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SHORT-TERM INSULIN INFUSED THROUGH THE PORTAL VEIN ENHANCES LIVER GLUCONEOGENESIS AND GLYCOGENESIS FROM [3-^{14}C]PYRUVATE IN THE STARVED RAT

M. Soley[1], R. Chieri[1] and E. Herrera[2]
[1]Cátedra de Fisiología General, Facultad de Biología, Universidad de Barcelona, Barcelona, Spain
[2]Departamento de Investigación, Centro Ramón y Cajal, Madrid, Spain

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Abstract—1. Insulin infusion through the portal vein immediately after a pulse of [3-^{14}C]pyruvate in 24 hr starved rats enhanced the appearance of [^{14}C]glucose at 2, 5 and 10 min and glucose specific activity at 1, 2 and 20 min in blood collected from the cava vein at the level of the suprahepatic veins.

2. Insulin infusion for 5 min decreased liver pyruvate concentration and enhanced both liver and plasma lactate/pyruvate ratio, and it decreased the plasma concentration of all amino acids.

3. When insulin was infused together with glucose, [^{14}C]glucose levels and glucose specific activity decreased in blood but there was a marked increase in liver [^{14}C]glycogen, glycogen specific activity and glycogen concentration, and an increase in liver lactate/pyruvate ratio.

4. The effect of insulin plus glucose infusion on plasma amino acids concentration was smaller than that found with insulin alone.

5. It is proposed that insulin effect enhancing liver gluconeogenesis is secondary to its effect either enhancing liver glycolysis which modifies the liver's cytoplasmic oxydoreduction state to its more reduced form, increasing liver amino acids consumption or both. In the presence of glucose, products of gluconeogenesis enhanced by insulin are diverted into glycogen synthesis rather than circulating glucose.

6. This together with results of the preceding paper (Soley *et al.*, 1985), indicates that glucose enhances liver glycogen synthesis from C_3 units in the starved rat, the process being further enhanced in the presence of insulin.

INTRODUCTION

Although it is generally accepted that insulin inhibits liver gluconeogenesis, this effect has been seen clearly only in the presence of gluconeogenic hormones such as glucagon and adrenaline (Exton *et al.*, 1971; Pilkis *et al.*, 1975; Claus and Pilkis, 1976; Hue *et al.*, 1978; Chisholm *et al.*, 1983). Reported short-term effects of insulin alone on liver gluconeogenesis are contradictory. In some *in vitro* studies when insulin was the only hormone present, gluconeogenesis was suppressed in rat liver (Mortimore, 1963; Haft, 1967) whereas in other studies no effect was found (Johnson *et al.*, 1972; Zahlten *et al.*, 1973; Claus and Pilkis, 1976; Hue *et al.*, Whitton *et al.*, 1978; Probst *et al.*, 1982; Chisholm *et al.*, 1983). The decrease in glucose release by liver cells during insulin treatment may be explained by diversion of newly formed glucose into liver glycogen, this synthesis from gluconeogenic substrates being enhanced by the hormone (Johnson *et al.*, 1972; Beynen and Geelen, 1981). Augmented incorporation of gluconeogenic substrates into circulating glucose soon after insulin administration has also been reported in starved mice *in vivo* (Rous, 1978). Portal vein glucose infusion enhances liver gluconeogenesis and glycogenesis from [3-^{14}C]pyruvate in the starved rat (Soley *et al.*, 1985), and based on the liver zonation model (Jungermann *et al.*, 1983), it is proposed that glucose stimulates gluconeogenesis as a secondary consequence of the enhanced glycolytic activity. Since these two pathways may be simultaneously affected by insulin, in the present study we determined whether insulin alone or insulin plus glucose affects liver [^{14}C]glucose formation from [3-^{14}C]pyruvate, using a methodology similar to that described for portal vein infusion of glucose in the starved rat (Soley *et al.*, 1985). Our study was extended to determine the appearance of label in liver glycogen and the circulating concentration of individual amino acids, as it has been consistently reported that insulin alone stimulates hepatic amino acid transport (Le Cam and Freychet, 1978; Dolais-Kitabgi *et al.*, 1981).

Correspondence should be addressed to: Dr E. Herrera. Departamento de investigación, Centro Ramón y Cajal, Ctra. Colemenar Km 9, 28034-Madrid, Spain.

MATERIALS AND METHODS

Conditions of the experimental animals and analytical determinations were basically as indicated in our previous paper (Soley *et al.*, 1985). In summary, 24 hr starved male Wistar rats, anesthetized i.v. with 60 mg/kg body weight of sodium pentobarbital, were given 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) through the portal vein. The surgical procedure was as previously described (Chieri *et al.*, 1966; Soley *et al.*, 1983). The pulse of [3-^{14}C]pyruvate was immediately followed by infusion of a

Table 1. Effects of insulin infusion through the portal vein on blood [^{14}C]glucose and radio-immunoassayble insulin and glucose concentrations 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)	Radio immunoassayble insulin (μU/ml)
Control					
1 min	42933 ± 2323	3067 ± 332	71 ± 4	4244 ± 531	179 ± 10
2 min	32390 ± 857	4580 ± 380	79 ± 4	5407 ± 442	97 ± 11
5 min	24330 ± 779	5350 ± 464	74 ± 4	7718 ± 894	70 ± 14
10 min	20064 ± 497	4959 ± 655	74 ± 6	6925 ± 1020	—
20 min	18456 ± 689	5134 ± 531	75 ± 6	6909 ± 620	—
Insulin					
1 min	46914 ± 3309	3818 ± 352	70 ± 6	6147 ± 464*	174 ± 23
2 min	34122 ± 1502	7329 ± 942**	80 ± 7	7908 ± 897*	249 ± 43**
5 min	24502 ± 1208	8426 ± 736**	89 ± 6*	9563 ± 1164	357 ± 22***
10 min	17715 ± 939*	7644 ± 1204*	77 ± 8	10279 ± 1651	—
20 min	11632 ± 1072***	5076 ± 941	41 ± 5***	12048 ± 1857**	—
Insulin + glucose					
1 min	41261 ± 2111	2830 ± 242	173 ± 21***	1779 ± 408***	339 ± 4***
2 min	28965 ± 1609	4256 ± 235	193 ± 19***	2309 ± 367***	342 ± 3***
5 min	20253 ± 756***	5134 ± 334	257 ± 13***	2401 ± 343***	344 ± 4***
10 min	14642 ± 652***	4216 ± 359	199 ± 16***	1996 ± 205***	—
20 min	10172 ± 402***	2430 ± 133***	227 ± 14***	1077 ± 62***	—

After receiving a pulse of [3-^{14}C]pyruvate, 24 hr starved rats were infused through the portal vein with either insulin (33.3 mU/min), insulin plus glucose (33.3 mU and 20.8 mg/min, respectively), 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 5–14 rats/group. Statistical comparisons between values for rat receiving either insulin or insulin plus glucose and controls are shown by asterisks: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

saline albumin solution (0.5 g bovine albumin of 0.9% NaCl) containing either Actrapid monocomponent bovine insulin (from Novo Industri A/S, Denmark) at the rate of 33.3 mU/min or insulin plus glucose (33.3 mU and 20.8 mg/min, respectively). Control animals were infused with only the saline-albumin solution. Infusions were always performed at the rate of 5 ml of the corresponding solution per hour. Blood aliquots were collected into heparinized syringes at the level of the suprahepatic veins at different times after the tracer, and they were used for glucose determination (Huggett and Nixon, 1957) and [^{14}C]glucose purification and analysis (Soley *et al.*, 1985) after deproteinization (Somogyi *et al.*, 1945). Plasma aliquots were employed for RIA-insulin determinations (Heding, 1972) by using a radioimmunoassay kit for rat, generously supplied by Novo Industri A/S (Denmark). Other plasma aliquots were studied for amino acid content with an autoanalyzer after deproteinization with 10% sulphosalycylic acid (Martín del Río and Latorre-Caballero, 1980) and for lactate and pyruvate determinations by means of enzymatic procedures (Passoneau, 1974; Passoneau and Lowry, 1974) after $HClO_4$ precipitation. At the last blood collection, a piece of liver was frozen in liquid N_2 and aliquots were used for glycogen purification (Good *et al.*, 1933) and analysis (Soley *et al.*, 1985). Other aliquots of frozen liver were used for lactate and pyruvate determinations (Passoneau, 1974; Passoneau and Lowry, 1974) after $HClO_4$ precipitation. Statistical analysis of the data was performed with Student's *t*-test.

RESULTS

As shown in Table 1, insulin infusion through the portal vein immediately after a pulse of [3-^{14}C]pyruvate in 24 hr starved rats enhanced the disappearance of total radioactivity in blood collected from the suprahepatic veins from the 10th min after de tracer. This effect was further enhanced when insulin plus glucose were infused and they produced statistically significant values from the 5th min compared with levels in control animals, infused with only the medium (Table 1). Values of [^{14}C]glucose in blood were significantly higher at 2, 5 and 10 min in rats infused with insulin than in their controls, whereas in rats infused with insulin plus glucose [^{14}C]glucose blood levels at 20 min were significantly lower than in controls (Table 1). Blood glucose concentration was higher in the insulin treated rats at 5 min and in the insulin plus glucose animals at 1, 2, 5, 10 and 20 min, than in controls whereas at 20 min it was lower than controls in the rats that received only insulin (Table 1). Blood glucose specific activity was augmented in the insulin treated rats at 1, 2 and 20 min after the tracer whereas it was decreased in those receiving both insulin and glucose at all the times studied (Table 1). Plasma RIA-insulin levels were greater than controls in rats receiving insulin from the 2nd min and in those receiving insulin plus glucose, from the 1st until the 5th min studied (Table 1).

Liver glycogen values in animals infused for either 5 or 20 min are shown in Table 2. Treatment with insulin alone did not affect the appearance of [^{14}C]-glycogen in liver at 5 min whereas it produced a significant decrease in this parameter at 20 min compared to control values. Infusion of insulin alone did not affect either liver glycogen concentration or [^{14}C]-glycogen specific activity at 5 or 20 min (Table 2). When insulin plus glucose were given there was a marked increase in liver [^{14}C]glycogen and glycogen concentration at 5 and 20 min and in liver [^{14}C]-glycogen specific activity at 5 min (Table 2).

Values of lactate and pyruvate concentrations in both liver and plasma at 5 min after the tracer administration are summarized in Table 3. Insulin treatment decreased liver pyruvate concentration and enhanced both liver and plasma lactate/pyruvate ratios compared with control values, whereas insulin plus glucose enhanced liver lactate concentration and lactate/pyruvate ratio without affecting these parameters in plasma (Table 3).

As shown in Table 4, at 5 min of insulin infusion

Table 2. Effects of insulin infusion through the portal vein in liver [^{14}C]glycogen and glycogen concentration after a pulse of [3-^{14}C]pyruvate

	[^{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	2252 ± 439	0.4 ± 0.0	5139 ± 944
Insulin			
5 min	1616 ± 521	0.7 ± 0.2	2110 ± 326
20 min	805 ± 106*	0.3 ± 0.0	3844 ± 480
Insulin + glucose			
5 min	8054 ± 1195***	1.5 ± 0.2*	5005 ± 583***
20 min	20492 ± 2609***	4.3 ± 0.7***	5978 ± 705

After receiving a pulse of [3-^{14}C]pyruvate, 24 hr starved rats were infused through the portal vein with either insulin (33.3 mU/min), insulin plus glucose (33.3 mU and 20.8 mg/min, respectively), 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 5–12 rats/group. Statistical comparisons between values for rats receiving either insulin or insulin plus glucose and control are shown by asterisks: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

there was a decrease in the plasma concentration of all amino acids, this change being statistically significant in most cases when compared with control values. Insulin plus glucose infusion produced a much smaller effect on plasma amino acid concentration than insulin alone, this change being statistically significant for only glutamine, glycine and methionine.

Table 3. Liver and plasma lactate and pyruvate concentrations in 24 hr starved rats infused with either insulin or insulin plus 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
Insulin	209 ± 24	14 ± 1**	13.5 ± 1.7**	3041 ± 191	117 ± 26	22.2 ± 3.3*
Insulin + glucose	445 ± 35**	38 ± 5	10.3 ± 1.3*	3744 ± 87	251 ± 21	13.8 ± 1.8

Rats received a pulse of [3-^{14}C]pyruvate after which they were infused with either insulin (33.3 mU/min) or insulin plus glucose (33.3 mU and 20.8 mg/min, respectively), or the medium (controls). 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 $HClO_4$ extract. Results are means ± SEM of 5–7 rats/group. Statistical comparisons between values for rats receiving either insulin or insulin plus glucose and controls are shown by asterisks: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 4. Plasma amino acids concentration at 5 min of insulin infusion through the portal vein in 24 hr starved rats

	Control	Insulin	Insulin + glucose
Aspartic acid	13.6 ± 1.7	9.9 ± 0.3	13.2 ± 0.9
Threonine	166.6 ± 29.8	97.4 ± 4.2	150.6 ± 8.6
Serine	174.6 ± 21.0	113.2 ± 7.2*	151.8 ± 5.9
Glutamic acid	88.0 ± 10.0	73.2 ± 7.1	103.1 ± 5.0
Glutamine	359.0 ± 37.4	215.0 ± 13.0*	191.3 ± 6.4**
Proline	82.8 ± 6.0	58.3 ± 4.3*	83.1 ± 3.8
Glycine	292.1 ± 36.1	166.2 ± 3.2*	185.2 ± 8.3*
Alanine	248.0 ± 10.0	185.0 ± 17.8*	239.2 ± 16.0
Cystine	57.7 ± 4.5	27.2 ± 1.0***	45.7 ± 8.9
Valine	109.0 ± 8.8	81.5 ± 3.5*	96.2 ± 3.9
Methionine	29.8 ± 0.9	20.4 ± 1.4***	25.8 ± 0.4**
Isoleucine	54.6 ± 4.3	38.8 ± 0.7*	49.7 ± 1.8
Leucine	77.4 ± 7.1	56.8 ± 2.8*	71.4 ± 2.8
Tyrosine	47.0 ± 5.5	33.5 ± 2.2	38.1 ± 2.8
Phenylalanine	42.4 ± 3.0	30.3 ± 1.8**	38.7 ± 2.8
Histidine	60.4 ± 3.6	47.1 ± 1.4**	63.7 ± 2.8

Rats received a pulse of [3-^{14}C]pyruvate after which they were infused with either insulin (33.3 mU/min or insulin plus glucose 33.3 mU and 20.8 mg/min, respectively), or the medium (controls). Blood was collected from the cava vein at the level of the suprahepatic veins at 5 min after the tracer. Plasma amino acids were measured after deproteinization with 10% sulphosalycylic acid by means of an autoanalyzer. Results are expressed as μmol per liter of plasma and correspond to means ± SEM of 5 rats/group. Statistical comparisons between values for rats receiving either insulin or insulin plus glucose and controls are shown by asterisks: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

DISCUSSION

Present results show that soon (2 min) after its portal infusion, insulin enhances liver [^{14}C]glucose production from [3-^{14}C]pyruvate. The effect was seen without a change in liver [^{14}C]glycogen appearance and before insulin produced a decrease in blood glucose concentration, indicating that it was not a secondary consequence of hypoglycemia. Thus it may be concluded that in this condition, insulin alone acutely increased liver gluconeogenesis. The effect was subsequently compensated by the hormonal effects enhancing peripheral glucose utilization, as shown by the progressive decrease in both [^{14}C]-glucose and glucose concentrations in blood which occurred after the 5th min of portal vein insulin infusion. Although these results are surprising, a short-term (3 min) insulin increase in [^{14}C]glucose production from [3-^{14}C]pyruvate was also observed *in vivo* by Rous (1978) in starved mice.The mechanism by which in certain conditions insulin alone enhances liver gluconeogenesis cannot be explained by changes in enzyme activities, as it has been shown that even after prolonged *in vitro* incubations of isolated hepatocytes, insulin alone does not affect liver gluconeogenic regulatory enzymes (Chisholm *et al.*, 1983; Pilkis *et al.*, 1983), although it antagonizes the effects of gluconeogenic hormones. The observed short-term gluconeogenic effect of insulin appeared in parallel with an increase in liver and plasma lactate/pyruvate ratio. Based on the liver zonation model for the flux direction of glycolysis and gluconeogenesis (Jungermann *et al.*, 1983), it is proposed that insulin's primary effect enhancing liver glycolysis (Beynen, 1982) modifies the liver's cytoplasmic oxydoreduction state to its more reduced form, causing a secondary enhancement of gluconeogenic activity. In the present study this effect has been visualized for the conversion of [3-^{14}C]pyruvate to glucose. The marked reduction of amino acid concentrations in blood collected at the level of the suprahepatic veins after 5 min of insulin infusion, however, indicates that an increase in liver consumption of amino acids may also being involved in this hormonal gluconeogenic effect, before the hormone has any effect on protein synthesis which requires longer treatments (Mohan and Bessman, 1981).

The combined effects of insulin plus glucose on these parameters further support our hypothesis, since this insulin action on glycolysis must be greater than that of insulin infused alone due to the greater availability of the substrate. In addition to the increased glycemia of these animals, this hypothesis is also supported by the increment in liver lactate concentration found after 5 min of insulin plus glucose infusion. In this condition, circulating [^{14}C]-glucose from [3-^{14}C]pyruvate was unchanged but newformed [^{14}C]glucose was clearly diverted for its deposit as liver [^{14}C]glycogen, the production of which was greatly enhanced. This glycogenic effect of insulin plus glucose was more pronounced than that of glucose infusion alone (Soley *et al.*, 1985), which, however, produced a parallel enhancement in the appearance of circulating [^{14}C]glucose from the same substrate. In relation with the recent findings of Newgard *et al.* (1983) on liver glycogen synthesis from glucose through a pathway involving C_3 units, our results support the possibility that enhanced glucose conversion to C_3 units (like that produced in the presence of insulin) causes a secondary increase in the rate of gluconeogenesis, probably by increasing the redox state. The product of this pathway is preferentially diverted to either circulating glucose, liver glycogen deposit or both, depending on different factors which must include circulating glucose concentration, insulin and other hormone levels, as well as the activity of the key enzymes involving in all these pathways. In conclusion, the general accepted view of the control of circulating glucose homeostasis should be now re-evaluated. Classically accepted antigluconeogenic factors such as insulin and glucose must now being recognized to enhance glucose synthesis from C_3 units, secondary to their action augmenting the glucose–lactate pathway. The fate of the newformed glucose (whether it is deposited as glycogen or released into circulation for use in extrasplachnic tissues) depends on many factors, but the overall mechanism explain what has been termed by the group of Katz (Riesenfeld *et al.*, 1981) the "glucose paradox" which corresponds to the well known fact that glucose is a poor direct precursor for glycogen synthesis even in the presence of insulin.

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