THE EFFECTS OF ANTIKETOGENIC AGENTS AND PYRUVATE ON THE OXIDATION OF PALMITATE IN ISOLATED HEPATOCYTES

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Received 8 March 1979
Revised version received 9 May 1979

1. Introduction

Malonyl-CoA, an intermediate in the fatty acid synthesis pathway, has been reported as a potent inhibitor of carnitine palmitoyltransferase I. Thus it has been postulated that this compound plays a central role in the regulation of fatty acid oxidation and ketogenesis [1–3].

We have reported that glucagon significantly stimulates the oxidation of fatty acids in hepatocytes isolated from fed or fasted-carbohydrate refed rats [4]. The high rates of fatty acid oxidation, in glucagon-stimulated cells, were accompanied by an increase in the level of long-chain acyl-CoA (most probably the extramitochondrial pool) and long-chain acylcarnitines [4]. It is difficult to explain these observations exclusively by an increase in the activity of carnitine palmitoyltransferase I due to lowered concentration of malonyl-CoA in the glucagon-stimulated cells.

Here the effect of a series of known antiketogenic agents and pyruvate on the oxidation of palmitate, in control and glucagon-stimulated cells, was investigated. The changes in the level of long-chain acyl-CoA, long-chain acylcarnitines and *sn*-3-glycerophosphate under different rates of fatty acid oxidation were determined. It has been found that the steady-state concentrations of long-chain acylcarnitines and long-chain acyl-CoA are positively correlated with the rates of palmitate oxidation under the conditions studied. It is suggested that an important regulatory site in the esterification pathway contributes to the regulation of fatty acid oxidation in the liver through the changes in the level of extramitochondrial acyl-CoA.

2. Materials and methods

[1-^{14}C]Palmitate was obtained fron NEM Chemicals GmbH, FRG. –[*methyl*-^{3}H]Carnitine was prepared according to [5]. Essentially fatty acid free bovine serum albumin, coenzyme A, palmitoyl-CoA, β-hydroxybutyrate dehydrogenase and *sn*-3-glycerophosphate dehydrogenase were purchased from Sigma Chemical Co, St Louis, MO. Other reagents used were of highest purity available.

The hepatocytes were isolated, from rats fed a usual laboratory diet, according to [6]. Ca^{2+}-free Krebs-Henseleit bicarbonate buffer was used as a suspension and incubation medium. The incubation was started by addition of 1 ml cell suspension (5–7 mg protein) to 1 ml buffer containing 0.58 mM bovine serum albumin and either 0.5 mM [1-^{14}C]palmitate (500 cpm/nmol) and 0.5 mM non-labelled carnitine or 0.5 mM palmitate and 0.5 mM [^{3}H]carnitine (4000 cpm/nmol) if not indicated otherwise. When present, lactate, pyruvate, ethanol, sorbitol and dihydroxyacetone were added to 10 mM, fructose to 5 mM, glycerol to 7.5 mM and glucagon to 4×10^{-8} M. All the determinations were made after 30 min incubation at 37°C since the rates of fatty acid oxidation and ketogenesis have been found to be linear for at least 1 h under the conditions used [4]. The measurements of acid-soluble products, $^{14}CO_2$, total ketone bodies, long-chain acylcarnitines and long-chain acyl-CoA were done as in [4–7]. *sn*-3-Glycerophosphate was determined with *sn*-3-glycerophosphate dehydrogenase. Carnitine was determined according to [8]; protein by the Lowry method [9].

The results are presented as mean ± SEM from

3–5 cell preparations. Student's *t*-test has been used for the statistical treatment of the results.

3. Results and discussion

Table 1 shows that all of the antiketogenic agents used in the experiments moderately decreased the rate of fatty acid oxidation in control hepatocytes isolated from fed rats. However, their inhibitory effects became apparent after the oxidation rate of palmitate had been stimulated by glucagon. All the agents used, except for pyruvate, increased the concentration of *sn*-3-glycerophosphate but to very different degrees, in agreement with [10].

The extent of inhibition of palmitate oxidation (to acid soluble products and CO_2) by different antiketogenic agents was very similar (39–47%) in spite of their different effects on *sn*-3-glycerophosphate concentration and redox situation both in the cytoplasm and in the mitochondria (note that paired substrates oxidized/reduced were used). This does not exclude the possibility that under certain conditions the concentration of *sn*-3-glycerophosphate may be rate-limiting for esterification. Glucagon decreased significantly the concentration of *sn*-3-glycerophosphate under all conditions except in the presence of glycerol, when the concentration of *sn*-3-glycerophosphate was extremely high (table 1). The K_m value for *sn*-3-glycerophosphate acyltransferase reported in the literature varies from 0.1–1 mM [11–13]. The observed concentration of this substrate was 0.86 mM in control cells and was decreased in the presence of glucagon to 0.46 mM (assuming the intracellular volume of 3 μl/mg protein [14]). Thus, it is difficult to predict how much influence such variations in *sn*-3-glycerophosphate concentration might have on the activity of glycerophosphate acyltransferase.

Table 1 and fig.1A show that there is a good correlation between the rate of palmitate oxidation and the level of long-chain acylcarnitines. The latter has been shown to vary depending on the intracellular

Table 1
The effect of antiketogenic agents and pyruvate on the metabolism of palmitate in isolated hepatocytes

	Additions	Acid-soluble products	$^{14}CO_2$	Total ketone bodies	Long-chain acyl-CoA × 10	Long-chain acylcarnitine × 10	*sn*-3-glycero-phosphate
A	None	14.7 (0.5)	5.8 (0.3)	88.4 (1.7)	14.1 (1.0)	22.1 (0.8)	2.58 (0.17)
	Lactate	12.7 (0.2)	4.6 (0.2)	72.4 (0.9)	12.2 (0.8)	14.0 (0.3)	3.40 (0.12)
	Pyruvate	18.1 (0.3)	7.1 (0.4)	125.8 (1.9)	18.3 (1.1)	22.3 (0.7)	1.09 (0.09)
	Glycerol	14.0 (0.4)	1.8 (0.1)	82.2 (1.3)	8.3 (0.4)	12.1 (0.3)	65.2 (2.75)
	Dihydroxy-acetone	13.6 (0.2)	3.6 (0.2)	78.0 (1.2)	11.1 (0.6)	15.2 (0.4)	6.90 (0.36)
	Sorbitol	13.6 (0.2)	2.8 (0.1)	71.8 (0.9)	9.0 (0.3)	12.0 (0.3)	17.90 (0.48)
	Fructose	13.3 (0.2)	4.1 (0.1)	78.0 (0.8)	10.0 (0.4)	15.1 (0.3)	2.96 (0.15)
	Ethanol	17.2 (0.5)	0.9 (0.1)	133.8 (2.0)	10.5 (0.5)	16.2 (0.3)	19.60 (1.25)
B	None	29.8 (0.6)[a]	7.3 (0.3)[c]	198.6 (4.1)[a]	24.0 (1.3)[a]	41.3 (0.9)[a]	1.39 (0.08)[a]
	Lactate	14.2 (0.8)[d]	6.0 (0.3)[b]	81.0 (1.0)[a]	16.3 (1.0)[c]	15.1 (0.3)[c]	2.00 (0.05)[a]
	Pyruvate	31.2 (0.6)[a]	7.6 (0.3)[d]	220.0 (4.8)[a]	26.2 (1.2)[a]	42.1 (1.0)[a]	< 0.7
	Glycerol	17.0 (0.4)[a]	3.2 (0.1)[a]	93.2 (1.2)[a]	10.0 (0.4)[c]	14.0 (0.4)[b]	60.30 (2.40)[d]
	Dihydroxy-acetone	15.0 (0.2)[a]	4.7 (0.1)[a]	84.0 (0.8)[b]	14.2 (0.5)[b]	19.3 (0.3)[a]	3.20 (0.30)[a]
	Sorbitol	16.2 (0.6)[b]	3.6 (0.1)[a]	86.3 (0.9)[a]	10.8 (0.3)[b]	14.0 (0.3)[a]	9.50 (0.75)[a]
	Fructose	15.4 (0.4)[a]	5.6 (0.1)[a]	84.4 (0.8)[a]	11.4 (0.2)[c]	16.0 (0.2)[c]	2.10 (0.07)[a]
	Ethanol	21.4 (0.4)[a]	1.2 (0.1)[d]	150.5 (2.1)[a]	13.2 (0.4)[b]	20.1 (0.3)[a]	10.70 (0.9)[a]

Different from the results obtained in the absence of glucagon with: [a] $p < 0.005$, [b] $p < 0.01$, [c] $p < 0.05$, [d] $p > 0.05$, NS

Acid-soluble products and $^{14}CO_2$ are expressed in nmol [1-^{14}C]palmitate. mg protein^{-1}, other results in nmol. mg protein^{-1}. (A) in the absence of glucagon; (B) in the presence of glucagon

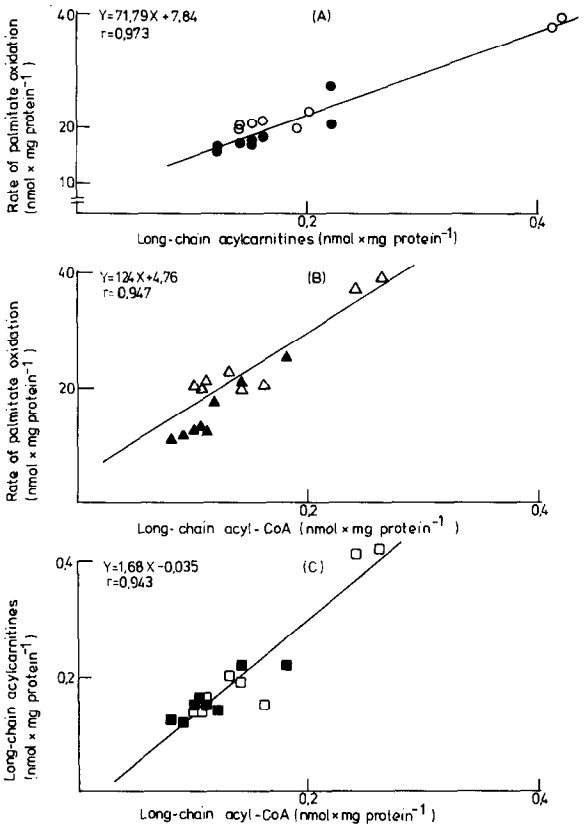

Fig.1. A. The correlation between the rate of palmitate oxidation and the level of long-chain acylcarnitines in the liver cells. B. The correlation between the rate of palmitate oxidation and the level of long-chain acyl-CoA in the liver cells. C. The correlation between the level of long-chain acylcarnitines and long-chain acyl-CoA in the liver cells. The results are the same as in table 1. The rate of palmitate oxidation is the sum of palmitate oxidized to acid-soluble products and $^{14}CO_2$. Linear regression analysis has been used to calculate correlation coefficients (r), interception and the slope. Open symbols, in the presence of glucagon; closed symbols, in the absence of glucagon.

concentration of carnitine [14] and the concentration of palmitate in the medium [4]. Under the conditions used in the present experiments, the concentration of intracellular carnitine and palmitate in the medium were approximately equal at a given time point in all the samples. The rate of palmitate uptake was almost identical under all conditions since the changes in the rates of oxidation were balanced by the changes in the rates of triacylglycerol synthesis (not shown). The carnitine transport into the cells is not significantly influenced by added agents since a specific carrier for carnitine transport apparently exists in the plasma membrane [14]. It seems likely then that the level of long-chain acylcarnitines is a reflection of a flux through the carnitine palmitoyltransferase I, which in turn, may be a consequence of changes in the level of long-chain acyl-CoA (fig.1C) and/or changes in the level of malonyl-CoA [3]. It is difficult to know how much weight one should put on the correlation found between the rate of palmitate oxidation and the level of long-chain acyl-CoA (fig.1B) since it was not possible to measure directly the extra- and intramitochondrial acyl-CoA. However, glucagon causes the increase in the long-chain acyl-CoA also under conditions where the flux through carnitine palmitoyltransferase is strongly inhibited by lowering the intracellular carnitine concentration and/or addition of (+) decanoylcarnitine (table 2). At low concentration of carnitine, (+) decanoylcarnitine inhibits the oxidation by ~75% and causes a small increase in long-chain acyl-CoA. Glucagon stimulates oxidation and increases the level of long-chain acyl-CoA by approximately the same factor in the presence and in the absence of the inhibitor. Carnitine does not affect the level of long-chain acyl-CoA in liver cells of fed rats (table 2). The results strongly suggest that the observed increase in the level of long-chain acyl-CoA, in the presence of glucagon, represents an increase in the extramitochondrial pool. The good correlation found between the level of acyl-CoA and the rate of oxidation favours therefore the hypothesis that the rate of fatty acid oxidation is regulated also at a site different from that of carnitine palmitoyltransferase I. If this enzyme was the only rate-limiting one and thus regulatory in the oxidation pathway, the level of long-chain acylcarnitines and the rates of oxidation would be expected to vary independently of the long-chain acyl-CoA level. The addition of cAMP to the medium perfusing the liver has been shown [15] to decrease the activity of the microsomal glycerophosphate acyltransferase when assayed in vitro. Also phosphatidate phosphohydrolase has been reported to be regulatory on the rate of esterification and the activity of this enzyme has been found to correlate with the level of *sn*-3-glycerophosphate under several conditions [16–18]. The changes in the rate of esterification may condition the level of long-

Table 2
The effect of glucagon on the changes in the level of long-chain acyl-CoA under different rates of palmitate oxidation

	−Glucagon		+Glucagon			
	Oxidation products	Long-chain acyl-CoA	Oxidation products	% Change[d]	Long-chain acyl-CoA	% Change[d]
Low carnitine	12.1 (1.0)	0.128 (0.016)	23.4 (1.3)[a]	+93	0.205 (0.018)[c]	+60
Low carnitine + (+) DC	3.1 (0.2)	0.148 (0.018)	5.6 (0.3)[a]	+81	0.230 (0.017)[c]	+55
High carnitine	21.1 (1.1)	0.132 (0.015)	39.0 (1.5)[a]	+85	0.211 (0.016)[c]	+60
High carnitine + (+) DC	14.6 (0.9)	0.138 (0.014)	24.0 (1.2)[a]	+64	0.218 (0.012)[b]	+58

Different from the results obtained in the absence of glucagon with: [a]$p < 0.001$, [b]$p < 0.005$, [c]$p < 0.02$, [d]% change due to glucagon

The hepatocytes were preincubated with or without 1 mM (−)carnitine for 45 min at 37°C, so that the intracellular concentration of carnitine was: 0.3–0.5 nmol. mg protein^{-1}, low carnitine; or 1.8–2.5 nmol. mg protein^{-1}, high carnitine. The oxidation products are presented as a sum of acid-soluble products and $^{14}CO_2$ in nmol palmitate. mg protein^{-1}; long-chain acyl-CoA in nmol. mg protein^{-1}. (+) Decanoylcarnitine ((+) DC) was added to 1 mM

chain acyl-CoA and this, in turn, the level of long-chain acylcarnitines and the rates of oxidation.

Acknowledgements

The author wishes to express appreciation to Drs David M. Gibson and Jon Bremer for many helpful discussions. This research was supported by grants from the National Institute of Health, AM 19299; Marion County Chapter of the American Heart Association, Indiana Affiliate.

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