. Epinephrine was far more effective than glucagon in stimulating both lipolysis and gluconeogenesis in Kupffer cells whereas glucagon is more effective in hepatocytes (8, 20). It is well known that cyclic AMP stimulates both gluconeogenesis (21) and lipolysis (22). Recently Wincek et al. (23) reported that epinephrine stimulates cyclic AMP formation in Kupffer cells while glucagon is ineffective. The present results are in agreement with this observation - that the marginal glucagon response observed in Kupffer cells may be due to its failure to stimulate cyclic AMP formation which is needed for triggering metabolic events.

To our knowledge this is the first report showing a hormonal response of gluconeogenesis and lipolysis in Kupffer cells. Although this response is relatively small it may contribute to the total metabolic activity in the liver.

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