Influence of oleate oxidation on pyruvate production and utilization in hepatocytes isolated from fed rats

Effect of 2-[5-(4-chlorophenyl)pentyl]oxiran-2-carboxylate

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1. Oleate (0.35 and 1.5 mM) decreases, in a concentration-dependent manner, lactate and pyruvate concentrations in hepatocytes, isolated from fed rats, incubated without exogenous substrate. 2. The glycolytic flux, estimated at 18 mM-glucose by [6-³H]-glucose detritiation and apparent production of lactate and pyruvate, is decreased by oleate. The measurement of glycolytic intermediates shows a cross-over at the phosphofructokinase level, which might result from an increased citrate concentration. All those effects are dependent on oleate oxidation in mitochondria, since they are suppressed by 1 μ M-2-[5-(4-chlorophenyl)pentyl]oxiran-2-carboxylate (POCA), an inhibitor of the mitochondrial entry of oleate, but not of its uptake by hepatocytes. 3. The decrease of lactate and pyruvate also results from an oleate-induced enhancement of pyruvate utilization by hepatocytes, as shown by the increase of ¹⁴CO₂ formation from [1-¹⁴C]- and [3-¹⁴C]-pyruvate, especially at low (0.4 mM) pyruvate concentration. Those oleate effects are also suppressed by POCA. They might be due to an enhanced flux through pyruvate carboxylase and pyruvate dehydrogenase, as a result of an oleate-induced increase in the mitochondrial concentrations of pyruvate and acetyl-CoA. 4. Thus oleate oxidation inhibits production of lactate and pyruvate in fed-rat hepatocytes, as it does in other tissues. But, in the liver, it also enhances the mitochondrial utilization of pyruvate. The physiological implications of those findings are discussed.

Fatty acids, ketone bodies and glucose are the main cell fuels. Some tissues, such as brain and blood cells, mainly depend on glucose for their energy supply. Other tissues, such as striated muscle, heart and liver, are able to use either glucose or fatty acids. In muscle and heart, glucose and fatty acids compete as energy donors. When both substrates are present, fatty acids are preferentially oxidized and, as a consequence, glycolysis and pyruvate oxidation are inhibited (News-holme & Randle, 1964; Randle *et al.*, 1964, 1978; Ashour & Hansford, 1983). Fatty acid oxidation results in an increase in cellular citrate and in mitochondrial [NADH]/[NAD⁺] and [acetyl-CoA]/[CoA] ratios, which respectively inhibit

glycolysis at the phosphofructokinase level and pyruvate oxidation (Garland & Randle, 1964; Bremer, 1969; Tsai *et al.*, 1973; Söling & Kleineke, 1976). Thus fatty acids exert a sparing effect on carbohydrates in muscle and heart.

In the liver of fed rats fatty acids may be esterified to triacylglycerols or oxidized to ketone bodies or CO₂. The balance between esterification and oxidation depends on malonyl-CoA, a powerful inhibitor of carnitine acyltransferase (McGarry *et al.*, 1978a). As their CoA derivatives, long-chain fatty acids inhibit acetyl-CoA carboxylase (Ogiwara *et al.*, 1978), decrease malonyl-CoA concentration (Cook *et al.*, 1978) and thus promote their own oxidation. Moreover, malonyl-CoA concentration is correlated with lipogenic activity, which depends on glycogen stores in hepatocytes incubated without exogenous substrates (McGarry

Abbreviation used: POCA, 2-[5-(4-chlorophenyl)-pentyl]oxiran-2-carboxylate.

et al., 1978b); thus carbohydrates appear to exert a sparing effect on fatty acid oxidation in the liver.

As far as we know, there is no evidence for a reciprocal effect of fatty acids on glucose utilization in the liver. A sparing effect of increased plasma fatty acids on liver glycogen content in the exercising rat was observed *in vivo* (Rennie *et al.*, 1976); however, it might be mediated by hormonal factors. We previously reported that 0.35 mM-oleate decreased lactate and pyruvate in hepatocytes isolated from fed rats (Demaugre *et al.*, 1983a). Nomura *et al.* (1983) obtained similar results with octanoate, a medium-chain fatty acid not directly used for triacylglycerol synthesis, but mainly transported into mitochondria by a carnitine-independent process and metabolized by β -oxidation. Effects of octanoate and oleate on accumulation of pyruvate and lactate may be due to a decreased production and/or an increased consumption of pyruvate. The purpose of the present work was to investigate more thoroughly the effects of oleate on glucose metabolism in hepatocytes isolated from fed rats. Experiments were performed at two oleate concentrations, 1.5 mM and 0.35 mM. The latter approaches concentrations that are observed in the plasma and portal vein of fed animals (Rémésy & Demigné, 1983). Since fatty acids may act either by themselves, or by their oxidation, their effects were compared in the presence and absence of an inhibitor of their entry into mitochondria, POCA (sodium salt). Used *in vivo*, this hypoglycaemic agent is the most powerful anti-ketogenic compound at present known (Wolf *et al.*, 1982). Its effects have been shown to result from an inhibition of carnitine acyltransferase (Bartlett *et al.*, 1981).

Materials and methods

Hepatocytes were isolated as reported previously (Demaugre *et al.*, 1978) from male Sprague-Dawley rats (200–250 g) fed on a standard diet containing 3% lipids (AO4; UAR, Villemoisson, France). Glucose (20 mM) was added to the perfusion medium in order to avoid glycogen depletion of hepatocytes during isolation procedures. Cells were incubated with constant shaking in Krebs-Henseleit bicarbonate buffer containing dialysed fatty acid-free albumin (final concn. 2%, w/v). The gas phase was O_2/CO_2 (19:1) and temperature 37°C. Unless otherwise indicated, incubations were performed with cell suspensions of 5×10^6 – 6×10^6 hepatocytes/ml. A preincubation of 30 min was performed with or without POCA before unlabelled or radiolabelled substrates were added. Results of experiments with effectors were always compared with controls performed on the same batch of cells.

Metabolic fates of [$1-^{14}C$]oleate (uptake, esterification to $^{14}CO_2$, formation of radioactive acid-soluble compounds) were studied as reported elsewhere (Demaugre *et al.*, 1983a). Glycolysis was estimated by the rate of formation of 3H_2O from 18 mM-[6- 3H]glucose under experimental conditions described by Hue *et al.* (1978). 3H_2O was separated from [6- 3H]glucose on columns of Dowex AG 1 (borate form) as described by Bontemps *et al.* (1978). 3H_2O initially contaminating [6- 3H]glucose was eliminated by evaporating the radioactive product before use, so that the radioactivity of the blank was less than 5% of that of incubated cells (Buc *et al.*, 1981). Under the experimental conditions described, neither POCA nor oleate has a significant effect on 3H incorporation into glycogen (results not shown). Oxidation of 18 mM-[6- ^{14}C]glucose was studied on the same batch of cells as used for glucose detritiation, by the same procedure as for [$1-^{14}C$]oleate oxidation to $^{14}CO_2$. The actual specific radioactivity of [3- ^{14}C]pyruvate derived from [6- ^{14}C]glucose cannot be determined because of the isotopic dilution by pyruvate derived from glycogen. So $^{14}CO_2$ production was expressed as c.p.m./ 10^6 c.p.m. of added [6- ^{14}C]glucose per 10^9 hepatocytes. Oxidation of [$1-^{14}C$] and [3- ^{14}C]pyruvate was measured as described by Buc *et al.* (1983) in diluted cell suspensions (300 000–400 000 hepatocytes/ml for [$1-^{14}C$]pyruvate decarboxylation and 600 000–700 000 hepatocytes/ml for [3- ^{14}C]pyruvate decarboxylation). [$1-^{14}C$]Pyruvate decarboxylation was measured during 6 min (it was linear for at least 10 min) and [3- ^{14}C]pyruvate decarboxylation during 15 min (it was linear for at least 20 min). Glycogen was determined as described by Chan & Exton (1976), and lipogenesis as described by Harris (1975). Glycolytic intermediates, ATP, ADP, malate, citrate, β -hydroxybutyrate and acetoacetate were assayed as described by Demaugre *et al.* (1983b) at the end of incubation in cell suspensions deproteinized with 0.5 M-HClO₄ and neutralized with KH₂PO₄.

Chemicals

All enzymes and coenzymes were purchased from Boehringer, Meylan, France; fatty acid-free albumin was from Miles; [$1-^{14}C$]oleate was from New England Nuclear; [6- 3H]glucose, [6- ^{14}C]glucose and [3- ^{14}C] and [$1-^{14}C$]pyruvate were from Amersham International. POCA was generously given by Dr. Gerhard Ludwig, Byle Gulden Lomberg Chemische Fabrik G.m.b.H., Konstanz, Germany.

Expression of results and statistical analysis

All results are expressed with reference to the number of incubated hepatocytes. Statistical calculations were performed by Student's paired *t* test.

Results

Effects of POCA on oleate metabolism

In hepatocytes isolated from fed rats, incubated without POCA for 60 min (Fig. 1), 55% of 1.5 mM- $[1-^{14}\text{C}]$ oleate taken up was esterified, and 45% was oxidized to radioactive acid-soluble compounds (mainly ketone bodies) and $^{14}\text{CO}_2$. With 0.35 mM- $[1-^{14}\text{C}]$ oleate (results not shown), 75% of the radioactive substrate was esterified and 25% oxidized, a difference that may result from the effects of acyl-CoA on acetyl-CoA carboxylase (Ogiwara *et al.*, 1978) and thus on malonyl-CoA concentration (Cook *et al.*, 1978). Increasing concentrations of POCA decreased the oxidation of 1.5 mM-oleate, and the maximum effect was already observed at 1 μM -POCA (Fig. 1). At that concentration, POCA decreased total oleate oxidation by 85%, and increased esterification in the same proportion, so that oleate uptake was not modified (see legend of Fig. 1). Similar effects were observed after 30 min of incubation with 1.5 mM-oleate, or after 60 min with 0.35 mM-oleate, but their extent was smaller in the latter situation (results not shown).

Effects of oleate on lactate and pyruvate concentrations

Fig. 1 shows that POCA increased lactate and pyruvate in cellular suspensions when it decreased oleate oxidation. POCA effects on lactate + pyruvate were maximum at 1 μM , as were those on oleate oxidation. POCA had no effect on pyruvate + lactate concentrations by itself, but it suppressed oleate-induced modifications; in 30 min incubations lactate + pyruvate ($\mu\text{mol}/10^9$ cells; mean $\pm$ S.D. for four determinations) was 373 ± 45 without addition, 121 ± 44 with 1.5 mM-oleate, 390 ± 43 with 1 μM -POCA and 375 ± 48 with 1.5 mM-oleate and 1 μM -POCA. Those data show that oleate decreases lactate + pyruvate insofar as the fatty acid may be oxidized in mitochondria. Oleate effects may result from decreased production and/or increased consumption of pyruvate.

Effects of oleate on glycolysis and production of lactate + pyruvate

Glycolysis was estimated at 18 mM-glucose by comparing detritiation of $[6-^3\text{H}]$ glucose, $^{14}\text{CO}_2$

Fig. 1. Effect of POCA on oleate metabolism and lactate + pyruvate content

Hepatocytes were incubated as described in the Materials and methods section without added glucose with 1.5 mM-oleate at different POCA concentrations. Incubation time was 60 min. Oleate uptake, $^{14}\text{CO}_2$, total acid-soluble compounds and triacylglycerol production from oleate are expressed as μmol of $[1-^{14}\text{C}]$ oleate. Similar results were obtained in a second independent experiment. The effects of 1 μM -POCA on lactate + pyruvate content, $[1-^{14}\text{C}]$ oleate uptake and $[1-^{14}\text{C}]$ oleate oxidation (radioactive acid-soluble compounds + $^{14}\text{CO}_2$) were repeatedly measured: lactate + pyruvate was 65 ± 15 and $350 \pm 70^{**}$, oleate uptake 215 ± 35 and 182 ± 32 , and oleate oxidation 100 ± 20 and $20 \pm 4^{**}$ ($\mu\text{mol}/10^9$ hepatocytes; means $\pm$ S.D. for three independent experiments) respectively without and with 1 μM -POCA. When results were significantly different from controls they are indicated as $^{**}P < 0.01$.

Table 1. *Influence of oleate and POCA on glucose metabolism*

Hepatocytes were incubated, as described in the Materials and methods section, with 18 mM-glucose. Incubation time was 15 min. Data are means $\pm$ s.d. for four independent experiments. Glycolytic flux was simultaneously measured in the same incubation by two different methods: $^3\text{H}_2\text{O}$ production from $[6\text{-}^3\text{H}]\text{glucose}$, and apparent lactate + pyruvate production calculated from differences of concentration between the end and the beginning of incubation. $^{14}\text{CO}_2$ production from $[6\text{-}^{14}\text{C}]\text{glucose}$ was measured on the same batch of cells. Results significantly different from controls (without oleate and POCA) are expressed by $**P < 0.01$ or $***P < 0.001$. Abbreviation: nd, not determined.

	Without effector			+ 1 μM -POCA	
	Control	+ 0.35 mM-oleate	+ 1.5 mM-oleate	Control	+ 1.5 mM-oleate
$[6\text{-}^3\text{H}]\text{Glucose}$ detritiation ($\mu\text{mol}/15\text{min}$ per 10^9 cells)	56.0 $\pm$ 14.8	45.5 $\pm$ 14.2**	30.4 $\pm$ 9.6**	55.3 $\pm$ 13.6	56.3 $\pm$ 11.9
Lactate + pyruvate accumulation ($\mu\text{mol}/15\text{min}$ per 10^9 cells)	204.9 $\pm$ 53.6	116.7 $\pm$ 24.9**	0.7 $\pm$ 32.4***	210.5 $\pm$ 53.2	184.4 $\pm$ 52.1
$[6\text{-}^{14}\text{C}]\text{Glucose}$ oxidation (c.p.m./15 min per 10^9 cells)	82500 $\pm$ 7400	nd	62500 $\pm$ 6500**	81500 $\pm$ 9400	85400 $\pm$ 7200

Table 2. *Effects of oleate and POCA on metabolic intermediates*

Hepatocytes were incubated, as described in the Materials and methods section, without added glucose. All results (glycogen as glycosyl equivalents) are expressed as $\mu\text{mol}/10^9$ hepatocytes. They are means $\pm$ s.d. for three to five experiments. Values that are significantly modified by oleate, without POCA, are shown by $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. Values that are significantly modified by POCA at 1.5 mM-oleate are represented by $\dagger\dagger P < 0.01$ and $\dagger\dagger\dagger P < 0.001$.

	Concn. ($\mu\text{mol}/10^9$ hepatocytes)			
	Without oleate		+ 1.5 mM-oleate	
	Control	+ 1 μM -POCA	Control	+ 1 μM -POCA
Glycogen + glucose	2707 $\pm$ 478	2638 $\pm$ 598	2849 $\pm$ 513	2741 $\pm$ 650
Hexose monophosphates	4.46 $\pm$ 0.32	4.34 $\pm$ 0.32	5.01 $\pm$ 0.28**	4.37 $\pm$ 0.23 $\dagger\dagger$
Fructose 1,6-bisphosphate	0.16 $\pm$ 0.03	0.17 $\pm$ 0.04	0.073 $\pm$ 0.017***	0.144 $\pm$ 0.024 $\dagger\dagger\dagger$
Dihydroxyacetone phosphate	1.31 $\pm$ 0.31	1.29 $\pm$ 0.31	1.09 $\pm$ 0.25***	1.28 $\pm$ 0.29 $\dagger\dagger\dagger$
Phosphoenolpyruvate	1.24 $\pm$ 0.19	0.96 $\pm$ 0.32	1.06 $\pm$ 0.16*	1.33 $\pm$ 0.18 $\dagger\dagger\dagger$
ATP	23.55 $\pm$ 3.50	22.72 $\pm$ 2.99	22.65 $\pm$ 2.78	23.45 $\pm$ 3.15
ADP	4.25 $\pm$ 1.47	3.89 $\pm$ 0.95	4.35 $\pm$ 1.33	4.28 $\pm$ 1.31
Malate	2.02 $\pm$ 0.54	1.59 $\pm$ 0.26	4.89 $\pm$ 0.48**	1.96 $\pm$ 0.36 $\dagger\dagger$
Citrate	3.13 $\pm$ 0.38	2.90 $\pm$ 0.51	6.91 $\pm$ 1.23***	2.71 $\pm$ 0.39 $\dagger\dagger$
β -Hydroxybutrate	10.84 $\pm$ 1.83	8.72 $\pm$ 1.64	59.36 $\pm$ 13.77***	10.72 $\pm$ 2.55 $\dagger\dagger\dagger$
Acetoacetate	24.31 $\pm$ 3.74	19.95 $\pm$ 1.21	54.36 $\pm$ 10.30***	23.0 $\pm$ 2.57 $\dagger\dagger\dagger$

production from $[6\text{-}^{14}\text{C}]\text{glucose}$ and the apparent production of lactate + pyruvate (Table 1). Without oleate, the latter was higher than glycolysis measured by glucose detritiation, which shows that glycogenolysis is not completely suppressed under the experimental conditions described (Kimmig *et al.*, 1983). Oleate decreased the three measured parameters, and POCA overcame the effects of oleate, which thus appear to result from oleate oxidation in mitochondria. However, the extent of the effects of oleate was quite different according to the method of glycolysis measurement: for instance, 1.5 M-oleate decreased $[6\text{-}^{14}\text{C}]\text{glucose}$ oxi-

dation to $^{14}\text{CO}_2$ by 20%, $[6\text{-}^3\text{H}]\text{glucose}$ detritiation by 50%, and completely suppressed the apparent production of pyruvate and lactate, which suggests an additional activating effect of oleate on pyruvate utilization.

Glycolytic intermediates were measured so as to localize the oleate-inhibited step(s) (Table 2): oleate increased concentrations of hexose monophosphates and decreased fructose 1,6-bisphosphate and triose phosphates. It increased the malate and citrate concentrations and ketone-body production, but did not modify ATP concentration. All those effects of oleate were suppressed by POCA.

Table 3. Effect of oleate and POCA on $^{14}\text{CO}_2$ release from [1- ^{14}C]- and [3- ^{14}C]-pyruvate

Hepatocytes were incubated as described in the Materials and methods section, with [3- ^{14}C]pyruvate and [1- ^{14}C]pyruvate. Data are means $\pm$ s.d. for four independent experiments. Results significantly different from controls are shown by * $P < 0.05$ or ** $P < 0.01$. The effects of oxalate on oleate-induced modifications of 0.4 mM-[1- ^{14}C]pyruvate oxidation to $^{14}\text{CO}_2$ were tested in two independent experiments. Results were (expressed as nmol of $^{14}\text{CO}_2$ released/ 10^6 cells): without oxalate, 4.59 and 7.05; with 200 μM -oxalate, 2.39 and 3.81 (respectively in the absence and presence of 1.5 mM-oleate).

[Pyruvate] ...	Label ...	Pyruvate oxidation (nmol of $^{14}\text{CO}_2$ /min per 10^6 cells)			
		0.4 mM		1 mM	
		[3- ^{14}C]	[1- ^{14}C]	[3- ^{14}C]	[1- ^{14}C]
Control		0.42 $\pm$ 0.10	4.65 $\pm$ 0.48	0.60 $\pm$ 0.09	7.15 $\pm$ 0.50
POCA (1 μM)		0.38 $\pm$ 0.09	4.42 $\pm$ 0.51	0.59 $\pm$ 0.08	7.05 $\pm$ 0.54
Oleate (0.35 mM)		0.65 $\pm$ 0.11**	7.19 $\pm$ 0.64**	0.77 $\pm$ 0.12*	8.85 $\pm$ 0.76*
Oleate (1.5 mM)		0.69 $\pm$ 0.10**	8.67 $\pm$ 0.82**	0.84 $\pm$ 0.12**	10.85 $\pm$ 1.04**
Oleate (1.5 mM)+ POCA (1 μM)		0.47 $\pm$ 0.09	5.71 $\pm$ 0.65	0.65 $\pm$ 0.10	8.35 $\pm$ 0.68

Effects of oleate oxidation on pyruvate utilization

The effects of oleate on pyruvate utilization, suggested by the results described above, were confirmed by measurements of $^{14}\text{CO}_2$ production from [1- ^{14}C]- and [3- ^{14}C]-pyruvate (Table 3). At 0.4 mM-pyruvate, a concentration similar to that observed *in vivo* or in the usual hepatocyte suspensions incubated for 30–45 min, oleate increased [1- ^{14}C]pyruvate oxidation to $^{14}\text{CO}_2$ in a concentration-dependent manner: the increase was 55% at 0.35 mM- and 85% at 1.5 mM-oleate. Similar results were previously reported in perfused liver by Scholz *et al.* (1978). The effects of oleate on [1- ^{14}C]pyruvate oxidation to $^{14}\text{CO}_2$ were also observed when pyruvate alone was replaced by a more physiological mixture of 0.2 mM-[1- ^{14}C]pyruvate and 2 mM-[1- ^{14}C]lactate (result not shown). Oleate also increased [3- ^{14}C]pyruvate oxidation to $^{14}\text{CO}_2$, by 55% at 0.35 mM-oleate, and by 65% at 1.5 mM-oleate. Oleate activated pyruvate oxidation less as the pyruvate concentration was higher: 1.5 mM-oleate increased 1 mM-[1- ^{14}C]pyruvate oxidation only by 50%, and 1 mM-[3- ^{14}C]pyruvate oxidation by 40%. POCA suppressed all effects of oleate on pyruvate oxidation.

For technical reasons (see the Materials and methods section), measurements of pyruvate oxidation were performed at low hepatocyte concentrations. The effects of pyruvate concentration on [1- ^{14}C]oleate oxidation to $^{14}\text{CO}_2$ have been investigated under those conditions. In diluted cell suspensions without added pyruvate, endogenous pyruvate concentration is very low, about 15 μM : the rate of [1- ^{14}C]oleate oxidation to $^{14}\text{CO}_2$ was then 0.04 ± 0.01 nmol/min per 10^6 hepatocytes, but it increased to 0.16 ± 0.03 in the presence of 1 mM-pyruvate (means $\pm$ s.d. for four experiments). At that low hepatocyte concentration, POCA de-

creased $^{14}\text{CO}_2$ production from oleate significantly only when pyruvate was added to the incubation medium (result not shown).

Discussion

The experiments described in the present paper show that, in hepatocytes isolated from fed rats, oleate inhibits glycolysis and activates pyruvate oxidation, effects which are suppressed by POCA, an inhibitor of fatty acid oxidation.

Validation of POCA as an inhibitor of long-chain fatty acid oxidation in isolated hepatocytes

Most POCA effects have been demonstrated *in vivo* (Wolf *et al.*, 1982) or *in vitro* on mitochondria (Bartlett *et al.*, 1981). When injected into animals, POCA considerably decreases ketogenesis, because as its CoA derivative it inhibits carnitine acyltransferase I and so decreases palmitoyl-CoA oxidation (Bartlett *et al.*, 1981). However, no results are at present available about POCA effects *in vitro* on intact cell preparations. The results presented here show that, in isolated hepatocytes, POCA is a very powerful inhibitor of long-chain fatty acid oxidation, since it decreases oleate oxidation by 90% under optimal conditions. Full effects of POCA on fatty acid oxidation were observed at 1 μM (Fig. 1). It is more effective than tetracyclglycidic acid, another inhibitor of carnitine acyltransferase, the maximal effects of which are obtained at 10 μM (Tutwiler & Dellevigne, 1979). In cells incubated without oleate, 1 μM -POCA modifies none of the measured metabolic parameters (glycolysis, pyruvate oxidation, ATP concentration, steady-state concentrations of glycolytic intermediates, malate and citrate). It has no effect on oleate uptake, which shows that, at

this low concentration, POCA does not compete with fatty acids for the cytosolic stores of CoASH. Moreover, POCA is specific towards the mitochondrial entry of long-chain fatty acids: it has no effect on ketogenesis enzymes and medium-chain fatty acid oxidation, as confirmed by its lack of action on $[1-^{14}\text{C}]$ octanoate oxidation to $^{14}\text{CO}_2$, radioactive acid-soluble compounds and ketone bodies (results not shown). Thus POCA appears as a powerful and specific tool in the study of the effects of long-chain fatty acid oxidation.

Effects of oleate on glycolysis

As was previously shown in muscle and heart (Randle *et al.*, 1964; Rennie & Holloszy, 1977), long-chain fatty acids decrease the glucose consumption of hepatocytes. This inhibition of glycolysis is dependent on oleate concentration and suppressed by POCA. Thus fatty acid oxidation is a prerequisite of oleate effects on glycolysis. As shown by the cross-over of glycolytic intermediates, phosphofructokinase is probably the inhibited step. ATP is not involved, since its concentration is modified neither by oleate nor by POCA. Oleate oxidation increases citrate concentration in whole cells (Table 2), and Siess *et al.* (1978) have reported that this increase is observed in both cytosolic and mitochondrial compartments. Thus it may be postulated that the oleate-induced increase in citrate inhibits phosphofructokinase either directly (Söling & Kleineke, 1976) or by a decreased concentration of its activator, fructose 2,6-bisphosphate (Van Schaftingen & Hers, 1981).

The effects of oleate on citrate concentrations are not mediated by long-chain acyl-CoA induced inhibition of acetyl-CoA carboxylase (Ogiwara *et al.*, 1978), since they are suppressed by POCA, which is without effect on oleate uptake and so probably does not decrease cytosolic acyl-CoA species. As a proof that POCA does not overcome acyl-CoA inhibition of lipogenesis, the latter (expressed as μmol of $^3\text{H}_2\text{O}$ incorporated by 10^9 hepatocytes in 60 min) was respectively 33.1 ± 6.4 without oleate and POCA, 29.4 ± 4.5 with $1 \mu\text{M}$ -POCA, 13.1 ± 2.5 with 1.5 mM -oleate and 8.4 ± 3.1 with both 1.5 mM -oleate and $1 \mu\text{M}$ -POCA (means $\pm$ S.D. for four determinations). Moreover octanoate, a medium-chain fatty acid which is mainly activated in mitochondria and is not appreciably esterified, modifies, similarly to oleate, the citrate concentration, lactate + pyruvate accumulation and glycolysis (results not shown), without inhibiting acetyl-CoA carboxylase or lipogenesis (Nomura *et al.*, 1983).

However, citrate-mediated inhibition of phosphofructokinase cannot completely account for the effects of oleate on the metabolic fate of glucose. Oleate (1.5 mM) inhibited $[6-^3\text{H}]$ glucose

detritiation by 50%, whereas it completely suppressed accumulation of lactate and pyruvate in hepatocyte suspensions (Table 1). But pyruvate production was not abolished by oleate, since $[6-^{14}\text{C}]$ glucose oxidation to $^{14}\text{CO}_2$ was only decreased by 20% under similar conditions. All the effects of oleate on the measured parameters were suppressed by $1 \mu\text{M}$ -POCA. It may be deduced from those results that oleate oxidation not only decreases pyruvate production, but also increases pyruvate consumption in mitochondria.

Effects of oleate on pyruvate oxidation

In hepatocytes isolated from fed rats, oleate increased $^{14}\text{CO}_2$ release from $[1-^{14}\text{C}]$ pyruvate more as pyruvate concentration was lower and oleate concentration higher (Table 3). Similar results were reported, at low pyruvate concentrations, in perfused liver (Scholz *et al.*, 1978) and isolated liver mitochondria (Dennis *et al.*, 1978). Those observations are unexpected in view of the known effects of oleate on pyruvate metabolism in muscle and heart and on pyruvate dehydrogenase activity in the liver (Wieland, 1983). Oleate is expected to raise the $[\text{acetyl-CoA}]/[\text{CoA}]$ and $[\text{NADH}]/[\text{NAD}^+]$ ratios (as shown by the $[\beta\text{-hydroxybutyrate}]/[\text{acetoacetate}]$ ratio in Table 2), which should inhibit pyruvate dehydrogenase by product inhibition and stimulation of pyruvate dehydrogenase kinase. Thus, either the $^{14}\text{CO}_2$ yield from $[1-^{14}\text{C}]$ pyruvate does not in fact reflect pyruvate dehydrogenase activity, or else other factors more than compensate for the effects of acetyl-CoA and NADH. $^{14}\text{CO}_2$ released from $[1-^{14}\text{C}]$ pyruvate may derive not only from pyruvate decarboxylation by the pyruvate dehydrogenase complex, but also from the recycling of $[1-^{14}\text{C}]$ -pyruvate via pyruvate carboxylase. $[^{14}\text{C}]$ Oxaloacetate may then release $^{14}\text{CO}_2$ by oxidation in the tricarboxylic acid cycle, or to a minor extent by decarboxylation in the phosphoenolpyruvate carboxykinase-catalysed reaction (Claus & Pilakis, 1977). However, as was also shown by Dennis *et al.* (1978) on isolated mitochondria, the inhibition of pyruvate carboxylase by oxalate in isolated hepatocytes did not prevent the fatty acid-induced increase of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ pyruvate (see legend of Table 3).

Though our results cannot exclude an enhanced recycling of $[1-^{14}\text{C}]$ pyruvate, it may be concluded that oleate actually increases the flux through pyruvate dehydrogenase at low pyruvate concentrations in isolated hepatocytes, as was also observed in perfused liver (Scholz *et al.*, 1978; Patel *et al.*, 1982) and in isolated liver mitochondria (Dennis *et al.*, 1978). But oleate decreases the flux through pyruvate dehydrogenase at high,

largely supraphysiological, concentrations of pyruvate (5 mM) (Scholz *et al.*, 1978).

When oleate is added to perfused liver (Patzelt *et al.*, 1973) or isolated mitochondria (Batenburg & Olson, 1975) it always decreases pyruvate dehydrogenase activity measured in homogenates at saturating pyruvate concentration. This effect was ascribed to a decrease of the active form of the complex. However, the fatty acid-induced modifications of the flux through pyruvate dehydrogenase measured in intact cells or mitochondria may depend on the pyruvate concentration, which determines pyruvate dehydrogenase activity as both substrate and inhibitor of pyruvate dehydrogenase kinase. Thus the effects of oleate on the flux through pyruvate dehydrogenase might be mediated by oleate-induced modifications of mitochondrial pyruvate concentration. Fatty acids were suggested by Scholz *et al.* (1978) and shown by Dennis *et al.* (1978) to increase mitochondrial pyruvate concentration as a result of an enhanced mitochondrial entry of pyruvate, a consequence of pyruvate exchange with fatty acid-derived acetoacetate through an antiporter previously described by Papa & Paradies (1974). At low pyruvate concentration, that oleate effect must be sufficiently potent to overcome the known inhibitory action of fatty acid oxidation on pyruvate dehydrogenase activity. At high pyruvate concentration, only the inhibitory properties, probably mediated by the increased $[NADH]/[NAD^+]$ and $[acetyl-CoA]/[CoA]$ ratios, are observed, as pyruvate transport seems to be maximal. In agreement with this hypothesis, we have shown that POCA suppressed oleate effects on $^{14}CO_2$ release from $[1-^{14}C]$ -pyruvate, and it decreased ketone-body production.

As shown in Table 3, oleate also increased $^{14}CO_2$ production from $[3-^{14}C]$ pyruvate, especially at low pyruvate concentration, an effect suppressed by POCA. That effect may also result from the increased mitochondrial concentration of pyruvate and the subsequent activation of both pyruvate dehydrogenase and pyruvate carboxylase, whose K_m for pyruvate is high (0.4 mM) (Scrutton *et al.*, 1969). Furthermore, under normal conditions the latter enzyme is not saturated by its activator, acetyl-CoA, the concentration of which is raised by oleate oxidation. Previous data of Buc *et al.* (1983) showed that inhibition of pyruvate carboxylase in a fatty acid-free medium decreased $^{14}CO_2$ production from $[3-^{14}C]$ pyruvate without obvious modifications of $^{14}CO_2$ release from $[1-^{14}C]$ pyruvate. It was deduced from those observations that pyruvate carboxylase activity was involved in the regulation of the tricarboxylic acid cycle. Results presented here do not allow us to conclude unequivocally that oleate oxidation

enhances the pyruvate-derived flux through the tricarboxylic acid cycle, since pyruvate dehydrogenase activation and acetyl-CoA formation from oleate modify acetyl-CoA concentration and specific radioactivity in isolated hepatocytes incubated with $[3-^{14}C]$ pyruvate. However, as suggested by Williamson *et al.* (1969) and Brocks *et al.* (1980), enhanced pyruvate carboxylase activity and subsequently increased mitochondrial oxaloacetate concentration may account for the raised citrate concentration in hepatocytes incubated with oleate.

As a whole, the simultaneous activation of pyruvate dehydrogenase and pyruvate carboxylase by oleate oxidation may explain the concomitant increase of $^{14}CO_2$ released from $[1-^{14}C]$ - and $[3-^{14}C]$ -pyruvate, and the increased pyruvate consumption observed in hepatocytes incubated with oleate.

Physiological considerations

Data presented here show that oleate oxidation inhibits glycolysis and increases the mitochondrial oxidation of pyruvate in hepatocytes isolated from fed rats. Those effects are already observed at an oleate concentration (0.35 mM) similar to the fatty acid concentration measured in portal vein (Rémésy & Demigné, 1983) and peripheral blood (Rémésy & Demigné, 1983; Rennie *et al.*, 1976) of rats fed on a standard diet containing 3% lipids. However, it is difficult to extrapolate our results *in vitro* to the much more complicated situation *in vivo* where liver cells receive a complex mixture of nutrients and hormones.

Glycolysis inhibition by fatty acid oxidation at the phosphofructokinase level, already described in heart and muscle, is relevant to the classical sparing effect of fatty acids towards glucose. However, the significance of the activation of pyruvate oxidation by physiological concentration of oleate in fed animals remains unclear. It may simply be a by-product of the increased pyruvate concentration in mitochondria primarily aimed at an enhancement of pyruvate carboxylation, a necessary step in the conversion of C_3 substrates into glycogen postulated by Newgard *et al.* (1983) to occur in re-fed animals.

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