Biochem. J. (1984) **219**, 773–780
Printed in Great Britain

Inhibition of gluconeogenesis in rat liver by lipoic acid

Evidence for more than one site of action

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(*Received 11 October 1983/Accepted 24 January 1984*)

Hepatocytes obtained from starved rats were incubated in oxygenated Krebs bicarbonate buffer containing 2% defatted bovine serum albumin. DL-α-Lipoic (dithiooctanoic) acid (1.0mM) caused striking reductions in hepatic glucose output in the presence of each of the following substrates: pyruvate, lactate, alanine, dihydroxyacetone, glycerol, and fructose. With lactate as substrate, 0.1–1.0mM-lipoate caused a concentration-dependent inhibition of gluconeogenesis. With the same substrate, e.g. lactate, 0.25–2.0mM-octanoate abolished the effect of lipoate in a dose-dependent manner. Additional experimental data are presented which support the concept that the antigluconeogenic effects of lipoic acid in liver can be attributed largely, if not entirely, to sequestration of intramitochondrial coenzyme A, presumably as lipoyl-CoA, bisnorlipoyl-CoA, or tetranorlipoyl-CoA.

α-(+)-Lipoic acid (6,8-dithio-octanoic acid) performs a crucial function in oxidative metabolism because it donates the prosthetic (lipoamide) group for the transacylation step in the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase reactions (Hayakawa *et al.*, 1964).

There have been reports (Singh & Bowman, 1970; Haugaard & Haugaard, 1970) that exogenous lipoic acid accelerates glucose uptake in rat tissues (perfused hearts from normal and alloxan-diabetic animals and excised hemidiaphragms from normal rats). This action may be ascribable to a primary reduction of acetyl-CoA levels in these tissues: decrease in acetyl-CoA leads to decrease in citrate levels, activation of phosphofructokinase and hence increased glycolysis (Singh & Bowman, 1970). In the experiments cited, lipoate also augmented insulin-mediated glucose utilization in rat heart and diaphragm (Singh & Bowman, 1970; Haugaard & Haugaard, 1970).

The observation that lipoic acid lowers acetyl-CoA levels in myocardium (Singh & Bowman, 1970) suggested that it might have a similar effect in liver and, consequently, might inhibit hepatic ketogenesis and gluconeogenesis in diabetic rats. In what follows, we present studies of the metabolic effects of lipoic acid in rat liver.

Materials and methods

General considerations

In preliminary experiments it was established that, at concentrations of 1.0mM or less, DL-α-lipoate did not affect either the measurement of acetyl-CoA or the assay of glucose by the glucose oxidase method. It was also ascertained (by measurement of the glycogen content of whole livers after 60min of perfusion with alanine) that there was no net synthesis of glycogen during the period of perfusion in livers from rats starved for 48h. Thus, the glucose which appeared in perfusate (or incubation medium) during the experiments reported below closely approximated the total quantity of glucose synthesized from added C_3 precursors.

Animals

Male Sprague–Dawley rats, 100–200g, which had been starved for 48h were used in these experiments.

Preparation of hepatocytes

Hepatocytes were prepared with collagenase (Sigma) by using published methods (Berry & Friend, 1969). The viability of hepatocytes was

confirmed by the ability of at least 95% of the cells to exclude Trypan Blue. Incubations (30min) were carried out in Krebs bicarbonate buffer (Krebs & Henseleit, 1932) [containing 2% defatted bovine serum albumin (Chen, 1967)], pH7.4 at 37°C. Erlenmeyer flasks (25ml), each containing 2ml of hepatocyte suspension or 80–100mg wet wt. of cells, were used for the incubations. Flasks were agitated in a metabolic shaker (80–100cycles/min) and continuously gassed with O_2/CO_2 (19:1). When the cells had been 'preincubated' for 20min, gluconeogenic substrate was added to each reaction flask to start the incubation. 'Zero-time' specimens were obtained by adding $HClO_4$ to some of the flasks at the end of the preincubation period.

Liver perfusion

Livers were perfused for 60min with oxygenated recirculating Krebs bicarbonate buffer (Krebs & Henseleit, 1932) which contained 3% defatted bovine albumin and enough outdated human red blood cells to bring the haematocrit to 20%. Perfusate volume was approx. 80ml. The perfusion technique used in these studies has been previously described (Exton & Park, 1967).

Sample collections

Liver cell suspensions were deproteinized with $HClO_4$ (0.2ml of 10% $HClO_4$ to 2.1ml of reaction mixture) at the end of incubation and centrifuged (150*g*, 10min) to obtain a clear supernatant. An aliquot of this supernatant was taken for immediate assay of glucose. The remainder of the fluid was neutralized (to pH3.5) with 5M-K_2CO_3, cooled and recentrifuged. The supernatant was then frozen. Metabolites were measured in thawed aliquots of this supernatant. In experiments with whole liver, portions of perfused livers were crushed in a tissue clamp previously cooled to −80°C in solid CO_2 and were stored at −70°C for subsequent analysis of intracellular metabolites. At the time of assay, the frozen tissue was weighed, powdered at −80°C, extracted with cold 10% $HClO_4$ (4ml per g of tissue) and neutralized wtih 5M-K_2CO_3.

Preparation of lipoic acid solution

Sodium lipoate (10mM) was prepared by adding 1ml of distilled water containing 20.63mg of DL-α-lipoic acid (Sigma) and 20mg of $NaHCO_3$ to 9ml of Krebs bicarbonate buffer (Krebs & Henseleit, 1932) containing 2% defatted bovine albumin.

Measurement of metabolites

Glucose was measured enzymically with glucose oxidase and peroxidase (Caraway, 1976), and glycogen by assay of the glucose liberated by acid hydrolysis (Walaas & Walaas, 1950). Other metabolites were measured enzymically with a Gilford recording spectrophotometer. These included acetoacetate (Mellanby & Williamson, 1974), β-hydroxybutyrate (Williamson & Mellanby, 1974), ATP (Lamprecht & Trautschold, 1974), AMP and ADP (Jaworek *et al.*, 1974), acetyl-CoA (Decker, 1974), coenzyme A (Michal & Bergmeyer, 1974), citrate (Dagley, 1974), pyruvate (Czok & Lamprecht, 1974), and lactate (Gutmann & Wahlefeld, 1974).

Estimation of [^{14}C]glucose synthesis from [1-^{14}C]lactate

DL-[1-^{14}C]Lactic acid, sodium salt (sp. radioactivity 51mCi/mmol), was procured from Amersham Corp., Arlington Heights, IL, U.S.A. Using the technique of Exton & Park (1967), we determined the rate of conversion of [1-^{14}C]lactate to [^{14}C]glucose by hepatocytes incubated in media containing labelled substrate (5mM-lactate with a specific radioactivity of approx. 3500c.p.m./μmol). A 0.5ml aliquot of supernatant from the centrifuged cell suspension was deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and centrifuged for 10min at 600*g* to obtain a protein-free filtrate (Nelson, 1944). The radioactivity from [^{14}C]lactate was extracted by the addition of 100mg of Dowex 50 (H^+ form) and 400mg of Duolite (OH^- form) to the filtrate (followed by 30min of shaking and 3–4min of centrifugation). Portions (0.5ml) of the resin-free supernatant were transferred to vials containing 10ml of dioxan scintillation fluid and counted. More than 90% of this radioactivity can be attributed to [^{14}C]glucose (Exton & Park, 1967).

Quantification of $^{14}CO_2$ formation from [1-^{14}C]lactate

The rate of $^{14}CO_2$ formation from [1-^{14}C]lactate by hepatocytes incubated with labelled substrate (5mM-lactate with a specific radioactivity of approx. 3500c.p.m./μmol) was determined in the following manner. Incubations were carried out in reaction flasks capped with perforated rubber stoppers through which plastic centre wells were inserted into the flasks. (Stoppers and wells were obtained from Kontes Scientific Glassware/Instruments, Vineland, NJ, U.S.A.) Incubations were terminated after 30min by addition of 0.2ml of 10% $HClO_4$ to each reaction mixture. The $^{14}CO_2$ liberated by $HClO_4$ was trapped in the centre well, which contained filter paper immersed in KOH. The centre well was then transferred to a vial and counted for radioactivity.

Statistics

Many of our studies involved the use of a control group and at least three different experimental conditions. In order to compare each of these

conditions with the control, Dunnett's *t* test was employed (Winer, 1971). This test permits a measure of total variance when a control group and two or more experimental groups coexist. When a control group and only one experimental group coexisted, Student's *t* test was used to compare them.

Results and discussion

Table 1(*a*) shows that lipoate inhibited glucose synthesis from six individual precursors. In the case of lactate, at least, the degree of inhibition was dose-related over the range 0.1–1.0mM-lipoate (Table 1*b*). In the presence of 1.0mM-lipoate, glucose formation from reduced substrates (lactate and glycerol) was depressed to the same extent as glucose formation from oxidized substrates (pyruvate and dihdyroxyacetone).

Table 2 contains two important observations. First, the inhibition of hepatic gluconeogenesis by lipoate was associated with a decrease in ketogenesis and in the molar ratio of β-hydroxybutyrate to acetoacetate in the incubation medium (Table 2*a*). Secondly, octanoate reversed the antigluconeogenic effect of lipoate in a dose-related manner (Table 2*b*). Production of ketone bodies and the β-hydroxybutyrate : acetoacetate ratio greatly exceeded control values in the presence of octanoate and increased in response to increments in octanoate concentration. These results can be explained if we assume: (1) that lipoate or one of its β-oxidative derivatives, e.g., bisnorlipoate or tetranorlipoate, suppresses endogenous fatty acid oxidation by sequestering intramitochondrial HSCoA in the form of lipoyl-CoA, bisnorlipoyl-CoA, or tetranorlipoyl-CoA; (2) that octanoate successfully competes with lipoate for HSCoA, thereby reversing its antigluconeogenic and antiketogenic effects. The first of these assumptions is supported by observations that rats and certain bacteria metabolize lipoate by β-oxidation of the valeric acid side chain (Harrison & McCormick, 1974; Chang *et al.*, 1975; Shih *et al.*, 1975; Spence & McCormick, 1976; Furr *et al.*, 1978). The second assumption is consistent with the finding that octanoate depressed the oxidation of lipoate in rat liver homogenates (Harrison & McCormick, 1974).

The data in Table 3 provide more direct evidence that lipoate combines with HSCoA to form lipoyl-CoA and that octanoate opposes this reaction. When livers from starved rats were perfused with lipoate, rapid and sustained decreases occurred in hepatic concentrations of HSCoA and acetyl-CoA (striking changes were apparent at 5min and persisted for at least 75min). When perfusion media contained equimolar amounts of lipoate and octanoate, there was an early decline in

Table 1. *Effect of lipoate on glucose formation from multiple substrates in hepatocytes from rats starved for 48h*

(*a*) Incubations (30min) of hepatocytes were carried out at 37°C and pH7.4 in Krebs bicarbonate buffer containing 2% defatted bovine serum albumin, 0–1.0mM-lipoate, and 5mM concentrations of each of the glucose precursors indicated. ***$P<0.001$. Glucose production is expressed per g wet wt. of hepatocytes/30min. Each number in parentheses gives the number of observations in a particular subgroup. The inhibitory effects of 1mM-lipoate were not reversed by 15mM-lactate (the mean of three measurements of glucose production in the presence of 1mM-lipoate and 15mM-lactate was 1.55μmol/g per 30min). (*b*) Shows the effect of increasing concentrations of lipoate on glucose production from lactate.

		Experimental group	Glucose production (μmol/g wet wt. per 30min, ±S.E.M.)
(*a*)	(*a*) I	5mM-Lactate	14.29±0.53 (6)
	II	5mM-Lactate+1mM-lipoate	4.14±0.70*** (6)
	(*b*) I	5mM-Pyruvate	17.58±0.48 (6)
	II	5mM-Pyruvate+1mM-lipoate	2.29±0.15*** (6)
	(*c*) I	5mM-Alanine	12.53±0.35 (12)
	II	5mM-Alanine+1mM-lipoate	1.14±0.27*** (12)
	(*d*) I	5mM-Glycerol	20.23±0.87 (23)
	III	5mM-Glycerol+1mM-lipoate	4.43±0.37*** (13)
	(*e*) I	5mM-Dihydroxyacetone	33.56±0.92 (6)
	II	5mM-Dihydroxyacetone+1mM-lipoate	10.55±0.01*** (6)
	(*f*) I	5mM-Fructose	63.45±4.55 (12)
		5mM-Fructose+1mM-lipoate	21.16±2.96*** (12)
(*b*)	I	5mM-Lactate	19.34±0.73 (6)
	II	5mM-Lactate+0.1mM-lipoate	15.62±0.27 (3)
	III	5mM-Lactate+0.25mM-lipoate	8.04±0.13 (3)
	IV	5mM-Lactate+0.5mM-lipoate	3.40±0.48 (6)
	V	5mM-Lactate+0.75mM-lipoate	1.50±0.10 (3)
	VI	5mM-Lactate+1.0mM-lipoate	0.74±0.22 (3)

Table 2. *Effects of increasing concentrations of octanoic acid on ketogenesis and on glucose formation from lactate in hepatocytes exposed to 1.0 mM-lipoate*

Hepatocytes were isolated from rats starved for 48 h, and incubated for 30 min in media containing Krebs bicarbonate buffer, 2% defatted bovine serum albumin, 5 mM-lactate, 0–1 mM-lipoate, and 0–2 mM-octanoate as indicated below. Groups II, III, and IV were compared with controls (group I) by Dunnett's *t* test. ** $P<0.005$; *** $P<0.001$. Glucose and ketone production are expressed per g wet wt. of hepatocytes/30 min. Mean square (MS) is the pooled sum of squares of intragroup variation divided by total degrees of freedom. (*b*) Shows the effect of increasing concentrations of octanoate on lipoate-induced inhibition of glucose production from lactate; (*c*) shows the effect of increasing concentrations of octanoate alone on the conversion of lactate to glucose.

		Experimental group	Glucose production (μmol/g wet wt. per 30 min, ±S.E.M.) (MS = 2.64)	Total ketone production (μmol/g wet wt. per 30 min) (MS = 3.46)	β-Hydroxybutyrate/acetoacetate molar ratio in medium (end of incubation) (MS = 0.04)
(*a*)	I	5 mM-Lactate	10.13 ± 0.22 (6)	2.96 ± 0.11 (6)	0.52 ± 0.02 (6)
	II	5 mM-Lactate + 1.0 mM-lipoate	1.15 ± 0.28*** (6)	1.37 ± 0.17 (6)	0.13 ± 0.01** (6)
	III	5 mM-Lactate + 1.0 mM-lipoate + 1.0 mM-octanoate	6.80 ± 0.11** (6)	12.98 ± 0.17** (6)	1.03 ± 0.09** (6)
	IV	5 mM-Lactate + 1.0 mM-lipoate + 2.0 mM-octanoate	11.81 ± 1.27 (6)	30.50 ± 1.49*** (6)	1.95 ± 0.12*** (6)
(*b*)	I	5 mM-Lactate	10.77 ± 0.68 (9)		
	II	5 mM-Lactate + 1 mM-lipoate	0.91 ± 0.12 (9)		
	III	Lactate + lipoate + 0.25 mM-octanoate	4.26 ± 0.20 (6)		
	IV	Lactate + lipoate + 0.5 mM-octanoate	6.82 ± 0.49 (6)		
	V	Lactate + lipoate + 1 mM-octanoate	9.27 ± 0.25 (3)		
	VI	Lactate + lipoate + 2 mM-octanoate	12.44 ± 0.17 (3)		
(*c*)	I	5 mM-Lactate	13.90 ± 0.34 (6)		
	II	Lactate + 0.25 mM-octanoate	19.73 + 1.20 (4)		
	III	Lactate + 0.5 mM-octanoate	21.65 ± 0.74 (4)		
	IV	Lactate + 1 mM-octanoate	20.53 ± 1.27 (5)		
	V	Lactate + 2 mM-octanoate	23.16 ± 0.75 (4)		

Table 3. *Effects of lipoic and octanoic acids on levels of acetyl-CoA, HSCoA, and citrate in perfused livers from starved rats*

Livers from rats starved for 48 h were perfused for 5 or 75 min with recirculating medium containing 3% defatted bovine serum albumin, 5 mM-lactate (initial concentration) and the other additions indicated. During the 75 min perfusions, lactate was added to the medium at 15 min rather than at time zero and glucose production was measured for 1 h following the addition of lactate. Whenever present, lipoate and octanoate were added at time zero. Tissue levels of acetyl-CoA were measured at 5 and 75 min. In another series of perfusions performed under the same experimental conditions, tissue levels of HSCoA and citrate were measured at 5 and 75 min. Values for each measurement are the means of observations in two or more livers, as indicated by the numbers in parentheses.

	Experimental group	Acetyl-CoA level (μmol/g dry wt.) 5 min	Acetyl-CoA level (μmol/g dry wt.) 75 min	HSCoA level (μmol/g dry wt.) 5 min	HSCoA level (μmol/g dry wt.) 75 min	Citrate level (μmol/g dry wt.) 5 min	Citrate level (μmol/g dry wt.) 75 min	Glucose production (μmol/100 g body wt./h)
I	5 mM-Lactate	0.154 (2)	0.162 (2)	0.793 (2)	0.475 (2)	0.246 (2)	1.327 (2)	112.4 (4)
II	5 mM-Lactate + 1 mM-lipoate	0.027 (2)	0.011 (2)	0.257 (2)	0.219 (2)	0.240 (2)	0.484 (2)	35.8 (2)
III	5 mM-Lactate + 1 mM-octanoate	0.240 (2)	0.156 (2)	0.305 (2)	0.366 (2)	0.344 (2)	1.968 (2)	130.1 (3)
IV	5 mM-Lactate + 1 mM-octanoate + 1 mM-lipoate	0.242 (2)	0.011 (2)	0.386 (2)	0.171 (2)	0.219 (2)	0.468 (2)	77.2 (2)
V	5 mM-Lactate + 2 mM-octanoate	0.240 (2)	0.135 (2)	–	–	–	–	128.2 (2)
VI	5 mM-Lactate + 2 mM-octanoate + 1 mM-lipoate	0.201 (2)	0.018 (2)	–	–	–	–	97.4 (2)

the level of HSCoA and a concurrent increase in that of acetyl-CoA, indicating the preferential formation and rapid β-oxidation of octanoyl-CoA. After 75min of perfusion with lactate/octanoate/lipoate, HSCoA and acetyl-CoA levels were extremely low. Extrapolation from the data in Table 2 on rates of ketogenesis in hepatocyte suspensions suggests that 1–2mM-octanoate added to recirculating liver perfusion media would be completely metabolized after 75min. Assuming, therefore, the virtual disappearance of octanoate from the medium by 75min, the low tissue levels of HSCoA and acetyl-CoA at this time point to the unopposed binding of HSCoA as lipoyl- or bisnorlipoyl-CoA, renewed suppression of endogenous fatty acid oxidation, and a marked delay in the oxidative removal of lipoyl-CoA or its thioester derivatives.

Table 4 shows that 1.0mM-lipoate caused equivalent (60–65%) reductions in $^{14}CO_2$ and [^{14}C]glucose formation from [1-^{14}C]lactate. Since this effect was seen in the absence of any increment in the lactate:pyruvate ratio (see Table 7), it is likely that lipoate inhibited the two principal pathways of pyruvate disposal, oxidation and carboxylation. We assume that conversion of pyruvate to glucose was impaired at the carboxylation step, because published data suggest that the measured acetyl-CoA concentrations of 0.01–0.02μmol/g dry wt. (see Table 3) would have been insufficient to stimulate pyruvate carboxylase (Utter & Scrutton, 1969; Toews *et al.*, 1970). On the other hand, the inhibition of pyruvate oxidation by lipoate is not explained by our data. It is unlikely that the 60–65% decrease in hepatic coenzyme A levels induced by lipoate (Table 3) would have interfered with pyruvate decarboxylation (Ngo & Barbeau, 1978). Depression of pyruvate carboxylase activity does not account for lipoate-induced inhibition of glucose formation from fructose, glycerol and dihydroxyacetone, compounds which enter the gluconeogenic pathway 'distal' to pyruvate decarboxylase.

A reasonable explanation for lipoate-induced inhibition of glucose synthesis from these compounds is that formation of lipoyl-CoA greatly decreased the amount of acetyl-CoA available for citrate synthesis. Consequently, the increments in citrate concentration observed in livers perfused with lactate/lipoate were exceedingly small (see Table 3). It is known that citrate inhibits phosphofructokinase and that coenzyme A decreases the concentration of citrate required for such inhibition (Parmeggiani & Bowman, 1963). In the presence of lipoate, hepatic coenzyme A levels were substantially depressed and thus the highest citrate level attained (0.48μmol/g dry wt.) was probably inadequate to block enzyme action. As a result, the direction of carbon flux was reversed at the level of fructose 6-phosphate and net glucose synthesis declined.

This conclusion is supported by the studies of Singh & Bowman (1970). These investigators perfused rat hearts with glucose or glucose and 1.0mM-lipoate and noted with the latter a decrease in citrate concentration (1.595 to 0.795μmol/g dry wt.), increased conversion of glucose to lactate, and a 'crossover' between fructose 6-phosphate

Table 4. *Effects of 1.0mM-lipoate on glucose production from lactate and on the conversion of [1-^{14}C]lactate to [^{14}C]glucose and $^{14}CO_2$ in hepatocytes from rats starved for 48h*

Hepatocytes were incubated for 30min in media containing [1-^{14}C]lactate (sp. radioactivity approx. 3500c.p.m./μmol) and 5mM-lactate. Production of [1-^{14}C]glucose and $^{14}CO_2$ were determined in the presence and absence of 1.0mM-lipoate. The experiments in which [^{14}C]glucose was measured were performed independently of those in which $^{14}CO_2$ was measured. Labelled lactate and glucose were separated by extracting the lactate-associated counts in the incubation medium with Dowex 50–Duolite. $^{14}CO_2$ was liberated from the medium by the addition of $HClO_4$ at the end of incubation and trapped in centre wells containing filter paper impregnated with KOH. (See the Materials and methods section for additional details.) In parts (*a*) and (*b*) groups I and II were compared by Student's *t* test. *** $P<0.001$.

		Experimental group	Glucose production (μmol/g wet wt. per 30 min, ±S.E.M.)	[^{14}C]Glucose production (c.p.m./g wet wt. per 30 min, ±S.E.M.)	$^{14}CO_2$ production (c.p.m./g wet wt. per 30 min, ±S.E.M.)
(*a*)	I	5mM-Lactate+[1-^{14}C]lactate (3534c.p.m./μmol)	8.09±0.27 (8)	36187±2405 (7)	
	II	5mM-Lactate+[1-^{14}C]lactate (3534c.p.m./μmol)+1.0mM-lipoate	3.96±0.42*** (8)	14674±3533*** (6)	
(*b*)	I	5mM-Lactate+[1-^{14}C]lactate (3637c.p.m./μmol)	8.84±0.25 (8)		16818±1268 (8)
	II	5mM-Lactate+[1-^{14}C]lactate (3637c.p.m./μmol)+1.0mM-lipoate	2.52±0.13*** (8)		6018±583*** (8)

Table 5. *Effects of lipoate on glucose and lactate formation from fructose and glycerol in hepatocytes from starved rats*

The experimental conditions used in these studies were the same as those described in Table 1. Glucose and lactate production are expressed in μmol/g wet wt. of hepatocytes per 30 min. In (*a*), *** denotes $P<0.001$ (when lactate or glucose production in the presence of lipoate is compared with that in the absence of lipoate). In (*b*), Groups II, III and IV are compared with Group I by Dunnett's *t* test; *** denotes $P<0.005$.

(*a*)

Experimental group	5 mM-Fructose		5 mM-Fructose + 1 mM-lipoate	
	Glucose production	Lactate production	Glucose production	Lactate production
	63.45 ± 4.55 (12)	41.74 ± 3.21 (9)	21.16 ± 2.96*** (12)	67.77 ± 3.64*** (9)

(*b*)

I 5 mM-Glycerol		II 5 mM-Glycerol + 1 mM-lipoate		III 5 mM-Glycerol + 1 mM-octanoate		IV 5 mM-Glycerol + 1 mM-lipoate + 1 mM-octanoate	
Glucose production	Lactate production	Glucose production	Lactate production	Glucose production	Lactate production	Glucose production	Lactate production
17.61 ± 1.32 (6)	5.30 ± 0.85	4.66 ± 0.45*** (6)	15.88 ± 0.26***	14.57 ± 0.61 (6)	3.94 ± 1.09	7.32 ± 0.54*** (6)	6.24 ± 1.25

Table 6. *Effect of lipoate on the conversion of glucose to lactate in hepatocytes from starved rats*

Incubations (30 min) were carried out at 37°C, pH 7.4, in Krebs bicarbonate buffer containing 2% defatted bovine serum albumin, 5 or 40 mM-glucose, and, in half of the flasks, 1 mM-lipoate. Lactate production is measured in μmol/g wet wt. per 30 min. ***$P<0.001$.

	Experimental group	Lactate production
(*a*)	I 5 mM-Glucose	0.28 ± 0.04 (5)
	II 5 mM-Glucose + 1 mM-lipoate	3.99 ± 0.09*** (3)
(*b*)	I 40 mM-Glucose	6.89 ± 0.89 (6)
	II 40 mM-Glucose + 1 mM-lipoate	23.31 ± 0.09*** (3)

Table 7. *Effect of 1.0 mM-lipoic acid on adenine nucleotide concentrations and intrahepatic lactate/pyruvate ratios in livers from starved rats perfused with 5 mM-alanine for 60 min*

The livers analysed in this Table were obtained from rats fasted for 48 h pre-perfusion. Adenine nucleotide concentrations were determined at the end of each perfusion and are expressed as μmol/g dry wt. ± S.E.M.

Experimental group	ATP	ADP	AMP	Total adenine nucleotides	Energy charge*	Lactate/pyruvate molar ratio
I 5 mM-Alanine	6.57 ± 0.47 (3)	2.85 ± 0.27 (3)	2.66 ± 0.54 (3)	12.07 ± 0.14 (3)	0.66 ± 0.04 (3)	20.4 ± 9.9 (5)
II 5 mM-Alanine + 1.0 mM-lipoate	7.69 ± 0.95 (3)	2.86 ± 0.05 (3)	1.73 ± 0.26 (3)	12.28 ± 0.82 (3)	0.74 ± 0.03 (3)	8.5 ± 6.0 (5)

* Energy charge, as defined by Atkinson (1968), equals [(ATP) + 1/2(ADP)]/[ATP + ADP + AMP].

and fructose 1,6-bisphosphate consistent with an increase in phosphofructokinase activity.

When livers were perfused with lactate/lipoate/octanoate, citrate concentration at 75min was 0.47μmol/g dry wt., i.e., equivalent to the concentration found after perfusion with lactate and lipoate. Yet octanoate clearly reversed lipoate-mediated blockade of gluconeogenesis. These results are compatible with the concept of early stimulation of gluconeogenesis, due to a high rate of octanoate oxidation, and late depression of glucose production, due to octanoate depletion and the unopposed action of lipoate.

Evidence is presented in Table 5 that lipoate antagonizes gluconeogenesis, in part, by augmenting glycolysis. When fructose or glycerol was used as the substrate in hepatocytes from starved rats, lipoate-mediated blockade of glucose synthesis was associated with an increase in lactate production sufficient to account for 30–40% of the inhibitory effect. (Measurement of lactate production underestimates glycolytic rate since pyruvate, the end product of glycolysis, is not only converted to lactate but also oxidized and transaminated to form alanine.) Addition of octanoate to hepatocytes incubated in a mixture of glycerol and lipoate partially relieved the depression in glucose output and concomitantly decreased lactate production. These effects can be attributed to octanoate-induced increases in hepatic acetyl-CoA and citrate levels. The failure of octanoate to restore normal glucose production in these experiments, as well as in studies employing other glucose precursors, is probably related to the continuous removal of octanoate by β-oxidation during the 30min hepatocyte incubations. As suggested previously, this would lead to progressive intensification of the antigluconeogenic action of lipoate. In other words, when lipoate, octanoate, and glycerol are simultaneously added to hepatocyte suspensions, gluconeogenesis is likely to be normal or increased during the first few minutes of incubation, when the effects of octanoate are predominant, but markedly diminished during the latter part of incubation, when the effects of lipoate predominate. This interpretation is consistent with the data in Table 2 on ketone body production in the presence of 1–2mM-octanoate. These data indicate that by the end of the 30min incubation period, the bulk of added octanoate has undergone partial β-oxidation.

Further evidence that lipoate stimulates glycolysis can be found in the data recorded in Table 6. When hepatocytes from starved rats were incubated with 5 or 40mM-glucose, the addition of 1mM-lipoate caused striking increases in lactate production.

Table 7 reveals that lipoate did not lower hepatic ATP concentrations or energy charge (Atkinson, 1968) or elevate lactate:pyruvate molar ratios in perfused livers from fasted rats. Maintenance of normal ATP levels under these conditions may be due, in part, to diminished utilization of ATP by energy-requiring processes (e.g., gluconeogenesis or conversion of endogenous fatty acid to fatty acyl-CoA) and to production of ATP by glycolysis.

In conclusion, using multiple glucose precursors, we have shown that lipoic acid powerfully inhibits hepatic gluconeogenesis. This effect appears to be the consequence of a single primary action of the drug, i.e., the sequestration of HSCoA as lipoyl-CoA. Schematically, the following sequence of events is believed to occur: formation of lipoyl-CoA causes reduction of HSCoA levels, reduction of acetyl-CoA levels, and hence impairment of citrate synthesis. Consequently, citrate levels do not increase sufficiently to repress phosphofructokinase. Low acetyl-CoA concentrations impair gluconeogenesis by preventing the activation of pyruvate carboxylase. By failing to inhibit phosphofructokinase, low citrate levels reverse gluconeogenic flux. This combination of circumstances (i.e. interference with glucose production and enhancement of glycolysis) tends to cause extreme reduction in net glucose synthesis. Octanoate overcomes the antigluconeogenic action of lipoate by reacting with HSCoA to form octanoyl-CoA, which promptly undergoes oxidation to generate acetyl-CoA (and citrate).

Several hypoglycaemic drugs, notably pent-4-enoate and methylene cyclopropylacetate (a metabolite of hypoglycin), resemble lipoate in that they bind intramitochondrial HSCoA and interfere with hepatic gluconeogenesis (Bressler *et al.*, 1969). Although these authors ascribe the decrease in new glucose synthesis solely to drug-induced suppression of fatty acid oxidation, the data presented above suggest that pent-4-enoate and hypoglycin may also diminish gluconeogenesis by derepressing phosphofructokinase.

I am grateful to Dr. Roger Bowman for his critical comments and encouragement. Ms. Barbara Hayes gave valuable technical assistance.

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