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INSULIN EFFECT ON *IN VIVO* GLUCONEOGENESIS FROM [3-^{14}C]PYRUVATE IN THE STARVED RAT

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Abstract—1. During a 24 hr fast rats received 4 subcutaneous injections of insulin, and 15 min after the last injection they were given an intravenous pulse of [3-^{14}C]pyruvate. The amount of [^{14}C]glucose in blood 2 min after the tracer did not differ between insulin treated and control animals, whereas at 5 and 10 min values were significantly lower in the former group.

2. At 10 min after the tracer, liver [^{14}C]glycogen specific activity and [^{14}C]fatty acid amount were higher in the insulin treated animals than in controls while plasma concentration of gluconeogenic amino acids was lower in the first group.

3. Similar changes but less pronounced and more retarded were found in 24 hr fasted rats given only one insulin dose 15 min before the [3-^{14}C]pyruvate pulse.

4. Results indicate that gluconeogenesis from pyruvate is not directly modified by insulin treatment. Effects found at 5 and/or 10 min after the tracer and reported effects after prolonged insulin treatments may be caused by one or all of the following possibilities: enhanced utilization of the new-formed glucose, reduced availability of gluconeogenic substrates, and counteracting action on gluconeogenic hormones.

INTRODUCTION

Insulin is generally considered an anti-gluconeogenic hormone (for a recent review see Kraus-Friedmann, 1984), but this effect has only been seen clearly in the presence of gluconeogenic hormones such as glucagon and adrenaline (Exton *et al.*, 1971; Pilkis *et al.*, 1975; Chisholm *et al.*, 1983). Reported short-term effects of insulin alone on liver gluconeogenesis are conflicting. In some studies insulin appeared to suppress this pathway (Jefferson *et al.*, 1968; Haft, 1967) whereas in other studies no change was detected (Chisholm *et al.*, 1983; Claus and Pilkis, 1976; Soling and Kleineke, 1976; Zahlten *et al.*, 1973; Hue *et al.*, 1978; Whitton *et al.*, 1978; Probst *et al.*, 1982; Johnson *et al.*, 1972). In some conditions an augmented incorporation of gluconeogenic substrates into circulating glucose was found after insulin administration (Rous, 1978; Soley *et al.*, 1985a). Therefore the effects of insulin on gluconeogenesis may be secondary to other hormonal metabolic actions which vary according to individual endocrine and nutritional conditions. Insulin is known to stimulate protein synthesis (Wool, 1964; Airhart *et al.*, 1982) and it has been proposed that this effect may alter the direction flux of gluconeogenic amino acids, depriving gluconeogenesis of adequate substrates (Mohan and Bessman, 1981). Insulin effects enhancing lipogenesis (Fain *et al.*, 1965; McCormick *et al.*, 1978) could also divert substrates from the gluconeogenic pathway. In the present experiments we studied simultaneously some parameters of these pathways in the starved rat shortly after *in vivo* administration of insulin in order to obtain a comparative index of how this hormone affects them.

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MATERIALS AND METHODS

Male Wistar rats weighing 200 + 11 g from our animal quarter were used without anaesthesia for the following processes. Rats were subjected to two kind of treatments:

(a) Some animals were food deprived for 24 hr during which time they received 4 subcutaneous doses of Actrapid monocomponent bovine insulin (from Novo Industri A/S, Copenhagen, Denmark) dissolved in 0.9% NaCl containing 0.5 g/l bovine albumin. The first three doses of insulin (0.6 IU/kg body weight) were given at 0, 6, and 19 hr and the fourth dose (1 IU/kg body weight) was given at the 24th hr. Test and control animals were maintained under the same nutritional and environmental conditions and controls received injections of the medium at the corresponding times. Fifteen min after the last injection all animals were given an intravenous injection of [3-^{14}C]pyruvate, as indicated below.

(b) After 24 hr starvation, other rats were given a single dose of insulin (1 IU/Kg body weight) or medium, and 15 min later they received an intravenous injection of [3-^{14}C]-pyruvate. The tracer ([3-^{14}C]pyruvate, specific activity 18.3 mCi/mmol, from The Radiochemical Center, Amersham, England) was given through a tail vein in the amount of 7 μCi containing 200 μmol of sodium pyruvate in 0.9% NaCl per 200 g body weight. Blood samples were collected 2, 5 and 10 min later from the tip of the tail, placed in heparinized receptacles, and used for deproteinization (Somogyi, 1945). Protein free supernatants were used for glucose estimation (Hugget and Nixon, 1957) and for [^{14}C]glucose purification by ion exchange column chromatography as already described (Soley *et al.*, 1985b). After the last blood collection, rats were killed with a guillotine, blood from the neck wound was placed in heparinized tubes for plasma separation, and an aliquot of liver was immediately placed into liquid nitrogen. Aliquots of plasma were used for deproteinization with 10% sulphosalycylic acid in 0.1 N HCl for amino acid analysis with a Beckman 121MB autoanalyzer (Martin del Rio and Latorre Caballero, 1980) while other aliquots were deproteinized with 10% $HClO_4$ for lactate and pyruvate determination by enzymatic procedures (Passonneau, 1974; Passonneau and Lowry, 1974).

One aliquot of the frozen liver was digested with 30% KOH for glycogen purification (Good *et al.*, 1933) and radioactivity and glucose concentration were quantified after acid hydrolysis following our previously validated procedure (Herrera *et al.*, 1969). Another aliquot of frozen liver was used for lipid extraction (Folch *et al.*, 1957) and the extracts were fractionated for fatty acid purification using a modified version of the method of Kerpel *et al.* (1961) as previously described (Carmaniu and Herrera, 1980). Another aliquot of frozen liver was deproteinized with 10% $HClO_4$ for lactate and pyruvate determinations (Passonneau, 1974; Passonneau and Lowry, 1974). Statistical analysis of the data was done by the Student's *t*-test.

RESULTS

Treatment with 4 doses of insulin

After receiving 4 successive doses of insulin over a 24 hr period of food deprivation, rats were given 200 μmol of [3-^{14}C]pyruvate (7 μCi) per 200 g body weight. As shown in Table 1, blood glucose concentration was greatly reduced in the insulin treated animals compared with controls. While the amount of [^{14}C]glucose in blood was the same in both groups 2 min after the tracer, it increased progressively with time in controls, remaining stable in insulin treated animals, this difference being statistically significant at 5 and 10 min. Blood glucose specific activity did not differ in the two groups at any of the times studied. At 10 min, liver [^{14}C]glycogen and glycogen concentration were the same in insulin treated and control rats (Table 1) while [^{14}C]glycogen specific activity and [^{14}C]fatty acid levels were significantly greater in the former group (Table 1). As shown in Table 2, total amino acids plasma concentration was significantly lower in the insulin treated rats than in their controls, this difference corresponding mainly to a general reduction in all gluconeogenic amino acids, which was significant for Thr, Ser, Gln, Pro, Glu, Gly and Ala. There were no other amino acid level variations in the two groups except for a significant reduction in Phe and an increase in Tau in the insulin treated animals versus their controls (Table 2). Plasma and liver concentrations of pyruvate and lactate remained similar in both groups (data not shown).

Treatment with 1 dose of insulin

In a preliminary experiment, 1 IU of insulin or medium was injected subcutaneously to 24 hr starved rats and blood was collected from the tip of the tail 5, 10, 15, 20 and 25 min later. Blood glucose concentration did not differ in the two groups at 5 and 10 min but was significantly lower in the insulin treated animals from the 15th min on ($P < 0.05$ or less). These findings led us to inject the tracer (200 μmol of [3-^{14}C]pyruvate, 7 μCi/200 g body weight) in other animals 15 min after receiving a subcutaneous injection of either 1 IU insulin or medium. As shown in Table 3, blood glucose concentration was significantly lower in insulin treated rats than in their controls throughout the period studied. The amount of [^{14}C]glucose in blood was similar in both groups 2 and 5 min after tracer administration, after which it gradually increased, the change being less pronounced in the insulin treated animals, and the difference between the two groups becoming

Table 1. Effect of subacute treatment with insulin (3 s.c. injections of 0.6 IU/kg body wt and 1 of 1 IU/kg during a 24 hr period) in the starved rat on *in vivo* utilization of [3-^{14}C]pyruvate

	Time after tracer (min)	Blood glucose (mg/dl)	Blood [^{14}C]glucose (dpm/ml)	Blood [^{14}C]glucose specific activity (dpm/mg)	Liver glycogen (‰)	Liver [^{14}C]glycogen (dpm/g)	Liver [^{14}C]glycogen specific activity (dpm/mg)	Liver [^{14}C]fatty acids (dpm/mg)
Control rats								
	2	88.9 ± 3.1	8713 ± 2269	10130 ± 2456	—	—	—	—
	5	95.9 ± 4.3	22492 ± 3053	24115 ± 3955	—	—	—	—
	10	108.4 ± 2.2	23550 ± 1975	23222 ± 2006	0.31 ± 0.05	360 ± 25	921 ± 265	343 ± 35
Insulin treated rats								
	2	39.9 ± 2.2***	7800 ± 3277	20292 ± 8658	—	—	—	—
	5	37.0 ± 1.8***	6980 ± 1895**	20223 ± 5618	—	—	—	—
	10	30.2 ± 1.4***	8763 ± 859***	29279 ± 2897	0.21 ± 0.01	399 ± 97	2085 ± 359*	1385 ± 220***

Rats were intravenously injected with 7 μCi/0.2 μmol of [3-^{14}C]pyruvate/200 g body wt 15 min after the last insulin injection and blood was collected at different times thereafter. Following the last blood collection rats were decapitated and liver immediately excised. Means ± SEM of 6–7 rats/group. Statistical comparisons between values from insulin treated and control rats are indicated by asterisks: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2. Effect in the starved rat of subacute treatment with insulin (3 s.c. injections of 0.6 IU/kg body wt and 1 of 1 IU/kg during a 24 hr period) on plasma amino acid concentrations

Amino acids (μmol/l)	Control rats	Insulin treated rats
Taurine	223.9 ± 7.4	316.1 ± 13.1***
Aspartic acid	23.7 ± 1.5	19.9 ± 1.3
Threonine	263.9 ± 8.0	206.7 ± 11.3**
Serine	194.9 ± 10.5	142.4 ± 4.2**
Glutamine	931.0 ± 35.5	733.6 ± 29.6**
Proline	129.7 ± 7.5	101.1 ± 4.6*
Glutamic acid	149.3 ± 5.4	73.4 ± 4.3***
Citrulline	59.0 ± 2.7	50.3 ± 4.4
Glycine	381.1 ± 24.3	239.7 ± 10.6***
Alanine	354.1 ± 19.6	290.9 ± 16.8*
Valine	177.0 ± 8.2	190.6 ± 9.2
Methionine	47.4 ± 3.1	50.2 ± 1.4
Isoleucine	87.4 ± 3.1	80.2 ± 3.1
Leucine	131.7 ± 4.4	143.0 ± 6.1
Tyrosine	75.1 ± 1.9	71.8 ± 4.0
Phenylalanine	85.1 ± 0.8	73.7 ± 2.4**
Ornitine	27.0 ± 1.6	27.0 ± 2.0
Lysine	355.8 ± 8.3	327.0 ± 13.4
1-Methylhistidine	5.5 ± 0.6	4.4 ± 0.3
Histidine	56.3 ± 5.8	58.0 ± 2.3
3-Methylhistidine	5.8 ± 0.4	6.7 ± 0.5
Tryptophan	111.6 ± 5.8	113.9 ± 6.4
Arginine	98.0 ± 3.9	87.0 ± 3.9
Total amino acids	3987.7 ± 1430	3446.2 ± 149.5*

Rats are the same as those of Table 1, and plasma amino acid values correspond to blood samples collected 10 min after the tracer. Means ± SEM of 6–7 rats/group. Statistical comparisons between the two groups are shown by the asterisks: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

significant at 10 min (Table 3). Blood [^{14}C]glucose specific activity did not differ between the groups at any of the times studied (Table 3). Radioactivity incorporation into liver glycogen and fatty acids, liver glycogen concentration, and [^{14}C]glycogen specific activity were not modified 10 min after the single insulin injection (Table 3). As seen in Table 4, total amino acid plasma concentration was significantly lower in rats treated with one dose of insulin than in controls. This difference corresponded to a specific reduction in gluconeogenic amino acids such as Ser, Gln, Pro, Glu, Gly and Ala, whereas there were no changes in the levels of other amino acids except for a significant decrease in Phe and an increase in Trp in the insulin treated animals versus their controls (Table 4). Plasma and liver pyruvate and lactate concentrations remained similar in insulin treated rats and their controls (data not shown).

DISCUSSION

Present results show that neither acute (15 min after 1 single dose) or subacute (after 4 doses during a 24 hr period) treatments with insulin in the starved rat modified the appearance of [^{14}C]glucose in blood shortly (2 min) after the intravenous injection of [3-^{14}C]pyruvate nor did they affect blood [^{14}C]glucose specific activity. Since endogenous pyruvate concentration did not differ in the insulin-treated and control groups after tracer administration, results do not seem to be affected by differences in the dilution of the administered radioactivity. Therefore present results indicate that gluconeogenesis from pyruvate is not modified by insulin treatment. This lack of effect may not be due to low sensitivity of the method since a similar one was used to compare the *in vivo*

Table 3. Effect of acute treatment with insulin (1 IU/kg body wt, s.c.) in the starved rat on *in vivo* utilization of [3-^{14}C]pyruvate

	Time after tracer (min)	Blood glucose (mg/dl)	Blood [^{14}C]glucose (dpm/ml)	Blood [^{14}C]glucose specific activity (dpm/mg)	Liver glycogen (‰)	Liver [^{14}C]glycogen (dpm/g)	Liver [^{14}C]glycogen specific activity (dpm/mg)	Liver [^{14}C]fatty acids (dpm/g)
Control rats	2	79.4 ± 5.9	5738 ± 1131	6983 ± 1351	—	—	—	—
	5	89.0 ± 7.7	13392 ± 3108	14717 ± 3124	—	—	—	—
	10	88.1 ± 8.1	22790 ± 2180	25067 ± 4324	0.02 ± 0.01	914 ± 354	29684 ± 7946	386 ± 111
Insulin treated rats	2	47.0 ± 5.9**	5079 ± 939	12815 ± 3886	—	—	—	—
	5	36.5 ± 3.9***	10200 ± 1820	26775 ± 6071	—	—	—	—
	10	45.8 ± 4.1***	11868 ± 1461**	22929 ± 3249	0.02 ± 0.01	405 ± 49	12125 ± 3768	551 ± 61

Rats were intravenously injected with 7 μCi/0.2 μmol of [3-^{14}C]pyruvate/200 g body weight 15 min after the insulin injection and blood was collected at different times thereafter. Following the last blood collection, rats were decapitated and the liver immediately excised. Means ± SEM of 6–7 rat/group. Statistical comparisons between values from insulin treated and control rats are indicated by asterisks: **$P < 0.01$; ***$P < 0.001$.

Table 4. Effect in the starved rat of acute treatment with insulin (1 IU/kg body wt, s.c.) on plasma amino acid concentrations

Amino acids (μmol/l)	Control rats	Insulin treated rats
Taurine	224.9 ± 6.6	202.8 ± 12.1
Aspartic acid	22.8 ± 2.1	18.4 ± 1
Theorine	181.6 ± 18.1	165.0 ± 7.3
Serine	170.6 ± 12.7	122.7 ± 5.2**
Glutamine	890.7 ± 54.3	622.1 ± 12.1**
Proline	101.0 ± 3.1	80.9 ± 5.2**
Glutamic acid	161.0 ± 21.8	91.2 ± 3.1*
Citrulline	37.6 ± 3.1	35.0 ± 1.9
Glycine	332.9 ± 5.2	243.2 ± 4.1***
Alanine	308.0 ± 24.3	226.1 ± 14.9*
Valine	144.2 ± 11.2	145.1 ± 1.7
Methionine	40.7 ± 3.5	33.7 ± 0.9
Isoleucine	70.8 ± 5.8	67.8 ± 1.7
Leucine	118.8 ± 10.7	115.0 ± 2.5
Tyrosine	71.3 ± 6.3	74.4 ± 2.5
Phenylalanine	76.4 ± 3.8	61.4 ± 2.6**
Ornitine	59.4 ± 18.2	27.8 ± 4.1
Lysine	356.4 ± 29.9	280.5 ± 16.5
1-Methylhistidine	4.8 ± 0.7	3.2 ± 0.5
Histhidine	58.1 ± 4.7	47.8 ± 1.3
3-Methylhistidine	4.7 ± 0.6	5.6 ± 0.8
Tryptophan	78.1 ± 7.7	108.4 ± 9.5*
Arginine	51.1 ± 16.8	62.2 ± 3.4
Total amino acids	3472.7 ± 202.8	2945.1 ± 83.3*

Rats are the same as those of Table 3, and plasma amino acid values correspond to blood samples collected 10 min after the tracer. Means ± SEM of 6–7 rats/group. Statistical comparison between the two groups are shown by the asterisks: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

gluconeogenic activities of fed and 24 hr starved rats (Zorzano and Herrera, 1984a) and pregnant versus virgin animals (Zorzano and Herrera, 1984b) and striking differences were already detected 2 min after the tracer. The delayed insulin effect of decreasing blood [^{14}C]glucose after the tracer may be interpreted as a result of enhanced utilization of the newly formed glucose produced by the hormone rather than reduced synthesis. Augmented liver [^{14}C]glycogen specific activity found in the rats treated with 4 doses of insulin is consistent with this possibility. These findings reflect the difficulties of many investigators (Johnson *et al.*, 1972; Zahlten *et al.*, 1973; Claus and Pilkis, 1976; Soling and Kleineke, 1976; Hue *et al.*, 1978; Whitton *et al.*, 1978; Rous, 1978; Probst *et al.*, 1982; Chisholm *et al.*, 1983; Soley *et al.*, 1985a) to find an inhibitory effect of insulin on liver gluconeogenesis when given alone and may indicate that reported inhibitory effects on this pathway are secondary consequences of other actions of the hormone such as counteracting the effect of gluconeogenic hormones (Exton *et al.*, 1971; Pilkis *et al.*, 1975; Chisholm *et al.*, 1983) and/or decreasing the availability of gluconeogenic substrates. The latter possibility fits well with the observed decrease in the concentration of gluconeogenic amino acids found here in the insulin treated animals. The insulin effect of decreasing the plasma level of amino acids is thought to mainly reflect their inhibited release from muscle (Ingle *et al.*, 1956; Felig, 1975); through this delayed insulin mechanism, the availability of gluconeogenic amino acids would decrease in the splanchnic bed, limiting their use for glucose synthesis. Fatty acid synthesis from pyruvate was negligible in our starved rats but subacute insulin treatment was found to enhance this parameter indicating that the hormone may also enhance the lipid synthesis from gluconeogenic substrates, indirectly decreasing their availability for glucose formation. In conclusion, present findings indicate that insulin does not directly affect *in vivo* gluconeogenesis, and it is suggested that reported changes found after insulin treatment on this pathway are secondary to hormonal effects in other areas. These secondary effects normally result in a decrease in glucose synthesis but in some conditions insulin may even enhance gluconeogenesis, as found by Rous (1978) in fasted mice and by ourselves in fasted rats (Soley *et al.*, 1985a) shortly after the portal injection of insulin. In view of the recent hypothesis concerning the reversible shift between glycolysis and gluconeogenesis through the hepatic zonation model (Probst *et al.*, 1982; Jungermann *et al.*, 1983), and the liver glycogen synthesis through the pathway involving glucose—C_3-compounds—glucose-6-phosphate—glycogen (Katz and McGarry, 1984; Newgard *et al.*, 1984), it is not surprising that insulin may enhance, inhibit, or not modify gluconeogenesis depending on the condition of the individual or the isolated tissue preparation receiving the hormone load, as well as its dose and the time of its administration. The concept of insulin as a classical antigluconeogenic hormone should therefore be seriously reevaluated.

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