Effects of lactate on pathways of glycogen formation in the perfused rat liver

Zi ZHANG and Jerry RADZIUK*

Department of Medicine and Physiology, University of Ottawa, and the Diabetes and Metabolism Research Unit, Ottawa Civic Hospital, 1053 Carling Avenue, Ottawa, Ontario K1Y 4E9, Canada

In order to investigate the roles of lactate as substrate and regulator of hepatic glycogen synthesis, two groups of rat livers were perfused with oxygenated blood for 2 h. The initial perfusate glucose and lactate concentrations of Group I and II were 245 ± 6.8 and 254 ± 12.9 mg/dl and 49 ± 2.6 and 54 ± 2.2 mg/dl respectively. Labelled glucose was added to the perfusate to assess direct glycogen formation. Either additional glucose (Group I) or lactate (Group II) was added (1 mg/min) to a recirculating liver-perfusion system. Initial lactate uptake and glucose formation was identical in the two groups of studies. For Group I, both glucose and lactate uptake by the liver fell to nearly zero, in spite of increasing glucose concentrations. However, with lactate infusion (Group II), its uptake by the liver was maintained at 0.89 ± 0.14 mg/min after 120 min. In total, 6.2 ± 0.7 mg (Group I) or 20.2 ± 3.9 mg (Group II) of glycogen was formed, 4.0 ± 0.7 mg or 9.2 ± 2.0 mg by direct synthesis from glucose and 2.2 ± 0.3 mg or 11.0 ± 2.1 mg by gluconeogenic formation, in Groups I and II respectively. With the provision of additional lactate, its uptake by the perfused liver tripled, as did glycogen synthesis. Glucose production doubled when lactate was added instead of glucose. Gluconeogenic formation of glycogen increased by 400%. Surprisingly, direct synthesis from glucose also rose by 130%. These data indicate that continued lactate uptake by the liver with gluconeogenic glycogen formation determines the amount of glycogen formed not only by this route, but also by direct synthesis from glucose.

INTRODUCTION

It is now established that in man (Radziuk, 1982, 1989c; Magnusson *et al.*, 1989), rats (Hems *et al.*, 1972; Shikama & Ui, 1978; Boyd *et al.*, 1981) and many other species at least half of the glycogen following a glucose load is formed by the gluconeogenic pathway. Various glucogenic substrates such as alanine, glutamine, lactate, fructose and glycerol are known to enhance hepatic glycogen formation (Williams & Landau, 1972; Walli *et al.*, 1974; Howard & Widder, 1976; Katz *et al.*, 1979; Youn *et al.*, 1986). Moreover, the gluconeogenic process appears to be necessary (Newgard *et al.*, 1983) to the formation of glycogen by both pathways.

Lactate is a metabolite which is ubiquitous in systemic metabolism. It increases after meals and, more specifically, after a glucose load (e.g. Radziuk & Inculet, 1983). Although it does not appear to arise from deep forearm tissue (Jackson *et al.*, 1973; Radziuk & Inculet, 1983), it has been shown to be formed by the gut during glucose loading in pigs (Radziuk, 1987) and in subcutaneous adipose tissue (Johnson & Fusaro, 1973; Hagstrom *et al.*, 1990). Moreover it has been estimated in man, by using the incorporation of ^{14}C from [^{14}C]bicarbonate into glycogen, that after a 100 g glucose load approx. 15 g of glycogen is synthesized by the gluconeogenic pathway: by using the ^{14}C recycled from C-1 in glucose divided by the circulating lactate specific radioactivities, a very similar estimate was obtained (Radziuk, 1989a,b). This suggests that lactate may be an important substrate for liver glycogen formation under many physiological circumstances.

Previous work in exercising rats (Johnson & Bagby, 1988) has demonstrated hepatic glycogen synthesis by the indirect pathway after exercise during glucose and lactate infusion. Moreover (Ryan & Radziuk, 1989), a synergistic effect of preceding exercise and additional lactate on hepatic glycogen formation was seen during post-exercise recovery, suggesting a regulatory role for lactate.

This study was therefore designed to assess more precisely lactate and glucose interactions in the formation of glycogen in an isolated perfused rat liver where the relevant variable may be controlled more carefully.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats weighing 200–250 g were fed on standard lab chow and fasted for 24 h before surgery.

Materials

The tracers used in these studies were [$1\text{-}^{14}\text{C}$]glucose (8.2 mCi/mmol) and [$\text{fructose-1-}^3\text{H}$]sucrose (11.7 Ci/mmol) from New England Nuclear, Boston, MA, U.S.A., which were pyrogen-free and were purified by h.p.l.c. (HPX-87P, Bio-Rad). The appropriate fraction was passed through a Millipore bacterial filter (0.2 μm pore size) into a sterile vial. The purity was periodically checked by h.p.l.c. D-Glucose was a product of BDH. Lactate and BSA were obtained from Sigma. All chemical reagents (NaCl , KCl , NaHCO_3 , CaCl_2 , KH_2PO_4 and MgSO_4) were purchased from Fisher Scientific Co.

Liver perfusion technique

The perfusion apparatus used was designed on the basis of previous work (Mondon & Burton, 1971; Hamilton *et al.*, 1974; Zhang *et al.*, 1982). Livers were perfused with well-oxygenated medium *in situ* and kept moist at constant temperature (38 °C), pressure and flow rate (11.5 ml/min) in this closed system.

Rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (65 mg/kg), and livers were isolated and perfused as described by Mondon & Burton (1971). The duodenum was separated and cannulated to allow free flow of bile. Perfusion was initiated within 5 s of starting the portal-vein cannulation and the hepatic artery was tied off immediately. Perfusion medium consisted of 9 parts of defibrinated rat blood and 1 part

* To whom correspondence and requests for reprints should be addressed.

of 3 % BSA/Krebs-Ringer bicarbonate buffer containing glucose (approx. 100 mg/dl) and lactate (approx. 40 mg/dl). The medium was pumped (Gilson Minipuls 3 roller pump) after passage through a membrane oxygenator saturated with O_2/CO_2 (19:1) (2.5 litres/min). Livers were isolated and transferred to the apparatus. After perfusion for approx. 25 min, perfusion with 60–70 ml of the final medium (glucose concn. 2.5 mg/ml and lactate 0.4 mg/ml) was initiated and continued for 2 h; 100 μ l of [$1-^{14}C$]glucose (10 μ Ci) was added to 100 ml of this perfusate.

Infusion studies

In order to maintain perfusate concentrations of glucose and lactate which are analogous to those seen after a glucose load by a meal *in vivo*, and to assess the relative roles of lactate and glucose in promoting hepatic glycogen synthesis, two protocols were followed: (i) glucose or (ii) lactate was added to the perfusion medium continuously at 1 mg/min. Lactate for infusion was prepared as previously described (Naylor *et al.*, 1984). Erythrocytes metabolize glucose to form lactate (Murphy, 1960). Two sets of control studies were therefore performed with the perfusion system with the liver excluded and (iii) glucose or (iv) lactate added at 1 mg/min. Perfusate samples (0.8 ml) were withdrawn from inflow and outflow at 0, 5, 10, 20, 30, 45, 60, 75, 90, 105 and 120 min. The data obtained from the control studies were used in the calculation to compensate for glucose utilization and lactate production by the red cells between the two sampling sites.

Oxygen saturation and bile production were monitored to assess liver function during the study. In four livers a detailed analysis was made by using the measurement of blood gases (i.e. pO_2 , pCO_2 , pH, HCO_3^- and O_2 saturation). The inflow and outflow samples were withdrawn at $t = 0, 10, 60$ and 120 min, these were compared to values from anaesthetized rats where samples were taken from the portal and hepatic veins.

Chemical analysis

Plasma from the perfusate was analysed for glucose and lactate (Yellow Springs Analyzer). Samples obtained at the inflow to the perfusion system were deproteinized, passed through an ion-exchange resin (Bio-Rad AG-2 $\times$ 8) to remove acid components and counted for radioactivity in a liquid-scintillation counter (Packard) to obtain total [^{14}C]glucose concentrations. ^{14}C label undergoes redistribution in the glucose molecule, as glucose is catabolized to pyruvate and lactate in the peripheral tissue and the liver and re-formed to glucose. The patterns of redistribution have been determined, and a method of extracting [$1-^{14}C$]glucose from the total [^{14}C]glucose radioactivity has been suggested, based on the measurement of recycled [$6-^{14}C$]glucose (Reichard *et al.*, 1963). The latter label is measured by dimedone precipitation of the formaldehyde arising from C-6 after periodate oxidation of the glucose molecule (Reichard *et al.*, 1963). The [$1-^{14}C$]glucose concentrations used in the calculations therefore represent label which has not passed through the Cori cycle. Liver samples were dissolved in preheated 30 % (w/v) KOH and extracted with ethanol. Glycogen was dissolved in HCl, hydrolysed and neutralized with NaOH. [$1-^{14}C$]Glucose (unrecycled label) and glucose concentrations in the hydrolysate were determined in a manner analogous to that for plasma.

Corrections for finite transit time

The predicted delay between inflow and outflow sampling is approx. 2 min. As a metabolite bolus traverses the perfusion tubing and the sinusoid labyrinth, it will be dispersed, so that its outflow will be distributed in time. Since an inflow can be considered as a continuous series of boluses, the cumulative

output at the outflow will be the sum of the distributed responses arising from all the boluses. Mathematically, this is expressed as a convolution integral (Sheppard, 1954):

$$C_o^i(t) = \int_{-\infty}^t h(t-\tau)C_i(\tau)d\tau$$

where $C_o^i(t)$ is the concentration of the metabolite (glucose or lactate) at time t at the outflow of the perfusion system. It corresponds to a single passage through the perfusion tubing and liver of metabolite at concentration $C_i(t)$; the latter is measured at the inflow site; $h(t)$ is the distribution function, which corresponds to an injection of a unit amount of a substrate which equilibrates in the same way as the metabolite in question, but is not removed. The substrate used was [$fructose-1-^3H$]sucrose, which is considered inert in the context of the studies described. Although it has been shown to enter lysosomes in a 24 h incubation with isolated hepatocytes (Thirion & Wattiaux, 1988), it is classically considered an extracellular marker in perfusions [see, e.g., Goresky (1983) and Goresky *et al.* (1985)]. It should also be noted that the time course of the studies with sucrose is approx. 5 min, so that minimal label, if any, is expected to enter the intracellular space. With this label, then, $h(t)$ was determined for a series of livers and averaged for use in the transit time correction used. When recirculation of label occurs, the curve is extrapolated to zero in a linear fashion. The integral was calculated by discretization and interpolation between the measured values of $C_i(t)$ and $h(t)$. The calculated sum yields $C_o^i(t)$, which corresponds to the metabolite concentration that would have been present at the outflow if the uptake or production of metabolite had not occurred in the liver. The difference between the calculated value $C_o^i(t)$ and the observed value $C_o(t)$, $C_o^i(t) - C_o(t)$, when multiplied by the perfusion flow rate, finally yields an estimate of uptake or production of the metabolite by the liver. Separate corrections were determined for the perfusion system in the presence of, or without, the liver.

Calculations

Hepatic uptake of glucose or lactate. As detailed above, this is calculated as $(C_o^i - C_o) \times$ perfusate flow rate, where C_o^i is the concentration at the outflow site calculated from the inflow concentrations, and based on the assumption that no removal or production of this metabolite has taken place in the distance between the inflow and outflow site. C_o is the observed outflow concentration. A negative uptake is a net production. Corrections are made by using the control data by adding the lactate produced in the absence of the liver to the lactate taken up by the liver. Similar corrections are made to glucose production or uptake.

Glycogen formed directly from glucose. Formation of glycogen linearly in time is assumed. The specific radioactivity of [$1-^{14}C$]glucose in the perfusate is averaged over the perfusion time. [$1-^{14}C$]Glucose (d.p.m., corrected for recycling) in the glycogen extracted from the liver is divided by this specific radioactivity to obtain the amount of glucose incorporated directly into glycogen.

Glycogen formed by new synthesis of glucosyl units. Glycogen formed directly from glucose (see above) is subtracted from total glycogen extracted.

RESULTS

Biochemical and physiological profile of rats in liver-perfusion studies

In these studies 13 rats were used with a mean weight of 243 ± 3 g. Liver weight was 8.2 ± 0.2 g. Plasma glucose and lactate

Fig. 1. Time course of labelled sucrose distribution

[fructose-1- ^3H]sucrose ($10\ \mu\text{l}$) was rapidly injected at $t = 0$ exactly at the inflow sampling site, and $200\ \mu\text{l}$ samples were withdrawn every 10 s at the outflow sampling site for 10 min. Results of a typical experiment are shown, with the dotted line indicating recirculating tracer. $h(t)$ is obtained by extrapolating to zero from the values observed before recirculation occurs.

after anaesthesia were $89 \pm 5\ \text{mg/dl}$ and $38 \pm 2\ \text{mg/dl}$ respectively. Bile production during perfusion was $1.94 \pm 0.15\ \mu\text{l/min}$ per g of liver ($n = 8$), comparable with that in intact anaesthetized rats ($1.74 \pm 0.10\ \mu\text{l/min}$ per g of liver; $n = 5$) and with values previously reported (Mondon & Burton, 1971).

Detailed analysis for four perfused livers demonstrated an oxygen saturation at the inflow of 80–86%, near that of the intact anaesthetized rat ($86.2 \pm 3.5\%$). At the outflow site, it varied from $27 \pm 4\%$ to $37 \pm 6\%$, compared with $57 \pm 6\%$ for the intact rat during the 2 h perfusion. The $p\text{O}_2$ and $p\text{CO}_2$ values at the inflow increased from 56 ± 1 to $88 \pm 4\ \text{mmHg}$ (intact value: $69 \pm 5\ \text{mmHg}$) and from 53 ± 1 to $95 \pm 1\ \text{mmHg}$ (intact value: $64 \pm 3\ \text{mmHg}$) respectively. At the outflow $p\text{O}_2$ increased from 21 ± 2 to $35 \pm 3\ \text{mmHg}$ (intact: $39 \pm 3\ \text{mmHg}$) and $p\text{CO}_2$ from 47 ± 1 to $96 \pm 3\ \text{mmHg}$ (intact: $65 \pm 2\ \text{mmHg}$). The pH value of the perfusate decreased from 7.33 ± 0.02 to 7.07 ± 0.02 (intact value: 7.18 ± 0.02). However, the HCO_3^- concentration remained stable at approx. $20 \pm 1\ \text{mM}$, compared with an intact-rat value of $24.1 \pm 0.03\ \text{mM}$.

Response to bolus injection of [fructose-1- ^3H]sucrose

A portion of label was rapidly injected at $t = 0$ exactly at the inflow sampling site. The label was monitored for 10 min at the outflow sampling site. The concentrations are given in Fig. 1. This function is used to quantify the dispersion that occurs in the transit across the perfusion tubing and the liver. With the liver excluded, the bolus response was similar, although not identical, indicating that in the perfusion system used dispersion occurs primarily in the perfusate.

Fig. 2. Glucose and lactate uptake by rat livers perfused for 2 h with additional glucose or lactate infusion at $1\ \text{mg/min}$

The isolated rat livers were perfused for 120 min with the infusion of additional glucose (a and b) or lactate (c and d) at $1\ \text{mg/min}$. Uptake of glucose (a and c) and lactate (b and d) by the livers (—) is compared with the control group in the absence of liver (·····). A negative uptake is a net production.

Table 1. Glucose and lactate uptake and glycogen deposition by the perfused rat liver

Total glucose production and lactate uptake during a 2 h liver perfusion are calculated from the areas under the curves determined during the perfusion (Fig. 2). Corrections for erythrocyte metabolism are made by considering the areas under the control curves (no liver included). Group I studies are for additional glucose infusion (1 mg/min) during the perfusion and Group II studies are for additional lactate infusion (1 mg/min). Values are means $\pm$ S.E.M. for 4 experimental analyses. Statistical analysis of the data was performed with Student's *t* test: * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$.

	Group I: glucose infusion	Group II: lactate infusion
Total glucose production (mg)	62.90 $\pm$ 13.43	120.71 $\pm$ 19.65*
Total lactate uptake (mg)	51.02 $\pm$ 5.51	150.96 $\pm$ 15.75***
Total glycogen formed (mg)	6.24 $\pm$ 0.65	20.17 $\pm$ 3.89**
Total direct glycogen formed (mg)	4.02 $\pm$ 0.65	9.22 $\pm$ 2.02*
Total indirect glycogen formed (mg)	2.21 $\pm$ 0.30	10.95 $\pm$ 2.11***

Glucose and lactate concentrations during perfusion

With glucose infusion at 1 mg/min, perfusate glucose concentrations increased from 245 ± 7 to 448 ± 13 mg/dl, similar to the increase in the absence of liver (from 269 ± 6.8 to 429 ± 4.1 mg/dl). The perfusate lactate concentrations without liver increased from 33 ± 0.9 to 72 ± 1.5 mg/dl, whereas, with the liver included, lactate levels decreased in the first 20 min from 49 ± 2.6 to 9 ± 0.6 mg/dl, and then remained constant. With lactate infusion at 1 mg/min, the perfusate glucose concentrations increased from 254 ± 12.9 to 333 ± 24 mg/dl, but decreased from 291 ± 19 to 207 ± 18 mg/dl in the absence of liver. The lactate concentrations increased slightly from 54 ± 2.2 to 63 ± 11 mg/dl, whereas in the control situation they rose from 35 ± 2.0 to 350 ± 2.0 mg/dl.

Glucose and lactate uptake

As shown in Fig. 2, initial lactate uptake and glucose production were identical in the two groups of studies, both averaging near 2.5 mg/min. This suggests nearly complete conversion of lactate into glucose. With glucose infusion at 1 mg/min, both decreased to less than 0.25 mg/min within 30 min (Figs. 2a and 2b). With infusion of lactate (Figs. 2c and 2d), its uptake by the liver was maintained, decreasing gradually from 2.0 ± 0.3 to 0.89 ± 0.14 mg/min. Glucose production never became zero, but fell from 2.8 ± 0.4 to 0.29 ± 0.33 mg/min.

Total lactate uptake (Table 1) increased from 51 ± 6 to 151 ± 16 mg when lactate was infused instead of glucose. Net glucose production over the perfusion period increased from 63 ± 13 to 121 ± 20 mg.

Glycogen deposition in the perfused liver

As shown in Table 1, when the perfusate was continuously supplemented with lactate instead of glucose, the total glycogen synthesized increased from 6 ± 0.7 to 20 ± 4 mg, even though the glucose concentration was lower in this case. Glycogen estimated to be formed by the gluconeogenic pathway increased 5-fold.

Interestingly, the amount of glycogen formed directly from glucose also increased by 130 %.

In summary, therefore, the presence of sufficient lactate promotes its uptake by the liver, with the net formation of glucose. Glycogen formation is tripled, with increase in fluxes through both the direct and gluconeogenic pathways.

DISCUSSION

Lactate appears to be a principle carrier of carbon from the peripheral tissues to the liver for the new synthesis of glucose or glycogen (Cori & Cori, 1929; Wood *et al.*, 1945; Topper & Hastings, 1949). This is of particular importance after carbohydrate meals, since it is the metabolic product of glucose whose concentration increases after its ingestion or intravenous administration (e.g. Radziuk & Inculet, 1983), largely owing to metabolism in the gut (Radziuk, 1989a) and in subcutaneous and adipose tissue (Johnson & Fusaro, 1973; Hagstrom *et al.*, 1990). Gluconeogenesis has been found to be a major pathway for liver glycogen synthesis (Hems *et al.*, 1972; Shikama & Ui, 1978; Radziuk, 1982; Magnusson *et al.*, 1989) in many species and is, moreover, essential to it (Newgard *et al.*, 1983). The peripheral lactate formation from glucose is in turn necessary to provide substrate for the gluconeogenic process (Landau & Wahren, 1988; Wajngot *et al.*, 1989). Both glucose and lactate therefore are substrates for hepatic glycogen synthesis after carbohydrate loading. The relative contribution of lactate to glycogen synthesis under different physiological circumstances varies from 14 % to 40 % of glycogen formed (e.g. Shulman *et al.*, 1985; Levitsky *et al.*, 1988; Salhanick *et al.*, 1989; Radziuk, 1989b; Katz *et al.*, 1989).

Both glucose and lactate (as well as other glucogenic substrates) can also modulate enzyme activities to enhance flux through a particular pathway. For example, activities of enzymes of glycolysis and gluconeogenesis have been shown to be modified by the addition of glucose, lactate or fructose to the perfusates in a liver-perfusion system (Wimhurst & Manchester, 1973). Glucose can inhibit the activity of glycogen phosphorylase *a* and promote that of glycogen synthetase *a* (Hers *et al.*, 1974; Hue *et al.*, 1975). Fructose 1-phosphate has also been demonstrated to be a competitive inhibitor of phosphorylase *a* (Kaufmann & Froesch, 1973).

The data presented here demonstrate that increasing lactate concentrations in the perfused liver system allows its increased uptake, with subsequent increases in glucose production and glycogen synthesis. With an increase in the availability of substrate, the gluconeogenic pathway of glycogen synthesis is enhanced (4-fold). Surprisingly, the direct formation of glycogen from glucose (carbon not recycled via glycolysis) also increases by 130 %. This suggests that lactate, a principle physiological substrate, also modulates the flux from glucose to glycogen.

That lactate uptake is rate-limiting for the synthesis of glycogen is indicated in Fig. 2(b), where all lactate formed by perfusate erythrocytes is removed by the liver. As lactate uptake increases (Fig. 2d), more glycogen is formed, in spite of lower glucose concentrations (333 ± 24 versus 448 ± 13 mg/dl). Increased gluconeogenesis takes place, since lactate can induce both pyruvate carboxylase and phosphoenolpyruvate carboxykinase activities, although pyruvate kinase is unaffected (Wimhurst & Manchester, 1973). In addition, high concentrations of lactate (20 mM) cause a decrease in fructose 2,6-bisphosphate, with the result that there is a relative inhibition of phosphofructokinase and stimulation of fructose 1,6-bisphosphatase (Hue *et al.*, 1982). Lactate promotes the gluconeogenic process, thus providing additional substrate for glycogen synthesis. However, it provides

a weaker glucose signal (Hers *et al.*, 1974; Hue *et al.*, 1975) for glycogen synthesis than does glucose infusion.

Since glycogen formation and the direct conversion of glucose into glycogen in particular are enhanced by lactate when glucose concentrations are lower, it is tempting to postulate that lactate could also modulate the activities of glycogen synthetase or phosphorylase. This is especially likely since glucokinase activity appears unaffected by perfusion with lactate (Wimhurst & Manchester, 1973).

The results presented here re-emphasize that the availability of physiologically generated gluconeogenic substrate (represented by lactate) from non-hepatic sources and its uptake by the liver appear essential in determining not only the net rate of glucose production but also the amount of glycogen formed. The availability of this substrate, moreover, promotes glycogen formation both by gluconeogenesis and directly from glucose. Since lactate concentrations change under circumstances such as glucose loading, meals or exercise, they may contribute to the regulation of hepatic glycogen repletion as well as serving as a substrate.

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