Biochem. J. (1982) **208**, 53–60
Printed in Great Britain

Effect of the fatty acid oxidation inhibitor 2-tetradecylglycidic acid on pyruvate dehydrogenase complex activity in starved and alloxan-diabetic rats

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(*Received 13 April 1982/Accepted 6 July 1982*)

Intravenous administration of the fatty acid oxidation inhibitor 2-tetradecylglycidic acid had no effect on the proportion of pyruvate dehydrogenase complex in the active form in heart, diaphragm or gastrocnemius muscles or in liver, kidney or adipose tissue of fed normal rats. The compound reversed the effect of 48 h starvation (which decreased the proportion of active complex) in heart muscle, partially reversed the effect of starvation in kidney, but had no effect in the other tissues listed. The compound failed to reverse the effect of alloxan-diabetes (which decreased the proportion of active complex) in any of these tissues. In perfused hearts of fed normal rats, 2-tetradecylglycidate reversed effects of palmitate (which decreased the proportion of active complex), but it had no effect in the absence of palmitate. In perfused hearts of 48 h-starved rats the compound increased the proportion of active complex to that found in fed normal rats in the presence or absence of insulin. In perfused hearts of diabetic rats the compound normalized the proportion of active complex in the presence of insulin, but not in its absence. Palmitate reversed the effects of 2-tetradecylglycidate in perfused hearts of starved or diabetic rats. Evidence is given that 2-tetradecylglycidate only reverses effects of starvation and alloxan-diabetes on the proportion of active complex in heart muscle under conditions in which it inhibits fatty acid oxidation. It is concluded that effects of starvation and alloxan-diabetes on the proportion of active complex in heart muscle are dependent on fatty acid oxidation. Insulin had no effect on the proportion of active complex in hearts or diaphragms of fed or starved rats *in vitro*. In perfused hearts of alloxan-diabetic rats, insulin induced a modest increase in the proportion of active complex in the presence of albumin, but not in its absence.

In animal tissues pyruvate dehydrogenase complex (EC 1.2.4.1 + EC 2.3.1.12 + EC 1.6.4.3) exists in interconvertible active (dephosphorylated) and inactive (phosphorylated) forms. Active complex (but not total complex) is decreased in concentration by starvation or induction of alloxan-diabetes, in rat heart, skeletal muscle, liver kidney and adipose tissue, resulting in a diminished rate of glucose oxidation (Wieland *et al.*, 1971*a*, 1972; Hennig *et al.*, 1975; Stansbie *et al.*, 1976; Hagg *et al.*, 1976; Randle *et al.*, 1978). *In vitro* the proportion of active complex in perfused rat heart and liver of fed normal animals is decreased by oxidation of fatty acids, or of ketone bodies (heart only) (Wieland *et al.*, 1971*b*, 1973; Kerbey *et al.*, 1976).

In the present study, the contribution of β-oxidation of long-chain fatty acids to inactivation of the pyruvate dehydrogenase complex in tissues of starved or diabetic rats has been investigated with the fatty acid oxidation inhibitor 2-tetradecylglycidic acid. This fatty acid analogue inhibits oxidation of long-chain fatty acids (but not short-chain fatty acids or ketone bodies) in rat heart, diaphragm and soleus muscles, kidney cortex, hepatocytes and epididymal adipose tissue (Tutwiler *et al.*, 1978, 1979; Tutwiler & Dellevigne, 1979; Pearce *et al.*, 1979; Bowden *et al.*, 1981). On the basis of the results of these studies, a concentration of 100 μM-2-tetradecylglycidate has been used in

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0306-3283/82/100053-08$01.50/1

experiments *in vitro* in the present study. The dose administered *in vivo* was calculated to give a circulating concentration of 100–200 μM.

Experimental

Materials

Male albino Wistar rats (250–350g) were used. Details of feeding, starvation and induction of alloxan-diabetes were as given by Kerbey *et al.* (1977). 2-Tetradecylglycidic acid (McNeil 3802) was generously given by Dr. G. Tutwiler of McNeil Laboratories, Fort Washington, PA 19034, U.S.A. Bovine insulin and bovine plasma albumin were from Sigma (London) Ltd., Poole, Dorset, U.K. Albumin was freed of fatty acids by the method of Chen (1967). Methylcellulose was from Eli Lilly Co., Basingstoke, Hants., U.K. Pyruvate dehydrogenase complex was purified from pig hearts by the method of Kerbey *et al.* (1979). Pyruvate dehydrogenase phosphate phosphatase was partially purified from ox or pig hearts (Severson *et al.*, 1974). Sources of other chemicals and biochemicals were as given by Kerbey *et al.* (1976, 1977, 1979).

Methods

Experiments in vivo. For injection, sodium 2-tetradecylglycidate was complexed with bovine plasma albumin (fatty acid-free) as described for sodium palmitate by Garland & Randle (1964*b*) and dialysed against 0.9% (w/v) NaCl. Rats were anaesthetized with sodium pentobarbitone (60mg/kg, intraperitoneal injection) and injected (via the tail vein) with either 4% (w/v) albumin or 2mM-sodium tetradecylglycidate/4% (w/v) albumin (3.5ml/kg). After 20min tissue samples were removed, freeze-clamped at liquid-N_2 temperature and stored under liquid N_2 (or at −80°C). Epididymal fat-pads, the left kidney (after clamping the renal vascular pedicle) and the heart were obtained from one rat; diaphragm and a sample of liver were obtained from a second rat; and gastrocnemius muscle was frozen *in situ* with a small tissue clamp at liquid-N_2 temperature after exposure by minimum dissection in a third rat.

In some experiments, fed and 48h-starved rats were given 2-tetradecylglycidic acid as a suspension in 0.5% (w/v) methylcellulose by intragastric tube as described by Tutwiler *et al.* (1979). The dose was 25μg/kg at 50, 26 and 2h before anaesthesia with sodium pentobarbitone and intravenous injection with sodium 2-tetradecylglycidate as described above. Control rats received methylcellulose alone. Tissue samples were taken 20min after injection as above.

Experiments in vitro. Hearts were perfused through the aorta at 38°C and a pressure of 7kPa (50mmHg) for 10min by recirculation of 100ml of perfusion medium. The perfusion apparatus incorporated an oxygenator through which the medium was recycled by means of a separate pump. Epididymal fat-pads were preincubated (30min) and incubated (30min) as described by Stansbie *et al.* (1976). Hemidiaphragms were incubated in groups of three to five in 10ml of medium for 30 or 75min at 38°C. The perfusion or incubation medium was bicarbonate-buffered saline gassed with O_2/CO_2 (19:1) (Krebs & Henseleit, 1932) modified to contain 1.8mM-$CaCl_2$. Concentrations of additions (glucose or fructose; insulin, sodium palmitate, sodium 2-tetradecyglycidate, bovine plasma albumin) are given in the Tables or text. Media containing albumin were dialysed at 0°C for 24h against a 10-fold excess of albumin-free medium to equilibrate ions and centrifuged for 30min at 38000*g* to remove insoluble material. At the end of perfusion or incubation, hearts, fat-pads or diaphragms were frozen with a tissue clamp at liquid-N_2 temperature and kept either under liquid N_2 or at −80°C.

Tissue extracts. Frozen tissue was powdered at liquid-N_2 temperature with a pestle and mortar and warmed to −10°C. Extracts for assay of enzymes were made at 0°C in phosphate/EDTA/dithiothreitol with a Polytron homogenizer (Kerbey *et al.*, 1976), except that for extraction of gastrocnemius and diaphragm phosphate concentration was 40mM. Extraction volumes were 25–50ml/g of powder (heart) or 5ml/g (other tissues). Extracts were clarified by centrifuging (Eppendorff 3200 centrifuge) for 2.5min (fat-pads) or 30s (other tissues). For assay of CoA and acetyl-CoA, $HClO_4$ extracts were prepared and neutralized as described by Randle *et al.* (1970).

Assays. Pyruvate dehydrogenase complex (active and inactive forms) and citrate synthase were assayed by methods given in Kerbey *et al.* (1976). CoA was assayed spectrophotometrically at 340nm by the NADH formed in the pyruvate dehydrogenase complex reaction. Concentrations in the cuvette were extract (33%, v/v), 0.1M-Tris/HCl, 0.35mM-EDTA, 0.7mM-$MgCl_2$, 3.5mM-2-mercaptoethanol, 1mM-thiamin pyrophosphate, 0.5mM-NAD^+, 1mM-sodium pyruvate, pH7.5. Reaction (at 30°C) was initiated with 50 munits of pig heart complex and was complete in 3min. There was a linear and stoicheiometric relationship between CoA added and NADH formed over the range tested (5–50nmol/ml of assay mixture). Acetyl-CoA was assayed by the method of Chase (1967), with use of *N*-ethylmaleimide to decrease free thiols in the tissue extracts to acceptable amounts ($A_{412} < 0.3$ in cuvette).

A unit of enzyme converts 1μmol of substrate into product/min at 30°C.

Results and discussion

Experiments with sodium 2-tetradecylglycidate in vivo

In rat heart *in vivo* total pyruvate dehydrogenase complex (sum of active and inactive forms) averaged 20.1 ± 1.0 units/g dry wt of tissue (mean ± S.E.M. for 64 observations) and was not changed by starvation or diabetes. The concentrations of total complex, active complex and the proportion of active complex (Table 1) were comparable with those obtained previously (Whitehouse *et al.*, 1974; Kerbey *et al.*, 1976; Sale & Randle, 1981).

Intravenous injection of sodium 2-tetradecylglycidate (7 μmol/kg body wt., complexed with albumin) had no effect on the concentration or proportion of active pyruvate dehydrogenase complex in the heart in fed normal rats. In rats starved for 48 h, injection of sodium 2-tetradecylglycidate increased the concentration and proportion of active complex to those of fed normal rats, but it had no effect in alloxan-diabetic rats (Table 1). Reasons for the ineffectiveness of sodium 2-tetradecylglycidate in diabetic rats are discussed in the following section on experiments *in vitro* in perfused heart. In kidney, as in heart, the compound has no effect on the activity of the complex in fed normal or alloxan-diabetic rats (Table 1). In 48 h-starved rats injection of sodium 2-tetradecylglycidate increased the concentration and proportion of active complex in kidney, but only to approx. 50% of that of the fed normal control.

The lack of an effect of intravenous sodium 2-tetradecylglycidate on the concentration and proportion of active pyruvate dehydrogenase complex in rat liver, epididymal adipose tissue, gastrocnemius muscle and diaphragm muscle is shown in Table 2. These tissues showed the expected effects of starvation and diabetes on the proportion of active complex. Intragastric 2-tetradecylglycidic acid (see the Experimental section) had no effect on the concentration or proportion of active complex or the response to intravenous sodium 2-tetradecylglycidate in heart, kidney, liver, adipose tissue or gastrocnemius muscle (results not shown).

There were no significant differences in the activity of the mitochondrial marker enzyme citrate synthase, in extracts of hearts or kidneys of fed normal, 48 h-starved or alloxan-diabetic rats (with or without sodium 2-tetradecylglycidate) (Table 1) or in extracts of liver, adipose tissue, gastrocnemius muscle or diaphragm muscle (results not shown).

Experiments with sodium 2-tetradecylglycidate in vitro

Perfused rat heart. The lack of effect of 2-tetradecylglycidate on the proportion of active complex in the heart in alloxan-diabetic rats as compared with 48 h-starved rats could be related to quantitative differences in insulin deficiency and/or fatty acid release and oxidation in the two conditions. The relative concentrations of plasma insulin in fed normal, 48 h-starved and alloxan-diabetic rats are approx. 100:24:1.5 (Randle *et al.*, 1966). In starved rats, plasma concentrations of ketone bodies

Table 1. *Effect of intravenous sodium 2-tetradecylglycidate on pyruvate dehydrogenase (PDH) complex activity in rat heart and rat kidney*

Rats (250–300 g) were anaesthetized and injected intravenously with 4% (w/v) albumin with or without 2 mM-sodium 2-tetradecylglycidate (TDG) (3.5 ml/kg). After 20 min the left kidney and the heart were removed, freeze-clamped and powdered, and extracts were prepared and assayed for citrate synthase and PDH complex. Total PDH complex (sum of active and inactive forms) was assayed after conversion with phosphatase. Activities are means ± S.E.M. for numbers of observations shown (*n*). *$P < 0.001$ for difference from fed normal rats; †$P < 0.001$ for difference from albumin alone; ‡$0.05 > P > 0.01$ for difference from fed normal; for other differences $P > 0.05$. For further details of methods used see the Experimental section.

Rat	Injection	Tissue	*n*	PDH complex (active form) (units/g dry wt.)	PDH complex (active form) (% of total complex)	Citrate synthase (units/g dry wt.)
Fed, normal	Albumin	Heart	12	5.33 ± 0.71	27.9 ± 3.8	609 ± 60.5
	Albumin/TDG		10	5.10 ± 0.86	29.0 ± 3.5	628 ± 50.5
Starved (48 h)	Albumin	Heart	16	0.48 ± 0.10*	2.3 ± 0.4*	576 ± 27.1
	Albumin/TDG		10	6.86 ± 1.05†	32.2 ± 4.9†	628 ± 47.6
Alloxan-diabetic	Albumin	Heart	7	0.24 ± 0.14*	1.5 ± 0.8*	695 ± 90.0
	Albumin/TDG		9	0.43 ± 0.14*	2.0 ± 0.7*	738 ± 76.7
Fed, normal	Albumin	Kidney	8	3.91 ± 0.56	38.2 ± 5.4	87 ± 3.8
	Albumin/TDG		7	4.99 ± 0.56	42.7 ± 6.3	95 ± 3.5
Starved (48 h)	Albumin	Kidney	8	0.35 ± 0.07*	4.0 ± 0.7*	89 ± 8.7
	Albumin/TDG		9	1.71 ± 0.24*†	19.4 ± 1.7†‡	99 ± 10.5
Alloxan-diabetic	Albumin	Kidney	6	0.00 ± 0.00*	0.0 ± 0.0*	87 ± 9.1
	Albumin/TDG		5	0.07 ± 0.04*	0.4 ± 0.4*	97 ± 7.0

Table 2. *Effect of intravenous sodium 2-tetradecylglycidate on pyruvate dehydrogenase (PDH) complex activity in rat liver, epididymal adipose tissue, gastrocnemius muscle and diaphragm muscle*

For details of intravenous treatment and assays see Table 1. Activities are means ± S.E.M. for the numbers of observations in parentheses. Total PDH complex activity (mean ± S.E.M. in units/g wet wt. for numbers of observations in parentheses) was: liver, 1.97 ± 0.08 (41); epididymal adipose tissue, 0.07 ± 0.003 (60); gastrocnemius, 0.34 ± 0.03 (31); diaphragm, 1.21 ± 0.04 (35). *$P < 0.01$, †$0.05 > P > 0.01$ for difference from fed normal. For other differences $P > 0.05$.

		PDH complex (active form) as % total complex in:			
Rat	Injection	Liver	Adipose tissue	Gastrocnemius	Diaphragm
Fed normal	Albumin	9.1 ± 3.1 (11)	15.7 ± 3.1 (14)	24.5 ± 2.6 (8)	5.9 ± 1.9 (6)
	Albumin/TDG	8.1 ± 1.5 (11)	14.2 ± 2.1 (14)	31.0 ± 4.9 (8)	4.8 1.5 (5)
Starved (48 h)	Albumin	3.6 ± 0.6 (7)	7.9 ± 1.3 (12)	3.1 ± 0.5 (4)*	1.3 ± 0.7 (6)
	Albumin/TDG	2.6 ± 0.5 (7)†	3.1 ± 1.0 (8)*	2.1 ± 1.1 (4)*	1.0 ± 0.8 (6)
Alloxan-diabetic	Albumin	1.7 ± 0.3 (4)	6.3 ± 0.9 (6)	4.5 ± 3.7 (4)*	0.3 ± 0.2 (6)*
	Albumin/TDG	2.5 ± 0.6 (4)†	6.4 ± 2.1 (6)†	5.6 ± 2.3 (4)*	0.7 ± 0.3 (6)*

and heart concentrations of free fatty acids, long-chain fatty acyl-CoA and triacylglycerols are higher than those of fed normal rats, but lower than those of severely diabetic rats (Garland & Randle, 1964*b*; Berry *et al.*, 1964; Denton & Randle, 1967*a*,*b*; Blackshear *et al.*, 1974, 1975).

These possibilities have been explored in the perfused rat heart. A low work load was used [aortic perfusion at 7 kPa (50 mmHg)], because sodium 2-tetradecylglycidate is known to inhibit pressure development and cardiac output at high work loads, but not at low work loads (Pearce *et al.*, 1979). In the absence of added fatty acids, lipid oxidation in perfused heart is dependent upon the breakdown of muscle triacylglycerols (increased to a greater extent by alloxan-diabetes than by starvation) (Garland & Randle, 1964*b*; Denton & Randle, 1967*a*,*b*). In the absence of added insulin, action of the hormone is likewise dependent on insulin sequestered in the tissue (most evident in hearts of fed normal rats; Randle *et al.*, 1966). In order to conserve effects of muscle lipids and tissue insulin, the perfusion period was restricted to 10 min.

Perfusion had no effect on total pyruvate dehydrogenase complex activity in rat heart (22.1 ± 0.29 units/g dry wt.; means ± S.E.M., 160 observations combined for fed normal, starved and alloxan-diabetic rats). In hearts of fed normal rats the proportion of active complex (and therefore its concentration) was not altered by perfusion (glucose alone) or by insulin or by sodium 2-tetradecylglycidate. Palmitate (with added insulin) decreased the proportion of active complex by approx. 60%, and its effect was reversed by 2-tetradecylglycidate (Table 3). In hearts of 48 h-starved rats, the proportion of active complex was increased 2.5-fold by perfusion with glucose (no insulin) compared with the value *in vivo*. Insulin had no effect on the proportion of active complex, whereas 2-tetradecylglycidate increased it to that of the fed control. Palmitate (in the presence of insulin) reversed the effect of 2-tetradecylglycidate (Table 3).

In hearts of diabetic rats, perfusion with glucose and albumin (20 g/l) had no effect on the proportion of active complex (compare Tables 1 and 3). Insulin increased the proportion of active complex approx. 20-fold, to a value comparable with that of 48 h-starved rats (Table 3). In the presence of insulin 2-tetradecylglycidate increased the proportion of active complex to that of the fed normal control. In the absence of insulin 2-tetradecylglycidate had no effect. The effect of 2-tetradecylglycidate in the presence of insulin was reversed by palmitate (Table 3). In an earlier study (Kerbey *et al.*, 1976) the proportion of active complex in hearts of diabetic rats perfused with medium containing glucose and insulin was comparable with that seen *in vivo* in the present study (Table 1). In that earlier study perfusion medium contained no albumin. As shown in Table 3, the proportion of active complex in hearts of diabetic rats perfused with glucose and insulin in the absence of albumin was comparable with that *in vivo*, and not significantly different from zero (i.e. there was no effect of insulin in the absence of albumin). Sodium 2-tetradecylglycidate [140 μM, on albumin (2 g/l)] increased the proportion of active complex to that of the non-diabetic control.

Diaphragm muscle. In vivo, 2-tetradecylglycidate had no effect on the proportion of active complex in diaphragm muscle from starved rats, whereas in heart muscle it completely reversed effects of 48 h starvation. In the first series of experiments *in vitro* (Table 4, lines 2 and 3), 2-tetradecylglycidate had no effect on the proportion of active complex in hemidiaphragms of 48 h-starved rats in the presence or absence of insulin. In the second series (Table 4, lines 5 and 6), with a higher molar ratio of 2-tetradecylglycidate/albumin (10:1) and a longer

Table 3. *Effect of sodium 2-tetradecylglycidate in vitro on pyruvate dehydrogenase (PDH) complex activity in perfused rat heart*

Hearts were perfused through the aorta for 10min at 38°C at 7kPa (50mmHg) as described in the Experimental section. Unless otherwise given concentrations in perfusion media were: albumin, 20g/l; sodium 2-tetradecylglycidate (TDG), 0.1mM; glucose, 5.5mM; insulin, 20 units/l; sodium palmitate, 0.5mM. Hearts were freeze-clamped and powdered, and extracts prepared and assayed for PDH complex. Total PDH complex activity (sum of active and inactive forms) was assayed after conversion with phosphatase and averaged 22.1 ± 0.29 units/g dry wt. (mean ± S.E.M. for 152 observations). For further details see the Experimental section. Activities are means ± S.E.M. for numbers of observations in parentheses. *$P < 0.01$ for difference from normal fed control; †$P < 0.001$ for difference due to TDG; ‡$P < 0.001$ for difference due to palmitate; §$P < 0.01$ for difference due to insulin. For other differences $P > 0.05$.

		PDH complex (active form) (% of total PDH complex)		
Rat	Perfusion	Glucose	Glucose/insulin	Glucose/insulin/palmitate
Normal fed	Albumin	28.7 ± 4.2 (8)	30.1 ± 2.8 (23)	12.3 ± 2.3 (12)‡
	Albumin/TDG	33.7 ± 2.4 (8)	33.7 ± 2.7 (18)	30.6 ± 2.8 (11)†
Alloxan-diabetic	Albumin	0.3 ± 0.2 (4)*	6.2 ± 1.9 (14)*§	2.8 ± 0.6 (4)*
	Albumin/TDG	1.7 ± 0.9 (5)*	29.3 ± 5.0 (16)†§	3.9 ± 0.5 (5)*‡
Starved (48h)	Albumin	11.6 ± 0.3 (8)*	9.0 ± 0.8 (8)*	—
	Albumin/TDG	26.0 ± 2.1 (8)†	29.0 ± 0.6 (8)†	6.5 ± 0.1 (8)*‡
Alloxan-diabetic	No albumin	—	1.9 ± 0.82 (6)*	—
	Albumin (2g/l)/TDG (140μM)	—	29.3 ± 4.35 (6)†	—
Normal fed	No albumin	—	26.6 ± 1.67 (6)	—

period of incubation, the compound induced a small (1.3-fold) increase in the proportion of active complex in hemidiaphragms of starved rats. The value attained was only one-third of that of the fed control. There was no effect of insulin *in vitro* on the proportion of active complex in hemidiaphragms of fed or starved rats (Table 4, lines 1–3). The association constants for the binding of 2-tetradecylglycidate to albumin are not available. By assuming association constants of 10^6, 10^5 and 10^3 for the three classes of binding sites (Spector *et al.*, 1969), free concentrations of 2-tetradecylglycidate were computed at 9μM (first series) and 48μM (second series).

Epididymal fat-pads. Sodium 2-tetradecylglycidate (0.6mM) with albumin (8.5g/l) failed to increase the proportion of active complex in epididymal fat-pads incubated for 30min with fructose (2g/l) and insulin (10 units/l) after 30min of preincubation (fructose alone) (results not shown).

Effect of sodium 2-tetradecylglycidate on concentrations of CoA and acetyl-CoA in rat heart

Reversal of the effect of added palmitate on the proportion of active complex in hearts of fed normal rats by 2-tetradecylglycidate (the present work) is attributed to inhibition of palmitate oxidation (see Pearce *et al.*, 1979). The lack of any effect of 2-tetradecylglycidate in the absence of palmitate reinforces this interpretation; in perfused heart of fed rats fatty acid oxidation accounts for no more than 14% of the oxygen consumption (Neely *et al.*, 1972). The concentration of active complex measured in the present study (6 units/g dry wt. at 30°C) is sufficient to account for the requisite rate of pyruvate oxidation (7.5μmol/min per g at 38°C; Neely *et al.*, 1972), allowing for the temperature difference. It is important that the measured concentration of active complex is compatible with the rate of pyruvate oxidation. There are examples in the literature where the measured concentration of active complex may be too small to account for rates of pyruvate oxidation in perfused heart.

It was important in interpreting the effects of 2-tetradecylglycidate on hearts of starved or diabetic rats to attempt to show whether the presence or absence of effects on the proportion of active complex is correlated with the presence or absence of inhibitory effects on the myocardial oxidation of fatty acids. It is difficult to measure this flux reliably by direct methods *in vivo* or *in vitro*, and we have therefore resorted to indirect methods. We have measured overt carnitine acyltransferase (EC 2.3.1.21) by the method of Saggerson (1982) at 80mM-K^+ in mitochondria (isolated from hearts of fed normal rats by the method of Kerbey *et al.*, 1976). Intravenous injection of sodium 2-tetradecylglycidate (as in Table 1) had no effect on overt carnitine acyltransferase. The values (in munits of carnitine acyltransferase/unit of citrate synthase; means ± S.E.M. for four mitochondrial preparations) were 17.5 ± 1.67 (uninjected control) and 20.5 ± 5.04 (2-tetradecylglycidate). There was no evidence in these short-term experiments for irreversible inhibition of overt carnitine acyltransferase by

Table 4. *Effects of sodium tetradecylglycidate and of insulin in vitro on pyruvate dehydrogenase complex activity in rat hemidiaphragms*

Rat hemidiaphragms were incubated at 38°C for the time shown as described in the Experimental section. Concentrations were: glucose, 2 g/l; insulin, 20 units/l; albumin, as shown; 2-tetradecylglycidate (TDG), as shown. Hemidiaphragms were frozen with a tissue clamp at liquid-N_2 temperature, powdered, and extracts prepared and assayed for pyruvate dehydrogenase complex activity (see the Experimental section). Total pyruvate dehydrogenase complex activity (sum of active and inactive forms) averaged 2.26 ± 0.08 units/g wet wt. (mean ± S.E.M. for 12 observations; no difference between fed and starved). Results are means ± S.E.M. with the numbers of observations in parentheses. *$P < 0.01$ for effect of starvation; †$P < 0.01$ for effect of TDG; ‡$P < 0.01$ for time of incubation.

	Incubation		Active complex/total complex (%)	
Rat	Medium	Time (min)	Glucose	Glucose/insulin
Fed	Albumin (8.5 g/l)	30	23.5 ± 1.50 (15)	27.0 ± 5.82 (6)
48 h starved	Albumin (8.5 g/l)	30	2.5 ± 0.51 (15)*	2.6 ± 0.66 (6)*
	Albumin (8.5 g/l)/TDG (0.6 mM)	30	3.0 ± 0.25 (15)*	1.3 ± 0.64 (6)*
Fed	Albumin (2 g/l)	75	—	34.1 ± 0.36 (6)
48 h starved	Albumin (2 g/l)	75	—	8.5 ± 0.65 (6)*‡
	Albumin (2 g/l)/TDG (0.3 mM)	75	—	10.9 ± 0.14 (6)†‡

2-tetradecylglycidate, in contrast with evidence for such inhibition in longer-term experiments using rat liver mitochondria by Tutwiler *et al.* (1980).

In the heart, oxidation of fatty acids increases the concentration of acetyl-CoA and decreases that of CoA, and comparable changes are induced by oxidation of endogenous fatty acids in hearts of alloxan-diabetic rats (Garland & Randle, 1964*b*). These changes are assumed to reflect changes in mitochondrial CoA and acetyl-CoA because in heart as much as 95% of CoA may be mitochondrial (Idell-Wenger *et al.*, 1978). We have therefore investigated effects of 2-tetradecylglycidate on heart concentrations of CoA and acetyl-CoA as an indirect measurement of rates of oxidation of fatty acids.

Results of assay. The results of measurements of heart concentrations of CoA and acetyl-CoA in critical experiments are shown in Table 5. *In vivo* the concentration of acetyl-CoA in the heart was increased 3–4-fold by 48 h of starvation and 11-fold by alloxan-diabetes. The concentration ratio of CoA/acetyl-CoA was decreased 3-fold by starvation and 9-fold by alloxan-diabetes (Table 5, lines 1, 3 and 5). In perfused heart the concentration of acetyl-CoA was increased 4-fold by alloxan-diabetes and the concentration ratio of CoA/acetyl-CoA was decreased 4-fold (Table 5, lines 7 and 9).

In vivo, injection of sodium 2-tetradecylglycidate had no effect on heart concentrations of CoA and acetyl-CoA or the concentration ratio of CoA/acetyl-CoA under experimental conditions in which it had no effect on the proportion of active complex (i.e. in fed normal or alloxan-diabetic rats; Table 5, lines 1, 2, 5 and 6). Under experimental conditions in which 2-tetradecylglycidate restored the proportion of active complex in the heart to that of the normal control, it decreased the concentration of acetyl-CoA, and increased the concentration ratio of CoA/acetyl-CoA, to values that were not significantly different from normal (i.e. in hearts of 48 h-starved rats *in vivo* and in perfused hearts of diabetic rats *in vitro*; Table 5, lines 3, 4, 7 and 8). In the hearts of 48-starved rats *in vivo* the increase in CoA concentration (143 ± 28 nmol/g dry wt.) was not significantly different from the decrease in acetyl-CoA concentration (83 ± 6.3 nmol/g wt.). In the perfused hearts of diabetic rats the increase in CoA (119 ± 24 nmol/g dry wt.) was significantly greater ($P < 0.05$) than the decrease in acetyl-CoA (40 ± 11 nmol/g dry wt.). The reason for this discrepancy is not clear. There was no significant decrease in the concentration of long-chain acyl-CoA with 2-tetradecylglycidate in perfused hearts of diabetic rats *in vitro* or in hearts of 48 h-starved rats *in vivo* (results not shown). However, interpretation of measurements of long-chain acyl-CoA is difficult because its distribution between mitochondrial and extramitochondrial pools is uncertain. If 2-tetradecylglycidate (as its CoA thioester) inhibits carnitine acyltransferase I, as suggested by Tutwiler *et al.* (1980) and Bartlett *et al.* (1981), it may decrease the mitochondrial pool of acyl-CoA and increase the extramitochondrial pool.

Concentrations of CoA and acetyl-CoA were not measured in other tissues because of known problems of compartmentation or because (for skeletal muscle) the methods available were too insensitive.

General discussion and conclusions

These results with 2-tetradecylglycidate appear to show that the effects of starvation and of alloxan-diabetes to decrease the concentration of active complex in rat heart are dependent on fatty acid oxidation. In starvation there was no obvious direct

Table 5. *Effect of sodium 2-tetradecylglycidate on concentrations of CoA and acetyl-CoA in rat heart in vivo and perfused rat heart in vitro*

Rats were injected intravenously with albumin or albumin/sodium 2-tetradecylglycidate (TDG) as described in Table 1, and hearts were excised and frozen after 20 min. *In vitro*, rat hearts were perfused for 10 min with medium containing glucose (5.5 mM) and insulin (20 units/l) with or without albumin (2 g/l)/TDG (100 μM), and frozen. Extracts were prepared and assayed for CoA and acetyl-CoA as described in the Experimental section. Results are means ± S.E.M. for the numbers of observations shown in parentheses (*n*). *$P < 0.01$ for difference from fed normal; †$P < 0.01$ for difference due to TDG. For other differences $P > 0.05$.

			Concn. (nmol/g dry wt.)		
Rat	Injection	*n*	CoA	Acetyl-CoA	Concentration ratio CoA/acetyl-CoA
Fed, normal	Albumin	(13)	281 ± 16.86	32.4 ± 6.19	8.7 ± 1.27
	Albumin/TDG	(8)	258 ± 12.38	28.6 ± 7.81	9.1 ± 1.87
Starved (48 h)	Albumin	(6)	297 ± 22.45	103 ± 4.05*	2.9 ± 0.19*
	Albumin/TDG	(6)	440 ± 17.36*†	19 ± 4.86†	23.5 ± 4.80†
Alloxan-diabetic	Albumin	(9)	339 ± 52.95	355 ± 61.90*	0.95 ± 0.17*
	Albumin/TDG	(10)	297 ± 13.76	318 ± 22.14*	0.94 ± 0.06*
	Perfusion				
Alloxan-diabetic	—	(11)	421 ± 12.69	51.3 ± 10.70*	8.2 ± 1.28*
	Albumin/TDG	(10)	540 ± 20.76†	11.6 ± 1.49†	46.5 ± 4.65†
Fed normal	—	(6)	419 ± 14.81	12.4 ± 2.52	33.9 ± 5.4

effect of decreased circulating insulin. Plasma insulin concentration is not changed by 2-tetradecylglycidate *in vivo* (Tutwiler *et al.*, 1978) and the hormone had no effect *in vitro* in the present study. Three factors may be responsible for the lack of an effect of 2-tetradecylglycidate on the concentration of active complex in the heart in diabetic rats *in vivo*. There may be antagonism by the high concentration of long-chain fatty acids (this was demonstrated in the perfused heart in the present study). In diabetic rats, ketone-body oxidation, which is not inhibited by 2-tetradecylglycidate (Tutwiler *et al.*, 1979), may decrease the concentration of active complex (Wieland *et al.*, 1971*b*). The third possibility is a requirement for insulin; the plasma insulin concentration in alloxan-diabetic rats is much lower than in starved rats.

In perfusion experiments in the present study insulin increased the concentration of active complex in hearts of diabetic rats to that in hearts of starved rats. The hormone also enabled 2-tetradecylglycidate to increase the concentration of active complex in hearts of diabetic rats. For reasons that are not apparent, the effect of insulin on the concentration of active complex (but not on the response to 2-tetradecylglycidate) was only seen in the presence of albumin. Apart from this there was no evidence for a short-term effect of insulin *in vitro* on the concentration of active complex in heart or diaphragm such as is observed consistently in adipose tissue (Jungas, 1971; Coore *et al.*, 1971; Weiss *et al.*, 1971). The insulin preparation used in the present study was effective in adipose tissue (results not shown).

In experiments by Ohlen *et al.* (1978), a much longer period of perfusion with insulin (30 min) increased the concentration of active complex in hearts of alloxan-diabetic rats to that of normal controls. The longer period of perfusion should result in substantial depletion of myocardial triacylglycerol and might also allow longer-term effects of insulin to be manifested. In the studies of Ohlen *et al.* (1978) diabetic rats were treated with insulin for 6 days and then deprived of the hormone for 36 h. In the present study rats were used 48 h after injection of alloxan. The effects of insulin deficiency in the heart preparation used by Ohlen *et al.* (1978) were much less pronounced than in the preparation used in the present study. The proportion of active complex was 13%, compared with 0.3–1.9% in the present study. Ohlen *et al.* (1978) found that insulin restored glucose uptake to normal, whereas in the preparation used by us the hormone fails to restore normal glucose uptake (Randle *et al.*, 1966).

The reason for the relative lack of effect of 2-tetradecylglycidate on the concentration of active complex in rat gastrocnemius, diaphragm, liver, adipose tissue and kidney is not clear. Except for partial reversal of effects of starvation in kidney *in vivo* and in diaphragm *in vitro*, no significant effects were seen. The molecular mechanisms which may regulate interconversion of the pyruvate dehydrogenase complex in these tissues are largely unknown and need investigation. In heart muscle three mechanisms are known which result in approx. 99% of the complex being in the inactive (phosphorylated) form in starved or diabetic rats. The kinase reaction is accelerated by increased concentration of protein factor(s), which may be pyruvate dehydrogenase kinase and/or a protein activator of the kinase (Hutson & Randle, 1978; Kerbey & Randle, 1981, 1982). Fatty acid oxidation may also activate

the kinase reaction by increasing mitochondrial concentration ratios of acetyl-CoA/CoA and of NADH/NAD^+ (Garland & Randle, 1964*a*; Pettit *et al.*, 1975; Cooper *et al.*, 1975; Pearce *et al.*, 1979; the present study). Reactivation of inactive complex by the phosphatase is inhibited by multisite phosphorylation (Sugden *et al.*, 1978; Sale & Randle, 1982). The present study appears to show that inhibition of fatty acid oxidation in heart counteracts the effect of all of these mechanisms and restores the concentration of active complex to normal.

I. D. C. was an Applied Health Sciences Fellow of the National Health and Medical Research Council of Australia. S. J. F. is a Medical Research Council (U.K.) Research Student. This work was also supported by grants from the British Diabetic Association and the Medical Research Council (U.K.). We thank Dr. A. L. Kerbey and Mrs. A. Kearns for their help with these studies.

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