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Int. J. Biochem. Vol. 17, No. 6, pp. 685–688, 1985
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0020-711X/85 $3.00 + 0.00
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GLUCOSE INFUSED THROUGH THE PORTAL VEIN ENHANCES LIVER GLUCONEOGENESIS AND GLYCOGENESIS FROM [3-^{14}C]PYRUVATE IN THE STARVED RAT

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(*Received* 11 *September* 1984)

Abstract—1. After a pulse of [3-^{14}C]pyruvate, 24 hr starved rats were infused through the portal vein with two different doses of glucose (7.8 or 20.8 mg/min) or the medium, and blood was collected from the inferior cava vein at the level of the suprahepatic veins.

2. The highest dose of glucose enhanced the appearance of [^{14}C]glucose in blood from the 2nd to the 20th min after tracer delivery. It also enhanced production of [^{14}C]glycogen and concentration of glycogen in the liver after 5 and 20 min. At 20 min of glucose infusion the appearance of [^{14}C]glyceride glycerol in liver as well as liver lactate concentration and lactate/pyruvate ratio were increased.

3. The low dose of glucose used enhanced liver values of [^{14}C]glycogen, [^{14}C]glycogen specific activity and glycogen concentration.

4. Our results support the hypothesis that in the starved rat glucose is converted into C_3 units prior to being deposited as liver glycogen and based on the liver zonation model (Jungermann *et al.*, 1983) it is proposed that glucose stimulated gluconeogenesis by shifting the liver to the cytosolic redox state as a secondary consequence of increased glycolytic activity.

INTRODUCTION

The classical belief that increments in circulating glucose above a "threshold" concentration promote its uptake by the liver to be directly deposited as glycogen has recently been questioned. Riesenfeld *et al.* (1981) found that glucose yielded very little glycogen formation by rat liver in *in vitro* preparations whereas gluconeogenetic substrates promoted substantial glycogen synthesis (Hems *et al.*, 1972; Katz *et al.*, 1979; Boyd *et al.*, 1981). It has also been shown that most *in vivo* conversion of exogenous glucose to liver glycogen occurs mostly via an indirect mechanism by which the gluconeogenic pathway, rather than the utilization of intact glucose, plays a major role in glycogen synthesis (Newgard *et al.*, 1983). In agreement with this new concept, we found a great increase in [^{14}C]glycogen in the liver of starved versus fed rats following *in vivo* injection of [3-^{14}C]pyruvate (Herrera *et al.*, 1969; Llobera and Herrera, 1980). Similar findings were reported with an *in situ* liver infusion technique (Soley *et al.*, 1983) which minimizes the problem of gluconeogenic substrate recycling that is known to vary with the animals' dietary status (Friedmann *et al.*, 1971; Okajima *et al.*, 1981). Parallel increments were found in the formation of liver [^{14}C]glycogen and [^{14}C]glyceride glycerol as compared with [^{14}C]glucose production from [3-^{14}C]pyruvate (Soley *et al.*, 1983), suggesting that in the starved condition both glycogen and glyceride glycerol synthesis are predominantly gluconeogenic processes. To determine how glucose affects these metabolic relationships in liver, in the present work we studied the effect of glucose on liver utilization of [3-^{14}C]pyruvate following its direct infusion in the portal vein to starved rats.

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MATERIALS AND METHODS

Male Wistar rats weighing 189–214 g were studied after 24 hr starvation. Animals anesthetized with an i.v. injection of 60 mg/kg body weight of sodium pentobarbital were given through the portal vein a pulse of [3-^{14}C]pyruvate (1 μCi, from The Radiochemical Center, Amersham; specific activity 15.8 mCi/mmol, containing 100 μmol of sodium pyruvate per 100 g body weight). Blood aliquots were subsequently collected at the level of the suprahepatic veins at different intervals. Surgery was based on the procedure described by Chieri *et al.* (1966), and utilized by us for gluconeogenic studies (Soley *et al.*, 1983). In summary, after laparotomy, one cannula (PE-10, Intramedic, Clay Adams, New Jersey, U.S.A.) was introduced through the ileocholic vein up to the level of the portal vein and another cannula (PE-50 Intramedic, Clay Adams, New Jersey, U.S.A.) was introduced through the right jugular vein passing by the superior cava vein up to the level of the suprahepatic vein. Following cannulation, a saline–albumin solution (0.5 g bovine albumin/liter of 0.9% NaCl) was infused through the cannula in the portal vein at the rate of 5 ml/hr for 5 min. A pulse of [3-^{14}C]pyruvate was them introduced through the cannula and a solution was infused of either glucose in saline–albumin (468 mg/5 ml/hr or 1248 mg/5 ml/hr) or saline–albumin for the controls (5 ml/hr). At different times after tracer administration, blood aliquots were collected into heparinized syringes from the cannula placed at the level of the suprahepatic veins. After the last blood collection, a piece of liver was placed into liquid N_2. The exact position of the cannulas was always verified by autopsy and values were not used unless

a suitable cannula location was determined. Blood aliquots were deproteinized (Somogyi, 1945) and supernatants were used for glucose determination (Huggett and Nixon, 1957) and [^{14}C]glucose analysis. For the latter we used a slightly modified version of our reported procedure (Herrera *et al.*, 1969). 1 ml portions of the supernatant were passed over microcolumns (i.e. 1 ml tuberculin syringes filled to the 0.3 ml mark with Dowex-1 1 × 2–400 from Sigma Co., St Louis, Missouri, U.S.A.; from the 0.3 to 0.6 ml mark with activated Duolite A-561, generously supplied by Diamond Shamrock, California, U.S.A.; and to the 0.9 ml mark with more Dowex-1 1 × 2–400) and eluted with double distilled water to obtain 3 ml of eluate. Aliquots of the eluates were used to count their radioactivity. Recovery of [^{14}C]glucose added to blood before deproteinization was above 99.63% whereas the recoveries of added [^{14}C]alanine, [^{14}C]pyruvate and [^{14}C]lactate were less than 0.37, 0.24 and 0.17%, respectively. Aliquots of the frozen livers were placed in 30% KOH at 100°C for 10 min and when cold, glycogen was precipitated with ethanol (Good *et al.*, 1933). After two more washes, glycogen precipitates were hydrolyzed with 5 N H_2SO_4 at 100°C for 2 hr, and after their neutralization, aliquots were used for radioactive count and glucose determination (Hugget and Nixon, 1957). Another aliquot of frozen liver was used for lipid extraction and purification (Folch *et al.*, 1957) and fractionation following a modified version of the method of Kerpel *et al.* (1961), as described previously (Carmaniu and Herrera, 1980). Radioactive measurements were performed in a PPO/POPOP based scintillation cocktail dissolved in xylene and Triton X-100 and samples were counted in a Nuclear Chicago (Isocap 300) counter provided with an external standard device. Quenched standards were always counted with the samples to determine the channel ratio for d.p.m. estimations. For lactate and pyruvate determinations, aliquots of plasma and frozen liver were homogenized and deproteinized in cold 6% $HClO_4$, supernatants being neutralized with saturated $KHCO_3$. Aliquots of the neutralized supernatants were used for lactate (Passonneau, 1974) and pyruvate (Passonneau and Lowry, 1974) determinations. Statistical analysis of the data was done with the Student's *t*-test.

RESULTS

After receiving a pulse of [3-^{14}C]pyruvate through the portal vein, 24 hr starved rats were infused through the same vein with either 7.8 (G-7.8) or 20.8 (G-20.8) mg glucose/min and compared with controls which received the medium. Blood samples collected at the level of the suprahepatic veins gave an index of liver utilization of the tracer. As shown in Table 1, total blood radioactivity decreased progressively after the tracer and was lower at 10 and 20 min in G-7.8 and at 20 min in G-20.8 compared with control values. [^{14}C]glucose values in blood increased until the 5th min after receiving [3-^{14}C]pyruvate in controls and in G-7.8, and until the 10th min in G-7.8 and G-20.8, and values in the latter group were significantly higher than those in controls after the 2nd min (Table 1). Blood glucose concentrations were higher in G-7.8 than in controls from the 5th min whereas they were augmented in G-20.8 from the 1st min studied (Table 1). Blood glucose specific activity was significantly higher in G-20.8 than in controls at 2 min whereas it was lower at 5, 10 and 20 min in that group and at 10 and 20 min in G-7.8 (Table 1). To determine the incorporation of radioactivity to liver glycogen and lipids, some animals from each group were killed at 5 and 20 min. As shown in Table 2, liver [^{14}C]glycogen and glycogen concentrations were much higher in both G-7.8 and G-20.8 than in controls at both 5 and 20 min after the tracer. Liver glycogen specific activity was significantly augmented at 5 min only in G-7.8, when compared with control values (Table 2). As shown in Table 3, the appearance of radioactivity in total lipids was higher at 20 min in G-20.8 than in controls, and this difference corresponded to the radioactivity present in the glyceride glycerol fraction, the formation of [^{14}C]fatty acids being almost negligible in all the groups (Table 3). Liver and plasma lactate and pyruvate concentrations were measured 5 min after the tracer in the G-20.8 and the control groups, and these values are summarized in Table 4, showing that liver lactate concentration and the lactate/pyruvate ratio were higher in G-20.8 than in controls whereas liver pyruvate and plasma lactate and pyruvate values were unchanged.

Table 1. Effects of glucose infusion through the portal vein in blood [^{14}C]glucose and glucose concentration after a pulse of [3-^{14}C]pyruvate

	Total radioactivity (d.p.m./ml)	[^{14}C]glucose (d.p.m./ml)	Glucose concentration (mg/dl)	[^{14}C]glucose specific activity (d.p.m./mg)
Control				
1 min	42933 ± 2323	3067 ± 332	71 ± 4	4244 ± 531
2 min	32390 ± 857	4580 ± 380	79 ± 4	5407 ± 442
5 min	24330 ± 779	5350 ± 464	74 ± 4	7718 ± 894
10 min	20064 ± 479	4959 ± 655	74 ± 6	6925 ± 1020
20 min	18456 ± 689	5134 ± 531	75 ± 6	6909 ± 620
Glucose (7.8 mg/min)				
1 min	43339 ± 2067	2566 ± 199	76 ± 5	3401 ± 180
2 min	31485 ± 1242	4691 ± 387	78 ± 3	5744 ± 409
5 min	22124 ± 750	4787 ± 196	96 ± 7**	5598 ± 565
10 min	17544 ± 746*	4967 ± 252	126 ± 4***	3982 ± 241*
20 min	15028 ± 599**	4103 ± 239	154 ± 13***	2701 ± 161***
Glucose (20.8 mg/min)				
1 min	46172 ± 1616	3626 ± 309	85 ± 5**	4206 ± 505
2 min	33469 ± 1277	6511 ± 409**	96 ± 6*	7203 ± 649*
5 min	23638 ± 534	7658 ± 490**	186 ± 13***	4569 ± 599**
10 min	18942 ± 681	8262 ± 542***	258 ± 16***	3614 ± 342**
20 min	15593 ± 662*	7162 ± 720*	364 ± 24***	2286 ± 317***

After receiving a pulse of [3-^{14}C]pyruvate, 24 hr starved rats were infused through the portal vein with either 7.8 or 20.8 mg/min of glucose, or the medium (controls). Blood samples were collected from the cava vein at the level of the suprahepatic veins at different times after the tracer. Results are means ± SEM of 8–16 rats/group. Statistical comparisons between values for rats receiving glucose and controls are shown by asterisks: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2. Liver [^{14}C]glycogen and glycogen concentration after a pulse of [3-^{14}C]pyruvate in 24 hr starved rats perfused with glucose through the portal vein

	[^{14}C]glycogen (d.p.m./g)	Glycogen concentration (‰)	[^{14}C]glycogen specific activity (d.p.m./mg)
Controls			
5 min	1784 ± 257	0.8 ± 0.1	1889 ± 274
20 min	2254 ± 439	0.4 ± 0.0	5139 ± 944
Glucose (7.8 mg/min)			
5 min	8861 ± 2799**	1.9 ± 0.4**	4246 ± 367***
20 min	5731 ± 884**	1.3 ± 0.2***	4746 ± 740
Glucose (20.8 mg/min)			
5 min	5386 ± 1249**	2.6 ± 0.6**	1376 ± 163
20 min	13098 ± 1811***	3.2 ± 0.5***	5021 ± 364

After receiving a pulse of [3-^{14}C]pyruvate, rats were infused through the portal vein with either 7.8 or 20.8 mg/min of glucose, or the medium (controls). Livers were frozen in liquid N_2 at 5 or 20 min after the tracer and glycogen was purified by ethanol precipitation. Results are means ± SEM of 5–16 rats/group. Statistical comparisons between values for rat receiving glucose and controls are shown by asterisks: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Table 3. Liver [^{14}C]lipids after a pulse of [3-^{14}C]pyruvate in 24 hr starved rats infused with glucose through the portal vein

	[^{14}C]total lipids (d.p.m./g)	[^{14}C]fatty acids (d.p.m./g)	[^{14}C]glyceride glycerol (d.p.m./g)
Controls			
5 min	3658 ± 376	198 ± 18	3192 ± 389
20 min	5390 ± 799	200 ± 40	5010 ± 773
Glucose (7.8 mg/min)			
5 min	—	—	—
20 min	4742 ± 457	280 ± 32	4279 ± 447
Glucose (20.8 mg/min)			
5 min	3421 ± 737	209 ± 43	3036 ± 724
20 min	7797 ± 438**	270 ± 47	7320 ± 433**

After receiving a pulse of [3-^{14}C]pyruvate, rats were infused through the portal vein with either 7.8 or 20.8 mg/min of glucose, or the medium (controls). Livers were frozen in liquid N_2 at 5 or 20 min after the tracer and lipids were extracted and purified (Folch *et al.*, 1957) for fractionation. Results were means ± SEM of 5–16 rats/group. Statistical comparisons between values for rats receiving glucose and controls are shown by asterisks: $**P < 0.01$.

Table 4. Liver and plasma lactate and pyruvate concentrations in 24 hr starved rats infused with glucose through the portal vein

	Liver			Plasma		
	Lactate (nmol/g)	Pyruvate (nmol/g)	Lactate/ pyruvate ratio	Lactate (nmol/ml)	Pyruvate (nmol/ml)	Lactate/ pyruvate ratio
Controls	225 ± 33	30 ± 4	5.7 ± 0.7	3495 ± 163	196 ± 26	13.4 ± 1.9
Glucose	368 ± 22*	29 ± 5	10.5 ± 1.9*	3799 ± 234	236 ± 32	11.6 ± 1.3

To maintain the rats as in the previous experiments, rats received a pulse of [3-^{14}C]pyruvate after which they were infused with glucose (20.8 mg/min) or the medium (controls) through the portal vein. Blood was collected from the cava vein at the level of the suprahepatic veins and livers were frozen in liquid N_2 at 5 min after the tracer. Lactate and pyruvate were measured by enzymatic procedures in $HClO_4$ extracts. Results are means ± SEM of 5–6 rats/group. Statistical comparisons between values for rats receiving glucose and controls are shown with asterisks: $*P < 0.05$.

DISCUSSION

Our results indicate that contrary to the generally accepted belief, increments in glucose levels in blood reaching the liver through portal circulation enhance gluconeogenesis in starved rats. Through this mechanism, glucose also enhances liver glycogen synthesis from glucogenogenic precursors. This conclusion is in contrast with the assumption that the portal vein drains most intestinally absorbed glucose into the liver to be deposited directly as glycogen prior to its release into systemic circulation (Cahill *et al.*, 1959; Perley and Kipnis, 1967). The recent demonstration by several laboratories that in the rat glucose is a poor precursor for liver glycogen synthesis both *in vitro* (Hems *et al.*, 1972; Riesenfeld *et al.*, 1981; Katz *et al.*, 1979; Boyd *et al.*, 1981) and *in vivo* (Newgard *et al.*, 1983) supports the hypothesis that the conversion of exogenous glucose to liver glycogen may occur mainly via an indirect mechanism in which the gluconeogenic pathway plays an important role. Administration of glucose through the portal vein in a dose (7.8 mg/min) that increases blood glucose concentration to values normally seen in fed animals augmented the appearance of liver [^{14}C]glycogen from [3-^{14}C]pyruvate in conditions where no changes in circulating [^{14}C]glucose was observed and coinciding with increments in liver glycogen content and [^{14}C]glycogen specific activity (Tables 1 and 2). These results suggest that in the transition from the starved to the fed state, glucose reaching the liver from dietary carbohydrates enhances glycogen synthesis from gluconeogenic substrates. This hypothesis agrees with the reported delay in liver glycogen

content increase following oral glucose administration to starved rats (Gödeken *et al.*, 1966), a time which was reduced when glucose was given simultaneously to gluconeogenic substrates (Olavarria *et al.*, 1968). The glucose effect of enhancing liver gluconeogenesis was dose-dependent because with the dose of 20.8 mg/min there was not only increased [3-^{14}C]pyruvate conversion into liver glycogen, as found with the 7.8 mg/min dose, but also augmented conversion to liver glyceride glycerol and circulating glucose. This result is in agreement with our previous findings on parallel increments in liver glycogen, glyceride glycerol synthesis and gluconeogenesis in starved rats studied under different experimental conditions and with different substrates (Soley *et al.*, 1983; Zorzano and Herrera 1983), and substantiates our previous proposal that in the starved state, both liver glycogen and glyceride glycerol synthesis are predominantly gluconeogenic processes.

While mechanism by which glucose stimulates its own synthesis in the starved rat is still speculative, an evaluation of our data together with that of Newgard *et al.* (1983) supports the possibility that glucose is converted to C_3 units prior to their deposit as liver glycogen and is in agreement with the liver zonation model for the reversible shift from glycolysis and gluconeogenesis recently proposed by Jungermann *et al.* (1983). The increased liver lactate/pyruvate ratio produced by glucose infusion through the portal vein indicates a shift to the cytosolic redox state in the liver which may be caused by increased glycolytic activity in the perivenous hepatic cells produced by the glucose load. This condition permits greater use of lactate by the periportal cells which could cause the diversion of dihydroxyacetone phosphate to glycerol 3-phosphate for glyceride glycerol synthesis as well as diversion of 1,3-biphosphoglycerate to glyceraldehyde-phosphate, producing a simultaneous enhancement of liver glycerolgenesis and gluconeogenesis from the same substrate, [3-^{14}C]pyruvate in our case. Present results with 7.8 mg glucose/min, indicate that the later glucose effect enhances the appearance of [^{14}C]glycogen in liver of the starved rate even without increasing the appearance of newformed glucose in blood, demonstrating the major role of C_3 units as substrates for liver glycogen synthesis in this condition.

Acknowledgements—We thank Caroline S. Delgado for her editorial help. This work was supported by a grant from the Comisión Asesora de Investigación Científica y Técnica del Ministerio de Educación y Ciencia, Spain.

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