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KUPFFER CELLS PLAY A MAJOR ROLE IN INSULIN-MEDIATED HEPATIC GLUCOSE UPTAKE IN VIVO

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SUMMARY: The effect of insulin on the *in vivo* glucose utilization by different hepatic cells was investigated using the euglycemic, hyperinsulinemic clamp, combined with the 2-deoxyglucose tracer technique. Rats were infused with insulin at a rate of 2.8 or 9.0 mU/min/kg for 220 min, resulting in plasma concentrations of the hormone of about 80 μU/ml and 340 μU/ml, respectively. Glucose use by the whole liver was elevated by more than 200 % following insulin. However, glucose uptake by the parenchymal cells was only elevated by 50-60 %. By contrast nonparenchymal cells were more responsive to insulin. Glucose uptake by endothelial cells was increased 100 % and Kupffer cells displayed the most marked response to insulin showing a 3- to 6-fold increase in glucose uptake. These data indicate that the sinusoidal nonparenchymal cells are the major sites of the insulin-mediated increased glucose utilization by the liver.

Despite the voluminous literature on various aspects of the regulation of hepatic glucose uptake and production, this subject is still under debate with contradicting experimental findings and interpretations (for review see refs. 1,2). It is generally accepted that the liver is an insulin sensitive organ, as it expresses increased glycogen synthesis and lipogenesis from precursors in response to insulin both *in vivo* in whole liver tissue and in hepatocyte cultures (2,3). Furthermore, insulin also causes a dose-dependent inhibition of hepatic glucose output. However, while insulin dramatically increases glucose utilization in adipose tissue and muscle, net hepatic glucose uptake takes place only under special experimental conditions, such as very high glucose concentrations (1,2). Most of the studies aiming to elucidate the regulation of hepatic glucose uptake by insulin have not considered the potential importance of hepatic nonparenchymal cells in this process. The nonparenchymal cells represent more than 30 % of total hepatic cells, and may account for up to 8 % of the total liver protein. Their functional characteristics, as important contributors to the reticuloendothelial system, are different from that of parenchymal cells (4,5). Recently, we have shown that following an overnight fast endothelial and Kupffer cells are important contributors to the basal glucose consumption in the liver *in vivo* (6) and *in vitro* (7).

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We also demonstrated that the glucose use by this cellular compartment was increased several-fold following immune stimulation by bacterial endotoxins (8), cytokines (9,10), or during *in vivo* phagocytosis (11). In the present paper we report the contribution and importance of nonparenchymal cells to hepatic glucose utilization in response to insulin.

MATERIALS AND METHODS

Male Sprague-Dawley rats (300-340 g, Charles River, Wilmington, MA) were used for the study. On the day preceding an experiment, rats were anesthetized and two catheters were placed in the jugular vein and one in carotid artery using aseptic surgical procedures (12). Animals were fasted overnight with free access to water. The experiments were performed in accordance with the NIH guidelines for the use of laboratory animals.

A euglycemic hyperinsulinemic clamp was performed to assess *in vivo* insulin action (12). Briefly, porcine insulin (Eli Lily, Indianapolis, IN) was dissolved in 0.9 % saline containing 0.3 % human serum albumin and administered intravenously (iv) as a primed-constant infusion. Insulin was infused at a rate of 2.8 or 9 mU/min/kg which resulted in steady-state plasma insulin concentrations of approximately 80 and 340 μU/ml after 180 min. We reported previously that under the same experimental conditions plasma glucagon concentrations were not altered by insulin infusion (12). Control animals were infused with 0.9% saline containing human albumin. To maintain euglycemia, 30 % glucose was also infused into hyperinsulinemic animals through the second iv catheter (1-2 ml/h), and flow rate was adjusted to sustain plasma glucose at about 5 mM. Blood glucose concentrations were determined at 10 min intervals after starting the insulin infusion (YSI glucose analyzer; Yellow Springs, OH). Control animals were infused with a comparable volume of saline.

Hepatic nonparechymal cells were isolated using a modified version of the collagenase perfusion (4) and pronase digestion method as we described previously (13). After the digestion and differential centrifugation, the nonparenchymal cells were subjected to centrifugal elutriation using a J2-21 centrifuge with J6B elutriator rotor (Beckman Instruments Inc.) (14). Hepatic endothelial cells were eluted at a flow rate of 23 ml/min, and a mixture of endothelial and Kupffer cells at 29 ml/min. Kupffer cells at higher than 90 % purity were eluted at flow rate of 45 ml/min (fraction 1), and large Kupffer cells with contaminating parenchymal cells were eluted at zero rpm, flow rate of 45 ml/min. The latter cell suspension was subjected to gradient centrifugation (Histopaque 1077) to eliminate contaminating parenchymal cells (15). Purity of this Kupffer cells preparation (fraction 2) was higher than 98 %. Viability of isolated parenchymal and nonparenchymal cells as assessed by the trypan blue exclusion was greater 90 and 98 %, respectively.

In vivo glucose utilization (Rg) in individual tissues and isolated cells was assessed by the determination of the accumulated phosphorylated metabolites of 2-deoxy-D-[U-^{14}C]glucose after its intravenous injection as described previously (16). The specific activity of the tracer and plasma glucose concentration during the 40 min *in vivo* labeling period were determined from serial arterial blood samples, and Rg calculated as described earlier (16).

The concentration of plasma insulin was measured by radioimmunoassay using porcine standards (ICN/Micromedic, Horsham, PA). Plasma glucose during the radioisotope labeling period was determined enzymatically (16).

Unless otherwise mentioned all chemicals and reagents were obtained from Sigma Fine Chemicals, St. Louis, MO.

For statistical analysis of the data the one-way ANOVA was employed followed by Student-Newman-Keuls test.

RESULTS AND DISCUSSION

Insulin infusion at a rate of 2.8 and 9.0 mU/min/kg resulted in plasma insulin concentrations of 81 ± 12 μU/ml and 337 ± 56 μU/ml, respectively. These values were determined at 180 min, however, we have previously demonstrated that this infusion regime produces steady-state plasma insulin levels between 1-3 h (12). The insulin concentration in saline-infused control animals was 27 ± 3 μU/ml (means ± S.E.M., n=5-9). The lower insulin concentration achieved in our experiments is comparable to that found in the hepatic sinusoids following carbohydrate intake (17), while the higher dose represents a pharmacological concentration which is known to completely supress hepatic glucose output in rats (1,17). Plasma glucose concentrations in both treatment groups were in the physiological range as measured during the radioisotope labeling period (180-220 min of insulin infusion), (Fig 1), indicating that glucose metabolism in these animals was in a steady-state. As expected, plasma decay of the radiolabel was accelerated following insulin treatments (Fig 1), suggesting that whole body glucose utilization was elevated, in accordance with our earlier findings (12,18).

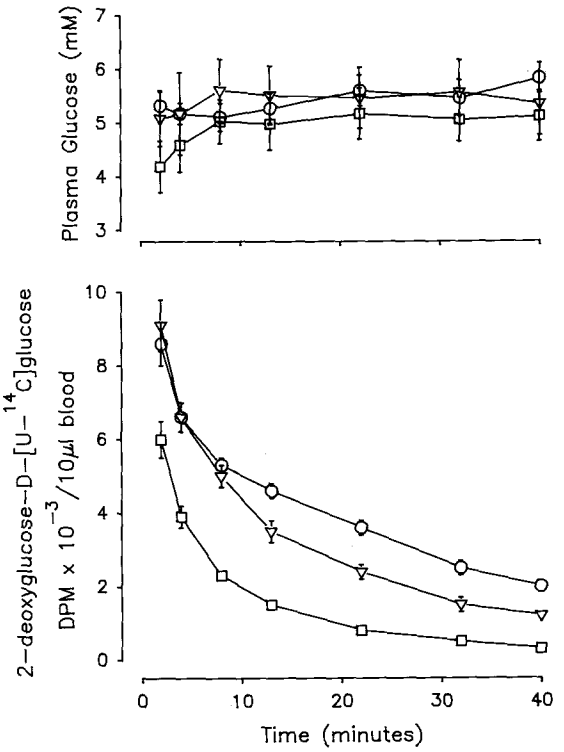

Fig. 1. Plasma glucose concentration and radioisotope decay during the 180-220 min period of hyperinsulinemic, euglycemic clamp. After 3h infusion of saline (○), or insulin at a rate of 2.8 mU/h/kg (▽), or 9 mU/h/kg (□), the tracer was injected as a bolus, and serial arterial blood samples were withdrawn to determine the concentration of plasma glucose and radioactive tracer. Symbols indicate means ± S.E.M., n=5-9.

Table 1. Glucose utilization rate (Rg) following *in vivo* infusion of insulin

Tissues and cells	Saline (30 μU/ml)	Insulin (80 μU/ml)	Insulin (340 μU/ml)
Whole liver[a]	23.9 ± 2.2	76.6* ± 11.9	64.5* ± 8.0
Parenchymal cells[b]	2.1 ± 0.3	3.5 ± 0.3	3.2 ± 0.5
Endothelial cells[b]	1.4 ± 0.1	2.6* ± 0.4	3.0* ± 0.3
Mixture[b]	2.8 ± 0.5	8.6* ± 1.9	10.6* ± 1.9
Kupffer cells[b]			
Fraction 1	3.3 ± 0.5	18.5* ± 4.3	13.6* ± 2.3
Fraction 2	3.6 ± 0.6	20.2* ± 4.4	12.0* ± 3.4
Testis[a]	168.1 ±10.6	153.9 ± 7.6	161.7 ± 9.9

Superscripts [a] and [b] indicate glucose metabolic rate (Rg) in units of nmol/min/g wet weight and nmol/h/10^6 cells, respectively. Mean ± S.E.M., n=5-9. Asterisks indicate statistically significant difference as compared with saline treatment, at the level of $p<0.05$. Lump constant was considered 0.5 for all tissues and cells ().

There are conflicting reports regarding the effects of glucose intake and insulin on net hepatic glucose uptake (1,2). It is accepted that insulin causes glycogen deposition in the liver, however, it is disputed whether blood glucose or gluconeogenic substrates (lactate and/or amino acids) are the primary precursors for this process. In our present experiments, under euglycemic conditions, insulin caused about a 3-fold increase in glucose utilization by the whole liver at both low and high concentrations of the hormone (Table 1). The parenchymal cells, which represent the dominant mass of the liver, however, increased their glucose use by only 50 to 60 %. This indicates that other cellular compartments of the liver are responsible for the insulin-stimulated increase in glucose uptake. Accordingly, hepatic nonparenchymal cells expressed a marked increase in their glucose utilization following insulin. Endothelial cells doubled, while the Kupffer cells (fraction 1) increased their glucose uptake by 300-460 % in response to the hormone (Table 1). The glucose metabolic response of the highly phagocytic large Kupffer cells (fraction 2) was similar to that of Kupffer cells of smaller size. Considering that the hepatic endothelial and Kupffer cells represent about 20-35 % and 10-15 % of total liver cells, respectively (4,5), our findings indicate that the nonparenchymal cells are the predominant contributors to the insulin-induced increase in glucose use by the liver. We estimate that at least two-thirds of the insulin-stimulated increase in glucose uptake is accounted for by the metabolic activity of nonparenchymal cells (Fig 2). The slightly lower response of whole liver and Kupffer cells to higher plasma levels of the hormone may indicate downregulation of insulin receptors on these cells in response to the sustained high plasma concentration of the hormone. No glucose metabolic response was found in the testis, an organ with a blood-tissue barrier.

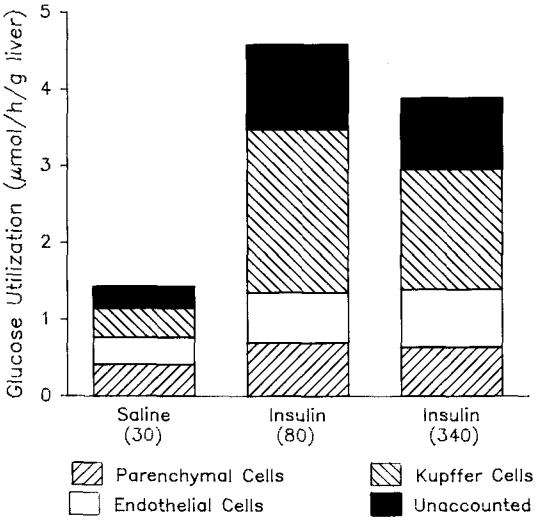

Fig. 2. Distribution of glucose utilization among parenchymal, hepatic endothelial and Kupffer cells following insulin infusion. In brackets mean values of plasma insulin concentration are shown. It was assumed that endothelial and Kupffer cells represents 4 and 4 % of total hepatic cellular protein, respectively.

The effect of insulin on hepatic nonparenchymal cells may have considerable physiologic importance. It has been shown that insulin increases glucose uptake and hexose monophosphate shunt activity in monocytes (19). Recently, we demonstrated increased glucose use and hexose monophosphate shunt activity in Kupffer cells under immune-stimulated conditions (13). This metabolic response primarily subserves increased bactericidal activity and protection against oxidative cellular injury (20). Due to the fact that endotoxemia or sepsis are accompanied by changes in the plasma concentrations of glucoregulatory hormones (21), including insulin (22), our present findings suggest that insulin may be an important factor in mediating the metabolic response of the hepatic resident macrophages and endothelial cells to bacterial infection. In agreement with our present findings it has recently been demonstrated that hepatic endothelial, Kupffer and fat storing cells express mRNA for insulin receptors (23).

In addition to the glucose metabolic effects, insulin may also be important in modulating other nonspecific immune functions of hepatic nonparenchymal cells, such as proliferation and growth by its growth hormone-like effects. The fact that insulin at low concentrations was as effective as at higher levels indicates that the metabolic effect is mediated by its interaction with the insulin rather than IGF-1 receptors on these cells. However, interaction of the hormone with the IGF-1 receptors and subsequent effects on cell metabolism and function may have also occured. It was also shown in mice that insulin inhibits the secretion of tumor necrosis factor, a cytokine secreted primarily by Kupffer cells during bacterial infection (24).

The data presented here indicate that insulin targets hepatic nonparenchymal cells *in vivo* resulting in marked changes in the metabolic, and presumably functional activities of these cells. Furthermore, the metabolic response of hepatic nonparenchymal cells, especially Kupffer cells, to insulin emphasizes that this cellular compartment is one of the major sites of insulin action in the liver.

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REFERENCES

1. Pagliassotti, M.J., and Cherrington, A.D. (1992) Annu. Rev. Physiol. 54, 847-860
2. Katz, J., and McGarry, J.D. (1984) J. Clin. Invest. 74, 1901-1909
3. Suzuki, M., Kojima, H., Nakano, T., Kashiwagi, A., Hidaka, H., Kosugi, K., Shigeta, Y., and Harano, J. (1991) Diabet. Res. Clin. Pract. 13, 163-172
4. Knook, D.L., Blansjaar, N., and Sleyster, E.C. (1977) Exp. Cell. Res. 109, 317-329
5. Brouwer, A., Wisse, E., and Knook, D.L. (1988) in The Liver: Biology and Pathobiology (I.M. Arias, W.B. Jacobi, H. Popper,, Schachter, D., and D.A. Shafritz, Eds.), pp. 665-683, Raven Press, New York
6. Mészáros, K., Bojta, J., Bautista, A.P., and Spitzer, J.J. (1991) Am. J. Physiol. 260, G7-G12
7. Spolarics, Z., Lang, C.H., Bagby, G.J., and Spitzer, J.J. (1991) Am. J. Physiol. 261, G185-G190
8. Spitzer, J.J., Mészáros, K., Bautista, A.P., Lang, C.H., and Spolarics, Z. (1990) in Cellular and molecular aspects of endotoxin reactions (Nowotny, A., Spitzer, J.J. and Ziegler, E.J. eds.) pp. 521-528, Elsevier Science Publishers
9. Spolarics, Z., Schuler, A., Bagby, G.J., Lang, C.H., Nelson, S., and Spitzer, J.J. (1991) J. Leukoc. Biol. 49, 309-312
10. Spolarics, Z., Schuler, A., Bagby, G.J., Lang, C.H., Nelson, S., and Spitzer, J.J. (1992) J. Leukoc. Biol. 51, 360-365
11. Spolarics, Z., Bautista, A.P., and Spitzer, J.J. (1991) Hepatol. 13, 277-281
12. Lang, C.H., and Dobrescu, C. (1989) Am. J. Physiol. 257, E301-E30812.
13. Spolarics, Z., Lang, C.H., Bagby, G.J., and Spitzer, J.J. (1991) Biochem. J. 278, 515-519
14. Figdon, C.G., Leemans, J.M.M., Bont, W.S., and Vries, J.E. (1983) Cell. Biophys. 5, 105-112
15. English, D., and Andersen, B.R. (1974) J. Immunol. Methods 5, 294-298
16. Mészáros, K., Lang, C.H., Bagby, G.J., and Spitzer, J.J. (1987) J. Biol. Chem. 262, 10965-10970
17. Cherrington, A.D., Pagliassotti, M.J., Myers, S.R., Adkins-Marshall, B., and McGuinness, O.P. (1991) J. Parenter. Enteral Nutr. 15, 71S-73S
18. Lang, C,H., Dobrescu, C., and Meszaros, K. (1990) Metab. 39, 1096-1107
19. Bieger, W., Weicker, H., and Michl, J. (1980) J. Clin. Endocrin. Metab. 50, 1121-1126
20. Spolarics, Z., Bautista, A.P., and Spitzer, J.J. (submitted for publication)
21. Spitzer, J.J., Ferguson, J.L., and Hirsch, H.J. (1980) Circ. Shock 7, 353-360
22. Yelich, M.R., and Filkins, J.P. (1980) Am. J. Physiol. 239, E156-E161
23. Zindy, F., Lamas, E., Schmidt, S., Kirn, A., and Brechot, C. (1992) J. Hepatol. 14, 30-34
24. Satomi, N., Sakurai, A., and Haranaka, K. (1985) J. Nat. Canc. Inst. (1985) 74, 1255-1260